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About the Journal

Overview

Pertanika Journal of Tropical Agricultural Science (JTAS) is the official journal of Universiti Putra Malaysia published by UPM Press. It is an open-access online scientific journal which is free of charge. It publishes the scientific outputs. It neither accepts nor commissions third party content.

Recognized internationally as the leading peer-reviewed interdisciplinary journal devoted to the publication of original papers, it serves as a forum for practical approaches to improving quality in issues pertaining to tropical agriculture and its related fields.

JTAS is a **quarterly** (*February, May, August and November*) periodical that considers for publication original articles as per its scope. The journal publishes in **English** and it is open to authors around the world regardless of the nationality.

The Journal is available world-wide.

Aims and scope

Pertanika Journal of Tropical Agricultural Science aims to provide a forum for high quality research related to tropical agricultural research. Areas relevant to the scope of the journal include: agricultural biotechnology, biochemistry, biology, ecology, fisheries, forestry, food sciences, genetics, microbiology, pathology and management, physiology, plant and animal sciences, production of plants and animals of economic importance, and veterinary medicine.

History

Pertanika was founded in 1978. A decision was made in 1992 to streamline Pertanika into three journals as Journal of Tropical Agricultural Science, Journal of Science & Technology, and Journal of Social Sciences & Humanities to meet the need for specialised journals in areas of study aligned with the interdisciplinary strengths of the university.

After 37 years, as an interdisciplinary journal of Agriculture, the revamped Journal, a leading agricultural journal in Malaysia now focuses on tropical agricultural research and its related fields.

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Pertanika is almost 40 years old; this accumulated knowledge has resulted in Pertanika JTAS being abstracted and indexed in SCOPUS (Elsevier), Thomson (ISI) Web of Knowledge [BIOSIS & CAB Abstracts], EBSCO & EBSCOhost, DOAJ, Agricola, Cabell's Directories, Google Scholar, MyAIS, ISC & Rubriq (Journal Guide).

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Pertanika policy prohibits an author from submitting the same manuscript for concurrent consideration by two or more publications. It prohibits as well publication of any manuscript that has already been published either in whole or substantial part elsewhere. It also does not permit publication of manuscript that has been published in full in Proceedings.

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An ISSN is an 8-digit code used to identify periodicals such as journals of all kinds and on all media–print and electronic. All Pertanika journals have ISSN as well as an e-ISSN.

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A decision on acceptance or rejection of a manuscript is reached in 3 to 4 months (average 14 weeks). The elapsed time from submission to publication for the articles averages 5-6 months.

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Most scientific papers are prepared according to a format called IMRAD. The term represents the first letters of the words **I**ntroduction, **M**aterials and Methods, **R**esults, **A**nd, **D**iscussion. IMRAD is simply a more 'defined' version of the "IBC" [Introduction, Body, Conclusion] format used for all academic writing. IMRAD indicates a pattern or format rather than a complete list of headings or components of research papers; the missing parts of a paper are: *Title, Authors, Keywords, Abstract, Conclusions*, and *References*. Additionally, some papers include Acknowledgments and Appendices.

The *Introduction* explains the scope and objective of the study in the light of current knowledge on the subject; the *Materials and Methods* describes how the study was conducted; the *Results* section reports what was found in the study; and the *Discussion* section explains meaning and significance of the results and provides suggestions for future directions of research. The manuscript must be prepared according to the Journal's **INSTRUCTIONS TO AUTHORS**.

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Authors are notified with an acknowledgement containing a *Manuscript ID* on receipt of a manuscript, and upon the editorial decision regarding publication.

Pertanika follows a **double-blind peer-review** process. Manuscripts deemed suitable for publication are usually sent to reviewers. Authors are encouraged to suggest names of at least three potential reviewers at the time of submission of their manuscript to Pertanika, but the editors will make the final choice. The editors are not, however, bound by these suggestions.

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Notification of the editorial decision is usually provided within ten to fourteen weeks from the receipt of manuscript. Publication of solicited manuscripts is not guaranteed. In most cases, manuscripts are accepted conditionally, pending an author's revision of the material.

As articles are double-blind reviewed, material that might identify authorship of the paper should be placed only on page 2 as described in the first-4 page format in Pertanika's **Instructions to Authors** given at the back of this journal.

The Journal's peer-review

In the peer-review process, three referees independently evaluate the scientific quality of the submitted manuscripts.

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What happens to a manuscript once it is submitted to *Pertanika*? Typically, there are seven steps to the editorial review process:

1. The Journal's chief executive editor and the editorial board examine the paper to determine whether it is appropriate for the journal and should be reviewed. If not appropriate, the manuscript is rejected outright and the author is informed.

2. The chief executive editor sends the article-identifying information having been removed, to three reviewers. Typically, one of these is from the Journal's editorial board. Others are specialists in the subject matter represented by the article. The chief executive editor asks them to complete the review in three weeks.

   Comments to authors are about the appropriateness and adequacy of the theoretical or conceptual framework, literature review, method, results and discussion, and conclusions. Reviewers often include suggestions for strengthening of the manuscript. Comments to the editor are in the nature of the significance of the work and its potential contribution to the literature.

3. The chief executive editor, in consultation with the editor-in-chief, examines the reviews and decides whether to reject the manuscript, invite the author(s) to revise and resubmit the manuscript, or seek additional reviews. Final acceptance or rejection rests with the Edito-in-Chief, who reserves the right to refuse any material for publication. In rare instances, the manuscript is accepted with almost no revision. Almost without exception, reviewers' comments (to the author) are forwarded to the author. If a revision is indicated, the editor provides guidelines for attending to the reviewers' suggestions and perhaps additional advice about revising the manuscript.

4. The authors decide whether and how to address the reviewers' comments and criticisms and the editor's concerns. The authors return a revised version of the paper to the chief executive editor along with specific information describing how they have answered' the concerns of the reviewers and the editor, usually in a tabular form. The author(s) may also submit a rebuttal if there is a need especially when the author disagrees with certain comments provided by reviewer(s).

5. The chief executive editor sends the revised paper out for re-review. Typically, at least one of the original reviewers will be asked to examine the article.

6. When the reviewers have completed their work, the chief executive editor in consultation with the editorial board and the editor-in-chief examine their comments and decide whether the paper is ready to be published, needs another round of revisions, or should be rejected.

7. If the decision is to accept, an acceptance letter is sent to all the author(s), the paper is sent to the Press. The article should appear in print in approximately three months.

   The Publisher ensures that the paper adheres to the correct style (in-text citations, the reference list, and tables are typical areas of concern, clarity, and grammar). The authors are asked to respond to any minor queries by the Publisher. Following these corrections, page proofs are mailed to the corresponding authors for their final approval. At this point, **only essential changes are accepted**. Finally, the article appears in the pages of the Journal and is posted on-line.

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ABSTRACTING/INDEXING

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Contents

Foreword

Welcome to the **Fourth Issue 2015** of the Journal of Tropical Agricultural Science (JTAS)!

JTAS is an open-access journal for the Tropical Agricultural Science published by Universiti Putra Malaysia Press. It is independently owned and managed by the university and run on a non-profit basis for the benefit of the world-wide science community.

This issue contains **13 articles**, out of which **one** is an invited review article, **one** is a review article, **six** are regular research papers and **five** are derived from the 2nd International Conference on Kenaf and Allied Fibres, ICKAF 2013. The authors of these articles are from **Australia**, **India**, **Nigeria** and **Malaysia**.

The invited review paper discusses in detail the topic of current and future challenges in conserving freshwater biodiversity in the molecular perspective (*Jane M. Hughes*). The relation of arbuscular mycorrhizal symbiosis and water stress is well discussed in the review article (*Navnita Sharma, Kuldeep Yadav, Jagbeer Cheema, Neetu Badda* and *Ashok Aggarwal*).

The first research paper by researchers from Universiti Putra Malaysia discussed the meat characteristics of red jungle fowl (*Gallus gallus Spadiceus*), Malaysian domestic chickens (*Gallus gallus Domesticus*) and commercial broilers (*Lokman I. H., Goh, Y. M., Sazili A. Q., Noordin M. M.* and *Zuki A. B. Z.*). The second research paper, written by researchers from Nigeria, discusses the influence of nut size, hydro priming duration and storage period of seedling emergence and early seedling vigour characteristics in cashew (*Anacardium occidentale* L.) (*Adebisi, M. A., Kehinde, T. O., Abdul-Rafiu, A. M., Amira. J. O., Oyewumi, A. A., Oni, O. D.* and *Onyeka, C. V.*). The next research paper describes the effects of extended heating time and post-urea treatment on formaldehyde emission and properties of phenolic *compreg* rubberwood (*Zaidon, A., Lee, S. H., Rasmina, H., Roslinda, S., Mariani Ayu, O.* and *Shuhaibah, S.*)

In the following research paper, a group of researchers from Universiti Putra Malaysia reports on the life table of *Cochlochila bullita* Stål (Hemiptera: Tingidae) on *Orthosiphon aristatus* (Blume) Miq. and *Ocimum basilicum* L. in laboratory conditions (*Tan Li Peng, Ahmad Said Sajap, Lee Han Jeen, Lee Seng Hua* and *Lum Wei Chen*) while another group of researchers from Universiti Sultan Zainal Abidin, Malaysia reports on glutathione functions on the physiological characteristics of corn plants to enhance Mn-induced corn production (*Nur Inani, Mohd Nozulaidi, Mohd Khairi, Abdulaziz Rabiu Abdulkadir* and *Md Sarwar Jahan*). The last research paper in this issue, which describes a new model sampling site for a secondary forest area, focusses on the colonisation of the dung beetle (Coleoptera: Scarabaeidae) of smaller body size in Bangi forest reserve, Selangor, Malaysia (*Muhaimin, A. M. D., Hazmi, I. R.* and *Yaakop, S.*).

I conclude this issue with five articles arising from the ICKAF 2013 international conference: a review of the compression moulding of natural fibre-reinforced thermoset composites; material processing and characterisations (*Ismail, N. F., Sulong, A. B., Muhamad, N., Tholibon, D., MdRadzi, M. K. F.* and *WanIbrahim, W. A. S.*); the effect of cutting speed on cutting torque and cutting power of varying kenaf-stem diameters at different moisture content (*Dauda, S. M., Ahmad, D., Khalina, A.* and *Jamarei, O.*); reinforcing mechanical, water absorption and barrier properties of poly(lactic acid) composites with kenaf-derived cellulose of thermally-grafted aminosilane (*Tee, Y. B., Rosnita, A. T., Khalina, A., Chin, N. L., Roseliza, K. B.* and *Khairul Faezah, M. Y.*); tensile strength of some natural-fibre composites (*Salleh, J., Mohd Yusoh, M. K.* and *Ruznan, W. S.*); and the effect of filler loading and NaOH addition on mechanical properties of moulded kenaf/polypropylene composite (*MdRadzi, M. K. F., Sulong, A. B., Muhamad, N., MohdLatiff, M. A.* and *Ismail, N. F.*)

I anticipate that you will find the evidence presented in this issue to be intriguing, thought-provoking and useful in reaching new milestones in your own research. Please recommend the journal to your colleagues and students to make this endeavour meaningful.

I would also like to express my gratitude to all the contributors, namely, the authors, reviewers and editors, who have made this issue possible. Last but not least, the editorial assistance of the journal division staff is fully appreciated.

JTAS is currently accepting manuscripts for upcoming issues based on original qualitative or quantitative research that opens new areas of inquiry and investigation.

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Invited Review Article

Current and Future Challenges of Conserving Freshwater Biodiversity: A Molecular Perspective

Jane M. Hughes

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ABSTRACT

As the world's population continues to grow, human water needs are growing accordingly, thus reducing the water available for sustaining our freshwater biodiversity. This is likely to be further exacerbated in areas where rainfall will decrease as a result of global climate change. Molecular ecologists have contributed substantially in recent years to our understanding of first, the levels and patterns of current biodiversity and second, to understanding patterns of connectivity among populations of aquatic species and their significance for their conservation and management. Both are critical for prioritisation of areas for protection and for designing rehabilitation programmes. In this paper, I attempt to synthesise our understandings to date. I argue that a multi-disciplinary approach that incorporates new technological approaches in acquisition of molecular data is the best way forward for our aquatic biodiversity. Molecular ecologists can contribute by collaborating with other ecologists, especially in the fields of species distribution modelling and conservation planning. This approach will help to prioritise conservation actions for the best possible outcomes.

Keywords: Freshwater, Aquatic biodiversity, Climate change, Aquatic species, Molecular ecologists, Biological connectivity.

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INTRODUCTION

As the world's population continues to grow, human water needs are growing accordingly, thus reducing the water available for sustaining our freshwater biodiversity. This is likely to be further exacerbated in areas where rainfall will decrease as a result of

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global climate change. Already, declines in freshwater biodiversity are far greater than in terrestrial systems (Sala, 2000; Strayer & Dudgeon, 2010). The major ways in which growing human demand for water will affect biodiversity include; increasing the number of dams, which already number over one million globally (Nilsson *et al*., 2005), and extraction of water for agriculture and aquaculture. These changes will have an array of negative impacts on biodiversity, but a major one is the impact on hydrological connectivity, which in turn will affect biological connectivity. Here, I use the term 'biological connectivity' to mean the connectivity between populations of a species, leading to gene flow, and also the connectivity between different parts of the habitat such as between rivers and floodplains, or between freshwater and marine conditions in the case of diadromous species. Connectivity can also refer to the flow of carbon and nutrients through the food web, but this form of connectivity will not be the focus of this review.

In order for competing needs to be managed, it is imperative that: first, we have accurate and efficient ways to assess our biodiversity. Currently, it is evident that species are going extinct more quickly than we can recognise them (Dudgeon *et al*., 2010). Second, we need accurate and efficient ways of assessing the current and historical connectivity among populations (as this will inform us of the potential for recolonisation following any local extinction – and also the degree of unique genetic diversity in populations that may represent adaptation to those environments). We also need to be able to assess the need for freshwater species to maintain connectivity with other habitats such as the floodplain or the estuary. Finally, given the competing needs of humans and aquatic biodiversity, we need to develop methods for prioritising which rivers, streams or reaches should best be preserved or protected in order to maximise protection of biodiversity. Headwaters are often less impacted by human influences than lower sections, but lower sections of rivers need to be maintained to sustain connectivity for migratory species (Pringle, 2001).

In this paper, I will:

1. Discuss recent advances in the assessment of biodiversity, the significance of cryptic species and how new information might affect prioritisation of regions for conservation.
2. Briefly discuss methods for assessing connectivity among intra-specific populations and determine if it might be possible to make generalisations about population connectivity based on a species' life-history and the habitat in which it lives (stream architecture).
3. Suggest some ways in which molecular approaches can be combined with other methods to understand migration patterns.
4. Finally, I will suggest future options that could improve our ability to conserve our freshwater biodiversity.

THE ROLE OF MOLECULAR APPROACHES IN ASSESSING BIODIVERSITY

Historically, aquatic biodiversity has been assessed using morphological information only (Jackson *et al*., 2014). While this has been the accepted method for many stream monitoring programmes, it has a number of drawbacks. One of the major ones is that there are few taxonomists sufficiently trained to be able to accurately identify many groups of invertebrates to species, especially as many invertebrates can only be identified as adults, but are usually collected as larvae from aquatic habitats. Furthermore, fewer taxonomists are being trained than in the past, so these skills are disappearing among our scientific community. For this reason, many programmes that monitor freshwater diversity identify organisms only to the family level (e.g., AUSRIVAS) (Smith *et al*., 1999). This clearly will be missing much of the diversity that exists below this taxonomic level and is likely to give a very inaccurate picture of the patterns of diversity across a landscape. The second problem is that there is a wealth of 'cryptic diversity' within freshwater taxa (Bickford *et al*., 2006; Jackson *et al*., 2014). By 'cryptic diversity', I mean species that are morphologically very similar, or even identical, but which are 'real species' in the sense that they have different geographic distributions, potentially different physiological tolerances, and in many cases, have been shown not to interbreed in nature (Baker *et al*., 2003; Cook *et al*., 2007). These 'cryptic species' are often identified initially from mitochondrial sequence data that are usually from the cytochorome oxidase 1 gene; the 'bar-coding gene' (Hebert *et al*., 2003). Having identified significant divergence in this gene, it is often possible for taxonomists to find distinguishing morphological characters, which before were just confusing, especially if multiple species occurred together, as for example in the genus *Paratya* in eastern Australia (Cook *et al*., 2006).

As more mitochondrial DNA work is amassed, large numbers of previously cryptic species are being recognised (Baker *et al*., 2004; Balint *et al*., 2011; Jackson *et al*., 2014). For example, *Caridina indisintcta*, described by Riek (1953) as a single species, has been shown to consist of a number of highly divergent lineages based on mtDNA. These lineages often co-occur in the same stream, making it difficult for taxonomists to determine whether they are looking at a single highly variable species or two or more coexisting species (Williams, 1977; Page *et al*., 2005). Allozyme analysis was used to demonstrate that the lineages did not interbreed where they co-occurred, as there were different alleles fixed in each lineage and no heterozygotes were detected at these loci (Woolschot *et al*., 1999). Furthermore, when a taxonomist examined species that had been identified using DNA, he was later able to describe morphological features that distinguished them (Page *et al*., 2005).

Many managers appear to think that geneticists are just describing additional intra-specific genetic variation when they talk about cryptic species, and that this

only represents differentiation between recently separated populations. There is a growing body of evidence, however, that many cryptic species are actually very 'old' (Bickford *et al*., 2006). Their morphologies may have remained unchanged due to unchanged selection pressures. In some cases, the features that differentiate species may not be visible in the form of different morphologies, but may, for example, involve discrete mating calls, pheromones, etc. One clear example of the importance of recognising cryptic species is the *Anopheles gambiae* species complex in Africa. There are seven cryptic species that vary in host preference and habitat – some only attack non-human hosts, thus posing no threat to humans. Recognition of these species allows management for human diseases to be focused only on those species that can impact humans (Besansky, 1999).

Another major issue with not recognising cryptic species is that real geographic distributions of the individual species are almost certainly a subset of the distribution of the single described morpho-species. Such is the case for the cryptic species of the freshwater mussel *Velesunio ambiguus* in eastern Australia, with two of the cryptic species being very widespread, while the other two have very limited distributions (Baker *et al*., 2002; Fawcett, 2008). The atyid shrimp *Paratya australiensis* consists of a complicated complex of cryptic species, with three species widespread across coastal and inland rivers systems, while other species are restricted to only a single or two nearby rivers (Figure 1) (Cook *et al*., 2006). Stuart *et al*. (2006) showed that 14 cryptic species could be recognised within two described species of the frog *Odorrana livida and Rana chalconota* in Southeast Asia. Each of the described species had a broad distribution, but the individual species distributions were much more limited, making them more prone to extinction, especially if managers were only working to protect certain populations of the nominal species. These authors were so far as to suggest that no widespread forest-dwelling frog species may occur in the region.

According to Dudgeon *et al*. (2006), extinction rates of freshwater animals in North America, based on data for unionid mussels, crayfishes, fishes and amphibians, are 4% per year, which is five times higher than the terrestrial average. This value could be much higher; However, this value could be much higher if cryptic species were taken into account, it is likely that many such species will go extinct as a result of human impacts even before they have been recognised formally.

Species distribution modelling uses a set of distribution records of a species, combined with a set of environmental variables to first, model the current distribution of each species. It then uses historical environmental records or future environmental predictions to predict past and future distributions. A study by Balint *et al*. (2011) sequenced mtDNA from nine aquatic insect species that inhabit alpine and subalpine regions and that are restricted to high altitude habitats because they are not tolerant of elevated ambient temperatures.

Within the nine formally described species, they identified potentially an additional 14 species. Using species distribution modelling, they predicted that three of the nine described species would go extinct by 2080, while 15 of the total 23 species would go extinct (Balint *et al*., 2011). This exemplifies the issue for aquatic diversity – as we have such poor assessments of it, knowing how to conserve and protect it is very difficult. These issues are exacerbated in tropical regions, where even less is known of the diversity (Dudgeon *et al*., 2006).

Conservation planning often uses outputs from species distribution modelling to prioritise the best areas to protect, given competing needs. However, conservation planning techniques have rarely been applied to cryptic species. This particular technique has been applied to freshwater fish species across northern Australia. They have assessed how regions that would be given priority would change depending on the taxonomic level at which the analysis was done. They used conservation planning tools to determine the areas that would be required to conserve 500 Km^2 of riverine habitat for each taxon. They did this in three ways, at the genus level, the described species level and the cryptic species level (identified using molecular data). There were 43 genera, 87 described species and a total of 143 species when molecular data were incorporated. They found that if the prioritisation were done at the genus level, only 60% of the total number of species (i.e., all the species identified using molecular data) would be conserved, and 1.5% of the total area would be required. If this was done at the described species level, still only 70% of the actual species would be preserved, and 2.5% of the total area would be required. By basing the analysis on all species, obviously all would be conserved, and this would require only an additional 1% of the total area. This suggests that conservation planners should take into account information on cryptic species, where it is available.

CONNECTIVITY AMONG POPULATIONS

It is extremely important to understand the way in which populations are connected in order to manage them effectively. Populations that are strongly connected with other populations are unlikely to suffer from chance local extinctions for long, because they will be quickly recolonised. In contrast, populations that are isolated from other populations of the same species are likely to suffer from reduced genetic diversity, as they will lose diversity through random genetic drift and if they do go extinct, recolonisation is much less likely. Over the last 20-30 years, ecologists have applied a range of techniques to understand dispersal among populations of freshwater species. Early studies used allozymes (Schmidt *et al*., 1995), while later studies used mitochondrial DNA sequence data (Schultheis *et al*., 2002) and more recently microsatellite data (Hughes *et al*., 2014). All these approaches rely on the very simple idea that if there is dispersal and gene flow between two populations, then they will tend

to contain a similar genetic composition, whereas if gene flow is limited between populations, gene frequencies will tend to diverge as a result of genetic drift and or selection (Slatkin, 1985). The degree to which populations are connected by gene flow and hence the genetic similarities among them has been empirically shown to be affected by a number of life-history factors and the habitats in which they live. For example, genetic similarity between populations has been shown to be greater for species with a flight stage in their life-history than for those that are solely restricted to the stream (Bohonak & Jenkins, 2003; Hughes, 2007) and species that occur in upstream sites tend to be more genetically differentiated than those that occur in lowland habitats (Hughes, 2007). In fact, Finn *et al*. (2011) demonstrated that for a group of freshwater insects and crustaceans, headwaters contained higher differentiation among sites than even populations in streams only slightly further down the stream hierarchy. If this finding is generally the case, then protecting headwater streams becomes even more important.

Not only is it important to know how much dispersal and gene flow occurs between populations, but it is also important to know the patterns of connectivity among populations. A number of models have been proposed to describe the way in which populations of riverine species are connected. The stream hierarchy model (Meffe & Vrijenhoek, 1988) proposes that riverine species should show patterns of connectivity that reflect the dendritic structure of the stream network. The highest levels of connectivity should be among sites within a stream, with connectivity decreasing with level in the hierarchy, and the lowest between sub-catchments or catchments. Such a model was originally suggested to apply to obligate freshwater fish, but also has been shown to apply to many other riverine species (e.g., crustaceans: Bunn & Hughes, 1997; stoneflies: Hughes *et al.*, 1999). The death valley model (Meffe & Vrijenhoek, 1999) was proposed for species that live in isolated waterholes that are rarely connected by surface flows. All populations are highly differentiated and there is no relationship between connectivity and position in a catchment network, nor with geographic distance separating them. The headwater model (Finn *et al*., 2007) was proposed for headwater specialists that have some abilities to move out of the stream. If their streams confluence outside their tolerance limits (i.e., at low altitudes), then connectivity is likely to be higher across the catchment divide than within the same catchment. The final model is panmixia, which describes systems where gene flow is very widespread and there is no relationship between connectivity and position in the catchment, nor with geographic distance. This model applied to some aquatic insects with strong-flying adults such as dragonflies and dytiscid beetles (Phillipsen *et al*., 2015).

Knowledge of the model that a species fits in terms of patterns of biological connectivity can assist managers in a number of ways. For example, if a species fits the stream hierarchy model, the outcomes of

different disturbance events can be predicted more successfully. If populations in a whole subcatchment are extirpated by, for example, a pollution event, then natural recolonisation is much less likely than if the same number of populations is removed patchily across the stream network, as might happen during a drought. If a species is effectively panmictic throughout the system, then local extinctions will have little impact, as they will quickly be recolonised from other parts of the system. Local extinctions in populations of headwater species within a sub-catchment will only be recolonised if there are potential source populations on the other side of the drainage divide. Species that fit the death valley model are likely to contain populations that are significantly diverged from one another, may be adapted to local conditions, and local extinctions are unlikely to be recolonised. Furthermore, translocation of individuals among these populations and transferring water between basins is likely to present risks to endemic populations such as extinctions of both types (Hughes *et al*., 2003), and introgression and loss of adaptive potential (Allendorf *et al*., 2001).

Hughes *et al.* (2013) proposed that it should be possible to predict the model that a given species should fit based on knowledge of the species life-history and the stream architecture/geography of its habitat. They developed a decision tree approach to assist managers with predicting patterns of connectivity. They tested this idea with data from 47 fish studies and 62 invertebrate studies. Predictions were correct for more than 70% of both fish and invertebrate cases, but there were still enough species that did not fit the predictions to suggest that data on individual species are still the best way to do this. Nevertheless, where there is no datum available for a given species, at least this approach may give managers a place to start.

ASSESSING CONNECTIVITY FOR SPECIES THAT MOVE BETWEEN HABITATS AS PART OF THEIR LIFE-HISTORY

Many species move quite large distances as part of their life-history. Possibly the most extreme of these are the diadromous species that spend part of their lives in fresh water and part in marine conditions. There are three main forms of diadromy: anadromy (where reproduction occurs in freshwater), then juveniles migrate to the sea and spend most of their lives in marine conditions before returning to the freshwater to breed, catadromy (where individuals go to the ocean to breed), but then return to freshwater as juveniles, where they spend most of their lives, and amphidromy, migration between marine and freshwaters habitats but not for the purpose of reproduction (Myers, 1949). Until recently, understanding these behaviours has been proven difficult for ecologists because it requires tagging large numbers of individuals that are likely never recaptured. Genetic markers are not useful because they can only give information concerning the eventual outcomes of successful reproduction.

In fish, this problem has been partly solved by using information contained in the fish otolith (or earbone). These structures are

composed of calcium carbonate and are laid down in a series of rings as the fish grows. While they are predominantly calcium carbonate, they also take up tiny amounts of other elements from the medium in which they occur (Hicks *et al*., 2100). The otolith thus contains a permanent record of where the fish has been residing. By combining the fact that the rings give an estimate of age with the fact that the composition will reflect where the fish was when that ring was laid down, it is possible to infer the history of an individual fish by running a transect through the otolith and analysing particular elements. This can be done using laser ablation mass spectrometry (Hughes *et al*., 2014). For examining diadromy, stable isotopes of Strontium have been shown to be particularly useful. Sea water has a very consistent ratio of ^{86}Sr to ^{87}Sr, while freshwater systems have ratios that reflect the geology of the surrounding catchments, so they often differ significantly from seawater and from one another. Hughes *et al*. (2014) studied a small amphidromous fish, which had been predicted to have little genetic variation across its range due to the fact that eggs and larvae were thought to go to the ocean and were thus likely to be mixed by ocean currents. Using Sr isotope ratios, they were able to show that some populations were composed totally of diadromous individuals, some populations were totally freshwater – and a small number contained a mixture of both. Even more surprisingly for an amphidromous species, populations in different river systems were highly genetically differentiated from one another. Further use of otolith chemistry showed that this was probably because although many populations spent time in marine conditions, this time was spent in individual estuaries rather than in the open ocean. This was inferred by multi-elemental analysis, which showed that populations differed in the core of their otoliths, a result that would not have been expected if they had come from the relatively homogeneous open ocean.

Studying within life-cycle migration is more difficult for invertebrates, as they do not possess otoliths. Some studies have used stable isotope signatures of soft body parts (Cook *et al*., 2007), which may show differences among streams and between marine and freshwater and hence aid in understanding migration. The drawback with using soft tissues, however, is that the signature only remains for a limited time, a period of weeks to months (Fraser *et al*., 1997). New work on hard parts of crustaceans such as eye-stalk and gastric mill has suggested that they retain signatures that assist with age determination (Kilada *et al*., 2012). These structures are also composed of calcium carbonate, so could presumably be used in the same way as otoliths to study migration patterns. So far, no one appears to have done this.

FUTURE CHALLENGES AND POSSIBILITIES

Deciding which catchments, rivers or stream reaches should be prioritised for

protection in the future will be a daunting task. However, some new technologies may make this slightly easier.

One possible answer to the taxonomic dilemma has come from recent advances in DNA sequencing. Next Generation Sequencing (NGS) allows the sequencing of many individuals in a single run. While the cost of these techniques was initially out of the range of most stream ecologists, prices are reducing all the time. It is possible, using the DNA bar-coding gene to analyse samples of sediment or water and to obtain mtDNA sequences from all (or at least many) of the organisms present in the sample (Shokralla *et al*., 2012; Thomsen *et al*., 2012). Depending on the technology used, more than single samples can be analysed in a single run. While the repeatability and feasibility of these approaches are still being determined, it is clearly a way of the future. The sequences obtained from the run can be combined with others of known described species from Genbank and put into a phylogenetic tree. This information can then be used to identify species in the sample – or even to identify sequences that may belong to new undescribed species. By using these techniques, ecologists should be able to obtain a reasonable idea of the species present at their site. There are still some problems with interpretation of these data though. The first is that at present, using these techniques to determine abundance of particular species may be problematic, for a few reasons. The first is that it is likely that more DNA sequences will be obtained from larger species. The second is that the polymerase chain reaction (PCR), which is used to amplify DNA fragments from the initial mixed sample, may preferentially amplify some species over others. Even so, this technique holds great promise for assessing biodiversity in the future, as demonstrated already for chironomids (Carew *et al*., 2013). These approaches are also likely to be useful for detecting the presence of threatened species that are difficult to catch and also to provide early warning of the presence of invasive species.

As we amass more information about the levels and patterns of connectivity among populations of aquatic species in different environmental settings and with different life-histories, especially by combining a range of approaches, we should be able to improve our generalisations about how best to manage them. For example, for amphidromous species that require access to the marine environment in order for larvae and eggs to develop, efforts should be made to maintain hydrological connectivity between river and ocean. Currently, this can be blocked in a number of ways: by building dams on the rivers so that larvae cannot get access to the estuary; secondly, if there is major pollution in downstream reaches that affects larvae, this could also restrict migration. Finally, in many high wave energy coasts such as in southern Australia, connections to the sea are intermittent. If flows are low, the waves deposit sand at the mouth and build bars, which stop the rivers from reaching the sea. As rainfall levels drop, as is predicted for southern Australia, and if significant water

is abstracted from the rivers along their way, then they might not open for a number of years. This will result in local extinctions of many diadromous species. Whether or not they can recover will depend on whether there are nearby source populations that can recolonise. This is not just an issue for southern Australia. Several of the world's large rivers (Ganges, Nile, Colorado) have already stopped flowing to the sea during prolonged dry periods (Strayer & Dudgeon 2010).

With more and more information available from DNA, both traditional mtDNA sequence data and those obtained from NGS, we are likely to identify many new species or at least new lineages that have apparently been isolated from the rest of their species for millions of years. Where possible, this information should be incorporated into conservation planning approaches, so that at least the cryptic diversity can be taken into account.

While currently conservation planners attempt to identify regions of high diversity and endemism based on described species, knowledge of the distributions of cryptic species may well identify different areas as being important. It will be very useful in the near future to consolidate all the information appearing on cryptic species to produce more meta-analyses that incorporate this. One approach may be to use what knowledge we have on the distribution of molecular diversity across sub-catchments and catchments to define molecular bioregions.

Finally, it appears that the freshwater biodiversity of tropical systems is particularly poorly known and should be given priority, possibly by setting up large collaborations across the world. Potentially, the DNA bar-coding project should contribute to this.

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REFERENCES

Allendorf, F. W., Leary, R.F., Spruell, P., & Wenburg, J. (2001). The problems with hybrids: setting conservation guidelines. *Trends in Ecology and Evolution, 11*, 613-622.

Baker, A. M., Hughes, J. M., Dean, J. C., & Bunn, S. E. (2004). Mitochondrial DNA reveals phylogenetic structuring and cryptic diversity in Australian macroinvertebrate assemblages. *Marine and Freshwater Research, 55*, 629-640.

Baker, A. M., Bartlett, C., Bunn, S. E., Goudkamp, K., Sheldon, F., & Hughes, J. M. (2003). Cryptic species and morphological plasticity in long-lived bivalves (Unionoida: Hyriidae) from inland Australia. *Molecular Ecology, 10*, 2707-2717.

Balint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., Theissinger, K., Pauls S. U., & Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nature Climate Change, 1*, 313-318.

Besansky, N. J. (1999). Complexities in the analysis of cryptic taxa within the genus *Anopheles*. *Parasitologia, 41*, 97-100.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., &

Das, I. (2006). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution, 22*, 148-155.

Bohonak, A. J., & Jenkins, D. G. (2003). Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters, 6*, 783-796.

Bunn, S. E., & Hughes, J. M. (1997). Dispersal and recruitment in streams: evidence from genetic studies. *Journal of the north American Benthological Society, 16*, 338-346.

Carew, M. E., Pettigrove, V. J., Metzling, L., & Hoffman A. A. (2013). Environmental monitoring using next generation sequencing: rapid identification of macroinvertebraet bioindicator species. *Frontiers in Zoology, 10*, 45.

Cook, B. D., Baker, A. M., Page, T. J., Grant, S. C., Fawcett, J. H., Hurwood, D. A., & Hughes, J. M. (2006). Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role of life-history transition in phylogeographic diversification. *Molecular Ecology, 15*, 1083-1093.

Cook, B. D., Bunn, S. E., & Hughes, J. M. (2007). A comparative analysis of population structuring and genetic diversity in sympatric lineages of freshwater shrimp (Atyidae: *Paratya*): concerted or independent responses to hydrographic factors. *Freshwater Biology, 52*, 2156-2171.

Cook, B. D., Bunn, S. E., & Hughes, J. M. (2007). Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable southern pygmy perch (*Nannoperca australis*). *Biological Conservation, 138*, 60-72.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z-I., Knowler, D. F., Leveque, C., Naiman, R. J., Prieu-Richard, A-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biology Reviews, 81*, 163-182.

Elith, J., & Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology and Systematics*, 677-697.

Fawcett, J. H. (2008). *Muddy waters: a molecular approach to clarifying freshwater mussel diversity in Australia*. (PhD thesis dissertation). Griffith University, Brisbane.

Finn, D. S., Blouin, M. S., & Lytle D. A. (2007). Population genetic structure reveals terrestrial affinities for a headwater stream insect. *Freshwater Biology, 52*, 1881-1897.

Finn, D.S., Bonada, N., Murria, C., & Hughes, J. M. (2011). Small but mighty: headwaters are vital to stream network diversity at two levels of organization. *Journal of the North American Benthological Society, 30*, 963-980.

Fraser, T. K., Ross, R. M., Quetin, I. B., & Monteur, J. P. (1997). Turnover of carbon and nitrogen during growth of larval krill *Euphausia superba* Dana: a stable isotope approach. *Journal of Experimental Marine Biology and Ecology, 212*, 259-275.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identification through DNA barcodes. *Proceedings of the Royal Society B- Biological Sciences, 270*, 313-321.

Hughes, J. M., Mather, P. B., Sheldon, A. L., & Allendorf, F. W. (1999). Genetic structure of the stonefly *Yoraperla brevis*: the extent of gene flow among adjacent montane streams. *Freshwater Biology, 41*, 63-72.

Hughes, J. M., Goudkamp, K., Hurwood, D. A., Hancock, M., & Bunn, S. E. (2003). Translocation causes extinction of a local population of the freshwater shrimp *Paratya australinesis*. *Conservation Biology, 17*, 1007-1012.

Hughes, J. M., Schmidt, D. J., McDonald, J. I., Huey, J. A., & Crook, D. A. (2014). Low inter-basin connectivity n a facultatively diadromous fish: evidence form genetics and otoliths. *Molecular Ecology, 23*, 1000-1013.

Jackson, J. K., Battle, J. M., White, B. P., Pilgrim, E. M., Stein, E. D., Miller, P. E., & Sweeney, B. W. (2014). Cryptic biodiversity in streams: a comparison of macroinvertebrate communities based on morphological and DNA barcode identifications. *Freshwater Science, 33*, 312-324.

Kilada, R., Bernard, S-M., & Remy, R. (2012). Direct determination of age in shrimps, crabs and lobsters. *Canadian Journal of Fisheries and Aquatic Sciences, 69*, 1728-1733.

Meffe, G. K., and Vrijenhoek, R. C. (1988). Conservation genetics in the management of desert fishes. *Conservation Biology, 2*, 157-169.

Myers, G. S. (1949). Usage of anadromous, catadromous and allied terms for migratory fishes. *Copeia, 2*, 89-97.

Page, T. J., Chooy, S. C., & Hughes, J. M. (2005). The taxonomic feedback loop: symbiosis of morphology and molecules. *Biology Letters, 1*, 139-142.

Phillipsen, I. C., Kirk, E. H., Bogan, M. T., Mims, M. C., Olden, J. D., & Lytle, D. A. (2015). Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. *Molecular Ecology, 24*, 54-69.

Pringle, C. (2001). Hydrologic connectivity and the management of biological reserves: a global perspective. *Ecological Applications, 11*, 981-998.

Riek, E. F. (1953). The Australian freshwater prawns of the family Atyidae. *Records of the Australian Museum, 15*, 11-121.

Schmidt, S. K., Hughes J. M., & Benn, S. E. (1995). Gene flow among conspecific populations of *Baetis* sp. (Ephemeroptera)- adult flight and larval drift. *Journal of the North American Benthological Society, 14*, 147-157.

Schultheis, A. S., Weight, L. A., & Hendricks, A. C. (2002). Gene flow, dispersal and nested clade analysis among populations of the stonefly *Peltoperla tarteri* in the southern Appalachians. *Molecular Ecology, 11*, 317-327.

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology, 21*, 1794-1805.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics, 16*, 393-430.

Smith, M. J., & Williams, W. D. (1980). Intraspecific variation within the Atyidae: a study of morphological variationwithin a population of *Paratya australiensis* (Crustacea: Decapoda). *Australian Journal of Marine and Freshwater Research, 31*, 397–407.

Smith, M. J., Kay, W. R., Edward, D. H. D., Papas, P. J., Richardson, K. S., Simpson, J. C. Pinder, A. M. Cale, D.J., Horwitz, P. H. J., & Davis, J. A. (1999). AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwater Biology, 41*, 269-282.

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society, 29*, 344-358.

Stuart, B. L., Inger, R. F., & Voris, H. K. (2006). High levels of cryptic diversity revealed by sympatric lineages of Southeast Asian forest frogs. *Biology Letters, 2*, 470-474.

Thomsen, P. F., Kilgast, J., Iversen, L. L., Wiuf, C., Rasmussen, F., Gilbert, M. T. P., Orlando, L., & Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology, 21*, 2565-2573.

Willaims, W. D. (1977). Some aspects of the acology of *Paratya australiensis* (Crustacea Decapoda Atyidae). *Australian Journal of Marine and Freshwater Research, 28*, 403-415.

Woolschot, L., Hughes, J. M., & Bunn, S. E. (1999). Dispersal among populations of *Caridina* sp. (Decapoda: Atyidae) in coastal lowland streams, south-east Queensland Australia. *Australian Journal of Marine and Freshwater Research, 50*, 681-688.

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Review Article

Arbuscular Mycorrhizal Symbiosis and Water Stress: A Critical Review

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ABSTRACT

Through evolutionary time, the ever changing environmental conditions have been faced by plants among which water stress is the most common. Nevertheless, a great deal of variations in responses of plants to water deficit and their sensitivity to water scarcity has been noticed. For perception, signalling and response to water stress, plants are supposed to have programmed capability. Under the conditions of water scarcity, improved resistance to drought has been provided by AM fungi by enhancing efficiency of water absorption, uptake of mineral nutrients, especially phosphorus, accumulation of osmoprotectants like proline and sugars, activity of antioxidant enzymes like SOD, CAT and POD, production of isoprenoids, stomatal conductance, chlorophyll contents, photosynthesis and decline in ABA content. Expression of drought related plant genes like *p5cs* genes, aquaporin genes, as well as *nced* genes, brings about the physiological response of mycorrhizal plants to drought stress. Moreover, the efficacy of AM in reducing the use of phosphorus fertilisers and enhancement of soil stability increase the value of mycorrhizae for sustainability and ecosystem services. Their appropriate management has prospective to ameliorate the effectiveness and sustainability of drought tolerance.

Keywords: Arbuscular mycorrhizal fungi, water stress, osmoprotectants, aquaporin genes, stomatal conductance

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INTRODUCTION

Water scarcity is one of the major abiotic stresses having detrimental effects on crop productivity throughout the world (Kramer & Boyer, 1995). On earth, 45% of agricultural lands are subjected to

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recurrent water deprivation leading to an average grain yield loss of 50% (Singh *et al*., 2012). During growing season, insufficient soil water and rainfall results in agricultural water deficit (Vadez *et al*., 2012). Estimates have shown an increase in intense rain events simultaneously decrease in the number of rain days. This has led to increased drought risk (Trenberth, 2011). Therefore, water deficit is a major limitation to crop production under rainfed conditions.

A period of dry weather which is harmful to crops is called drought. Drought stress is experienced when ambient temperature is high and relative humidity of air and soil humidity are low. During drought, plants predispose themselves in order to keep the water potential high in their tissues. This is termed as dehydration avoidance and the avoidance which determines plant predisposition is called drought resistance (Blum, 2005). In plants, a series of biochemical, physiological and morphological injuries occur due to water stress (Gong *et al*., 2013). Different factors like duration of exposure of plants to the drought, genetic resistance and stage of growth determine the effect of water stress on plant growth (Song *et al*., 2011). Nevertheless, several anti-stress mechanisms like accumulation of osmolytes have been used by plants in order to reduce osmotic pressure through enrichment of antioxidant enzyme activities for scavenging free radicals and increase in length of roots to absorb much water (Huang *et al*., 2011).

Due to population explosion, the greatest challenge faced by agricultural community is to satisfy the demands placed on agriculture for food and fibre supply. In order to meet the challenge, a wide variety of efforts focusing on agro ecosystem and soil biological system as a whole is required to understand the stability of process. Keeping in view the concept of sustainability, a vehicle for sustainable agriculture has been hiding secretly for decades in the rhizosphere in the form of plant growth enhancing microbes. Arbuscular Mycorrhizal Fungi (AMF) is one of the ancient, diverse and beneficial groups of such soil microbes (Smith & Read, 2008). Nearly 250,000 plant species form Arbuscular mycorrhizal associations. The AMF are susceptible to alteration in plant and soil conditions and form an interface between plant roots and soil (Power & Mills, 1995).

Molecular sequencing and palaeobotanical data implies that glomalean fungi belonging to glomeromycota formed associations with the first land plants nearly 460 million years ago. On the basis of morphology, 150 - 200 species of AM fungi have been well known so far. Highly branched fungal structures called arbuscules characterise the symbiosis, which grows intracellularly without piercing the host cell membrane (Brundrett, 2004). The intra-radical mycelium of these soil fungi proliferates in the root cortex of the host plant. Meanwhile, extra-radical AM hyphae provides increased surface area by extending into the soil around the root for absorption of nutritional elements like phosphorus, nitrogen, copper, zinc, etc. and convey them

to host (Smith & Read, 2008). Plants native to arid and semi-arid ecosystems have their roots highly colonised with AMF, which indicates the significance of AM symbiosis for performance under scarcity of water (Chaudhry *et al*., 2005). Studies have shown the transfer of water to host plant by extra-radical mycelium under low moisture conditions (Khalvati *et al*., 2005).

As AMF species richness and AM hyphal length increase, nutrient capture, productivity in microcosms and plant biodiversity increases considerably (Vander Heijden *et al*., 1998). The use of AMF inoculums in agriculture, site remediation, horticulture or landscape restoration dated back to almost two decades. Thus, the idea of exploiting AMF as biofertiliser is not new (Hamel, 1996). For maintaining soil health, nutrient uptake, fertility, plant community development and above ground productivity under phosphorus limitation conditions, AMF is extremely important (Smith & Read, 1997).

Different ways through which AMF management could be helpful for sustainable ecosystems including restoration and agro ecosystems had been described by researchers during the early 1990s (Pfleger & Linderman, 1994). Although AMF has several utilities, practical application of AMF has not yet been able to reach main stream markets. Even *Rhizobium* is more familiar to farmers for its practical applications while AMF is almost unknown. The reasons are (1) production limitations and (2) knowledge gaps. Being obligate symbionts, they are difficult to produce. Modern production systems are dependent on soil based systems (plots or pots), where contamination by other AMF or other microbes is unavoidable (Gianninazzi & Vosatka, 2004). Undoubtedly, production is a limitation for the current use of AMF. Therefore, discovery and development are still awaited for its large scale production and use.

DROUGHT TYPES AND CAUSES

Being a natural hazard, the effects of drought differ from one region to another. Frequently, it is referred to as a creeping phenomenon which can be classified as:

1. Meteorological drought
2. Hydrologic drought
3. Agricultural drought

Meteorological drought occurs when if precipitation is less than the seasonally normal or climatologically accepted for a long period of time over a wide area. In particular, drought affects the economy rigorously but it may affect only a few farmers or a small community if it occurs in a small region. A method of computing numerical drought index and index number was developed by Palmer (1964, 1965) for the assessment of severity of meteorological drought.

If meteorological drought occurs for a long time, it may lead to hydrologic drought, which is a step ahead of meterological drought and is usually marked by a shrinkage of above ground water bodies like drying up of rivers, streams, etc., as well as a decline in ground water levels. As compared

to meteorological drought, hydrologic drought is far more reaching as it affects industry, agriculture and hydroelectric power generation and if it persists, irrigable lands have to be deserted. Another category of drought is agricultural drought, which occurs at the time of growing season when rainfall and soil moisture are not sufficient to sustain healthy crop production that causes severe wilt and crop stress. Agricultural drought is independent of meteorological drought; it may subsist even if there is no meteorological drought (http://drought.unl.edu).

PLANTS' STRATEGIES TO SURRVIVE WATER DEFICIT

To survive water scarcity, different mechanisms have been developed by plants like avoidance, escape and tolerance to cell or tissue dehydration (Ludlow & Muchow, 1990).

Drought Escape

Drought escape involves the accomplishment of life cycle prior to the adverse effects of drought (Wu *et al*., 2010). In arid regions, annual plants escape against water deficit by producing seeds at the time of water availability followed by intermittent rainfall.

Drought Avoidance

Plants avoid drought through changes in their anatomy, orientation and area of leaves or by increasing resistance towards stomata and cuticle to transpiration (Jones & Corlett, 1992). Despite water scarcity, plants can maintain their normal growth to avoid drought. This is generally achieved by increasing water use efficiency (WUE), which is measured as photosynthetic carbon gain over transpirational water loss, while high WUE may decrease development and growth rate (Arntz & Delph, 2001).

Drought Tolerance

The strategy of drought tolerance of primitive terrestrial plants, including bryophytes and lichens, which remain conserved all through the evolution of angiosperms is by restricting intense levels to resurrection plants (Ingram & Bartels, 1996). The main mechanism to sustain cell turgor is osmotic adjustment which enables water uptake, and thus helps in maintenance of plant metabolism (Gunasekera & Berkowitz, 1992).

MYCORRHIZAE AMELIORATING WATER STRESS IN PLANTS

In terrestrial ecosystems, mycorrhizae are the most important symbiosis between AM fungi and 80% of terrestrial plants (Brundrett, 2009). It is a mutualistic association in which a biochemical communication between two symbionts helps the spores to recognise the presence of host plant. After recognising the host, hyphae is produced into the apoplastic space of cortical cells inside the host root to form arbuscules which are highly branched structures meant for exchanges of carbon and nutrients between two partners, whereas the bulbous structures that arise as terminal or intercalary swelling of hyphae (called vesicles) are meant for storage (Miransari, 2010). Arbuscules have been

considered as the only structures which define the features of arbuscular mycorrhizal fungus (Gianinazzi *et al*., 1979). They are temporary structures having turnover rate of up to 2 weeks, varying in their morphology according to the species of AM fungi (Morton, 2000). However, vesicles are not formed by all genera of AM fungi as the members of *Scutellospora* and *Gigaspora* are devoid of vesicles. When they mature, vesicles may act as reproductive structures. Besides arbuscules and vesicles, a variety of structures have been seen to be produced by AM fungi, like:

- Appressoria: These are the first fungal structures (Tawaraya *et al*., 2007) formed on epidermal cell wall after the first contact with host (Garriock, 1989).
- Auxillary cells: They are the swollen structures produced by extra-radical hyphae terminally. They are a morphological distinguishing feature between the members of Glomeromycetes and Gigasporaceae (Morton & Benny, 1990).
- Intra-radical mycelium: Morphologically, the mycelium may be straight, coiled and show a Y or H shaped branching having the function of transportation of the substances which have been absorbed by extra-radical hyphae.
- Extra-radical mycelium: It performs the functions of absorption and translocation of nutrients, propagation, spore production and production of other structures, as well as to look for new roots of different or same plant for infection (Smith & Smith, 2011).
- Sporocarps: They are meant for accommodation of spores and specialised hyphae or they may sometimes be found enclosed in an outer layer called Peridium.
- Spores: These are unicellular, multinucleate structures formed at the tip of sporocarp sometimes inside or outside the root and on decaying plant material or on the plant itself. They may be produced singly called azygospores or chlamydospores or may be grouped in the sporocarps.

Occupying a protected ecological niche, AMF constitutes an approach to minimise the use of chemical fertilisers mainly in phosphorus nutrition, thus improve nutritional status of both associates (Almagrabi & Abdelmoneim, 2012). Plants get the benefits of increased nutrient uptake and AMF gets carbohydrates from plants in turn. It is now well known that AMF protects the plants from the detrimental effects of biotic and abiotic stresses and enhances plant production and growth (Song *et al*., 2011). Under drought stress conditions, mycorrhizae help the plants to perform well through direct uptake and transport of water with the help of external hyphae (Auge, 2004), stomatal conductance regulation (Goicoechea *et al*., 1997) and osmotic adjustment (Wu & Xia, 2006). Protection of plants from the detrimental effects of

drought by AM is well documented (Auge, 2001; Zhang *et al*., 2014).

IMPACTS OF WATER STRESS ON AMF COLONISATION

As compared to other climates, spore production and species richness of AM fungi in arid climates are lower and found to decrease with the increase in aridity (Pond *et al*., 1984). However, comparable species richness of arid climates to that of other communities has been revealed by using different sophisticated culture techniques (Morton *et al*., 1995). In response to declining soil moisture, production of resilient spores and opportunistic rapid growth of mycelium may be the feature which allows AM fungal communities to perform under dry conditions (Jacobson, 1997).

Being obligate biotrophs, hyphal spread after spore germination occurs at slow pace or is inhibited by water scarcity which makes clear the adverse effects of drought on AM colonisation. Inhibition of AM colonisation due to water stress has been seen in foxtail millet roots (Gong *et al*., 2014) which has been attributed to reduced availability of carbon from host plants (Subramanian & Charest, 1995). Other pot-based experiments have confirmed the same in *Triticum aestivum* (Al-Karaki *et al*., 2004). Nevertheless, some reports have shown the promotion of colonisation in drought conditions. Under field conditions, persistent drought may promote more extensive colonisation as examined by Kuhn (1991) and Kuhn *et al*. (1991) on a fallow agricultural site in Germany, whereas some workers have also examined the promotion in spore production under short-term temporary decline in soil moisture (Jacobson, 1997).

AMF INDUCED METABOLOMIC AND BIOCHEMICAL RESPONSES TO WATER DEFICIT

Under the condition of water scarcity, water potential may increase as a result of less stomatal conductance and more diffusive resistance to carbon dioxide. Therefore, water potential is required to be reduced in order to maintain uptake of water from the soil, which in turn is achieved by different mechanisms of osmoregulation. The mechanism of osmoregulation causes a decrease in osmotic potential due to accumulation of compatible solutes (Munns, 1988), as discussed below:

Proline

It is the most commonly distributed non-protein amino acid, N-storage compound, osmosolute and a hydrophobic protectant for cellular structures and enzymes in higher plants accumulated as a universal metabolic response to osmotic adjustment under water stress (Szabados & Savoure, 2009). Proline is commonly synthesised in chloroplasts or mitochondria of plants by glutamate synthetic pathway. A key enzyme, n1-pyrroline-5-carboxylate synthetase converts glutamate firstly into n1-pyrroline-5-carboxylate and then another enzyme n1-pyrroline-5-carboxylate reductase (P5CR) transforms n1-pyrroline-5-carboxylate into

proline (Szabados & Savoure, 2009). An alternate pathway for proline synthesis is ornithine synthetic pathway, where proline is synthesised in mitochondria from ornithine. Enzyme ornithine-d-aminotransferase (OAT) causes transamination of ornithine to form glutamate semialdehyde and n1-pyrroline-5-carboxylate which is converted into proline (Szabados & Savoure, 2009). In mitochondria, proline dehydrogenase causes catabolism of proline into n1-pyrroline-5 carboxylate. Hence, the net proline accumulation in plants involves one proline catabolic enzyme and two proline synthetic enzymes.

There is a positive correlation between proline accumulation and AMF induced drought tolerance in plants. During the period of inhibited growth, proline serves as nitrogen and energy source (Kala & Godara, 2011). As compared to non-AM plants, higher proline accumulation in AM plants has been seen in *Allium sativum* (Borde *et al*., 2012) and *Oryza sativa* (Ruiz-Sanchez *et al*., 2011). Some studies have also shown a decrease in proline content in *Cyclobalanopsis glauca* (Zhang *et al*., 2014) and *Zea mays* (Abdelmoneim *et al*., 2014) as compared to their non-AM counterparts. Building up of lower proline in AM plants has been ascribed to less injury by water deficit (Auge & Moore, 2005). Furthermore, underwater stress mycorrhization in soybean causes more proline accumulation in roots as compared to shoots (Porcel *et al*., 2004).

Sugars as Osmoprotectants

Among osmotic solutes, sugars are equally important as they play an important role in stabilising cell turgor pressure. Higher photosynthetic rates in AM plants may cause increased building up of carbohydrates which act as excellent osmoprotectants to lower osmotic potential (Khalvati *et al*., 2005). AM symbiosis increases plant growth which in turn causes increased transport to the organs for consumption to meet the growth demands. Furthermore, AM fungi also utilise carbohydrates produced by the plant. Therefore, the overall increased utilisation rates do not point toward accumulation of carbohydrates. Studies have shown an increase in sugars in mycorrhizal plants exposed to drought in *Cyclobalanpsis glauca* (Zhang *et al*., 2014) and in *Poncirus trifoliata* (Qiangsheng *et al*., 2006). High sugar content of *Poncirus trifoliata* confirms a high natural physiological metabolism of AM plants under water stress and well watered conditions leading to accumulation of carbohydrates resulting in decrease of osmotic potential of host cells. In contrast, less sugar content has been noticed in water stress exposed mycorrhizal plants as in *Casuarina equisetifolia* (Zhang *et al*., 2010) and *Glycine max* (Porcel *et al*., 2004). In case of *Glycine max,* lower accumulation of sugars may be due to utilisation of photosynthates by fungus and their non-availability for storage (Schellembaum *et al*., 1998). Successful avoidance of drought stress due to AM may also be responsible for lower sugar accumulation as stated by Auge (2001).

Defense against Oxidative Stress

Oxygen generated during photosynthesis in chloroplasts can accept the electrons passing through photosystems forming superoxide radicals, which are scavenged by various antioxidant defense mechanisms under steady state conditions (Foyer & Noctor, 2005). Different abiotic stresses including drought, salinity, heavy metals, UV radiations, high temperature, herbicides, pathogen attacks, air pollution, heavy metals, nutrient deficiency cause disturbance in equilibrium between scavenging and production of ROS leading to increase in intracellular levels of ROS that suddenly cause damage to cell structures. Antioxidant defense system including enzymatic systems like superoxide dismutase (SOD) which breaks down H_2O_2 and O_2^- to O_2, with different substrates at the cost of H_2O_2 are detoxified by peroxidase (POD), whereas H_2O_2 is detoxified by catalase (CAT) (Mohammadi *et al*., 2011) and non-enzymatic defense system including flavonoids, glutathione and ascorbate (Mohammadi *et al*., 2011). Different studies have shown increases in the activities of antioxidant enzymes in plants inoculated with mycorrhizae during drought conditions. Increases in CAT and peroxidase activities have been seen in mycorrhizal *Plukenetia volubilis* during water stress conditions (Tian *et al*., 2013). The SOD and CAT of leaf and POD activities of root were higher in mycorrhizal *Allium sativum* plants as compared to non-mycorrhizal ones when exposed to drought (Borde *et al*., 2012). In addition, an increase in non-enzymatic molecules like glutathione and ascorbate has been noticed in *Avena nuda* seedlings subjected to SO_2 (Huang *et al*., 2008) when exposed to drought stress.

Isoprenoids

Isoprenoids represent a large group of plant compounds which have highly diverse and complex chemical structures. They are synthesised from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Isoprenoids may be non-volatile like carotenoids, which are terpene pigments in the plants helpful in dissipation of excess of energy during photosynthesis (Demming-Adams & Adams, 2006). Carotenoids act as precursor for the synthesis of ABA and strigolactones. It has been investigated that plant abscissic acid content is related to water deficit tolerance, while strigolactones help to promote AM colonisation in roots and are secreted by the host itself (Bouwmeester *et al*., 2007). Volatile isoprenoids including sesquiterpines, isoprenes, monoterpenes produced in response to abiotic factors like oxidative stress, irradiation or temperature, as well as biotic factors like pathogens, pests, insects or herbivores, may provide protection against abiotic stress (Penuelas & Munne-Bosch, 2005). Different workers have reported induction of high levels of ABA (Meixner *et al*., 2005) and apocarotenoids in host roots by mycorrhizae (Walter & Strack, 2011). Thus, AM induces synthesis of isoprenes in roots as both are isoprene derived compounds. In addition, it has been seen that production of essential isoprenoids by leaf has been favoured by

AM symbiosis under water stress but a decrease in volatile isoprenoids of roots has also been noticed. This has been attributed to more carbon demand by AM fungi which affect the amount of carbon allocation and carbon partitioning among various classes of isoprenoids (Asensio *et al*., 2012).

IMPACTS OF AMF ON GROWTH AND YIELD

Under drought stress, AM induced improvement in morphology, growth and yield has been reported in different plants. Improved plant growth and enhanced yield have been attributed to better uptakes of Cu and P under drought stress conditions (Al-Karaki & Clark, 1998). After relief from drought, it has been found that AM plants recovered from wilting more quickly than non-AM plants (Gemma *et al*., 1997). In case of wheat, leaf drop and necrosis (Bryla & Duniway, 1997) due to drought was reduced in AM plants. Biomass, panicle number per plant, grain number per panicle, grain yield and weight increased in AM inoculated wheat under water stress (Al-Karaki *et al*., 2004). In *Citrus tangerine* and *Poncirus trifoliate*, plant height, stem diameter, leaf number, leaf area, root and shoot dry weights increased due to mycorrhizal colonisation under water stress and well watered conditions than corresponding plants without AM (Qiangsheng *et al*., 2006; Wu & Xia, 2006). In rice, AM inoculation causes increase in root and shoot fresh weights after a drought stress period of 15 days (Ruiz-Sanchez *et al*., 2010). Stimulation of plant height, stem diameter and root fresh weight of *Cucumis melo* under drought conditions has been confirmed by Huang *et al*. (2011). In marigold, flower parameters and growth criteria have been stimulated by mycorrhizal fungus under drought stress (Asrar *et al*., 2011) due to increased phosphorus nutrition (Bethlenfalvay *et al*., 1988) and water uptake (Faber *et al*., 1991). Increases in growth parameters and grain yield due to inoculation with AM have been observed in foxtail millet under water stress (Gong *et al*., 2014).

IMPACTS OF AMF ON PHYSIOLOGOICAL PARAMETERS

Arbuscular Mycorrhizal fungi affect shoot physiology and tissue water content by altering influx and efflux rates of water. The AM fungal symbiosis has impact on stomatal conductance, leaf water potential, abscissic acid content, photosynthetic pigments and photosynthesis. Enhancement in the gas exchange rates including stomatal conductance, transpiration and CO_2 assimilation in mycorrhizal plants as compared to their non-AM counterparts has been investigated by different researchers (Khalvati *et al*., 2005). The AM that induced changes in different physiological parameters is discussed below:

Abscisic Acid

Arbuscular Mycorrhizal symbiosis influences stomatal conductance and other physiological traits during drought stress by some non-nutritional chemical signals like phytohormone abscisic acid (ABA)

(Ludwig-Muller, 2010). Among the different responses of plants to drought stress, abscisic acid is the most significant stress signal transduction pathway (Zhang *et al*., 2006) as it regulates root hydraulic conductivity (Aroca, 2006), expression of different aquaporins (Aroca *et al*., 2006), as well as transpiration rate (Zhang *et al*., 2006). In response to water deficit causing stresses, endogeneous level of abscisic acid increases in vegetative tissues of plants (Zhang *et al*., 2006). The relationship between abscisic acid content and water stress tolerance has been clearly demonstrated by Kulkarni *et al*. (2000). Abscisic acid minimises water loss through transpiration by promoting stomatal closure and then causes alleviation of damage due to stress by activation of many stress responsive genes leading to more stress tolerance by plants (Zhang *et al*., 2006). Drought stress inducible genes can be categorised into functional and regulatory genes. Functional genes help in acquiring drought tolerance through late embryogenesis abundant proteins, proteases, enzymes for biosynthesis of osmolytes, detoxification enzymes and water channels or other transporters.

Expression of functional genes is regulated by regulatory genes which involve protein kinases, phosphatases, transcription factors and also those involved in biosynthesis of abscisic acid (Shinozaki & Yamaguchi-Shinozaki, 2007). Abscisic Acid regulates transcription of genes with the help of cis and trans-acting factors, as well as MYC-like and MYB-like deoxyribonucleic acid elements. It has been reported that abscisic acid levels increased in mycorrhizal maize (Danneberg *et al*., 1992) and soyabean (Meixner *et al*., 2005) as compared to non-AM plants under normal conditions. It has been reported that when subjected to drought stress, AM plants showed lower levels of abscisic acid than non-AMF ones revealing that AM plants experience less intense water stress (Doubkova *et al*., 2013). When subjected to osmotic stress by polyethylene glycol mycorrhizal larch plants had lower abscisic acid as compared to non-AM plants (Rincon *et al*., 2005). Adequate balance between root water movement and transpiration during drought and its recovery are maintained by AM plants as compared to non-AM plants due to better and faster regulation of abscisic acid levels by AM plants (Aroca *et al*., 2008).

Leaf Water Potential and Stomatal Conductance

The index of water status of the entire plant is known as leaf water potential, which corresponds to a major trait showing improved resistance of plants to water stress through better hydration. Higher water use efficiency due to mycorrhization has been reported by many workers (Ruiz-Lozano & Aroca, 2010). During water stress, an increase in water use efficiency due to mycorrhizae has been seen in *Allium sativum* (Borde *et al*., 2012). At the same time, a lack of constructive effect of mycorrhizae on water use efficiency in *Knautia arvensis* (Doubkova *et al*., 2013) has also been found. Higher relative water content and water use efficiency help in the movement

of water to the evaporating surfaces so that stomata in leaves can be maintained in open state (Nelsen & Safir, 1982). During lethal periods of water stress, osmotic adjustment in the leaves of mycorrhizal basil plants was found to be more as compared to non-mycorrhizal plants (Kubikova *et al*., 2001). Similarly, during water stress, a decline in leaf water potential has been postponed by AM in plants (Subramanian *et al*., 1997) and after relief from water stress, leaf water potential returns back to its normal level much faster in mycorrhizal than non-mycorrhizal maize plants (Subramanian *et al*., 1997).

Often water status of AM plants is found related to gas exchanges including stomatal behaviour and transpiration fluxes (Zhu *et al*., 2012). As pointed by Auge (2001), leaf water potential and stomatal conductance are functionally linked with each other. Stomatal conductance during water stress remains unchanged for a longer time in mycorrhizal plants as compared to non-mycorrhizal plants (Duan *et al*., 1996). Different rates of stomatal conductance and transpiration have been shown by AM and non-AM plants during water stress. It has been confirmed many times that altered rates of stomatal conductance and transpiration have been observed in mycorrhizal lettuce, rose, soybean and wheat. Stomatal opening increased in AM plants as compared to their non-AM counterparts (Auge, 2001). Increased stomatal conductance has also been found in mycorrhizal maize (Zhu *et al*., 2012).

Photosynthetic Pigments

A decrease in chlorophyll content due to water stress is a typical symptom of oxidative stress in water stressed plants. It has been observed by different researchers as in *Tagetes erecta* (Asrar & Elhindi, 2011), *Citrus* (Wu & Xia, 2006) and *Zea mays* (Zhu *et al*., 2012; Abdelmoneim *et al*., 2014). The chlorophyll content of mycorrhizal plants has found to be more as compared to non-AM plants (Mathur & Vyas, 1995; Gemma *et al*., 1997). Moreover, the rise in photosynthetic pigments due to mycorrhizae has been confirmed by Abdelmoneim *et al*. (2014). During water stress, mycorrhization resisted the reduction of chlorophyll (Asrar & Elhindi, 2011; Zhu *et al*., 2012; Abdelmoneim *et al*., 2014). It has been well recognised that chlorophyll concentration is related to photosynthetic rate and chlorophyll fluorescence. Thus, in AM plants, an increased rate of chlorophyll has been associated with the increased rate of photosynthesis or with higher Mg and N which are major constituents of chlorophyll (Mathur & Vyas, 1995). Meanwhile, application of AM helps the plants to overcome photodestruction and photoinhibition of pigments under the conditions of water stress by increasing the content of carotenoids, as they help in protection of photosynthetic apparatus against the harm caused by single oxygen. Therefore, quenching and deactivation of excited triplet state of chlorophyll can be brought about by carotenoids (Foyer & Harbinson, 1994).

Photosynthesis

Higher rates of photosynthesis have often been shown by AM plants in comparison to their non-AM counterparts, which are consistent with the effects of mycorrhizae on chlorophyll content and stomatal conductance, as discussed earlier. In AM colonised *Bouteloua gracilis*, higher photosynthesis was related to a decrease in both liquid phase and gas phase resistance to CO_2 transported in leaves (Allen *et al*., 1981). An increase in the number of photosynthetic units by AM symbiosis has been suggested by some researchers. AM fungi are also known to increase the rate of photosynthetic export and storage. During drought, different effects have been shown by different AM fungi on photosynthesis, as confirmed by Ruiz-Lozano and Azcon (1995). When comparing different *Glomus* species, it was found that one species decreased the photosynthetic phosphorus use efficiency while the other increased it (Ruiz-Lozano & Azcon, 1995). During drought stress, different mycobionts influence host photosynthesis differently (Dixon *et al*., 1994). With the help of chlorophyll fluorescence, it has been found that water stress interrupts transport of electrons in photosynthetic apparatus and causes the destruction in structure and function of PSII reaction centre (Baker, 2008). Reduced availability of CO_2, which results in inefficient use of CO_2 and high susceptibility of photo damage (Powles, 1984), poor ability to manage excess of radiation is one of the main photosynthetic limitations imposed by water stress (Chaves *et al*., 2003). Photo protective mechanisms help in regulation of excitation energy reaching reaction centre of photosystem by dissipation of thermal energy (Demmig-Adams & Adams, 2006). These photoprotective mechanisms also help in repair of oxidative damage by scavenging oxidative molecules (Fernandez-Marin *et al*., 2009). Drought stress decreases the quantum efficiency or photochemical efficiency of PSII, which is given by the ratio of (Fv/Fm) (Borkowska, 2002). This ratio also provides a way to monitor environmental stress (Krause & Weis, 1991). Mycorrhizal symbiosis has been shown to alleviate the undesirable effects of drought stress on photochemical efficiency and PSII reaction centre (Baker, 2008) in maize (Zhu *et al*., 2012).

ROLE OF VAM IN MINERAL NUTRITION

Drought stress and the nutritional status of plants are correlated to each other. It has been confirmed that AM plays an important role in improving nutritional status of host plants. The concentration of phosphorus may influence host water balance but it is not easily accessible to the plants as it cannot flow freely in soil. In addition to phosphorus, zinc and copper are the other elements which are also fixed in the soils. In a study on clover plants planted on five compartments having an air gap, Li *et al*. (1991) observed that more than a half of the overall copper and zinc content was absorbed by extension hyphae. The AM symbiosis has been reported to improve the absorption of silicon, nickel, copper

and calcium (Gong *et al*., 2000). Enhanced phosphorus and nitrogen nutrition is the most recognizable benefit of AM fungi for plants. Further details of this are discussed below:

Phosphorus Nutrition

Phosphorus deficiency can greatly limit plant growth as it is extremely important for plant growth. From the reserves in the soil, the most important way of assuring adequate supply of phosphorus to the land plants is through mycorrhizal associations (Al-Amri *et al*., 2013). In the phosphorus deficient soils, the principal role of AM symbiosis in efficiently absorbing phosphorus has been shown (Smith *et al*., 1986). Using gel electrophoresis (Abdel-Fattah, 2001) and ultra-cytochemistry (Gianinazzi-Pearson & Gianinazzi, 1978), an active enzyme alkaline phosphatase has been identified in arbuscular mycorrhizae (Abdel-Fattah, 2001). Localisation of alkaline phosphatase in the vacuoles of mature arbuscules (Gianinazzi *et al*., 1979) reveals the involvement of this enzyme in acquisition of phosphorus in mycorrhizal plants. The AMF can be of great benefit to the plants growing in tropical soil having high capacity to immobilise phosphorus, as well as having low phosphates (George *et al*., 1995). High content of phosphorus may lead to inhibition of AM colonisation in roots and can decrease vesicle and entry point formation (Amijee *et al*., 1989), as well as the length of AM associated external hyphae (Abbott *et al*., 1984). Symbiotic efficiency of AM fungi reduces in the concentration of soil phosphorus suboptimal for functioning of mycorrhizae and competition occurs between mycobiont and host for scarce phosphorus. Meanwhile, low mobility of phosphorus in the soil and quick uptake of phosphate into the roots results in the formation of phosphate depletion zone (Abdel-Fattah, 1997). This phosphate depletion zone in the roots of non-mycorrhizal plants goes beyond root hair cylinder which points towards the unavailability of phosphate to plants directly. The absorption and transportation of phosphorus beyond the depletion zones into root tissues can be brought about by external hyphae of AMF (Wu *et al*., 2010).

Improved hydraulic conductivity and quick recovery from water stress confirm the water stress tolerance of the plants which has been attributed to better phosphorus nutrition (Bryla & Duniway, 1997). As compared to amply watered plants, significant reduction in the phosphorus levels has been noticed in mycorrhizal and non-mycorrhizal plants and the rate of decrease was found to be higher in non-mycorrhizal plants as compared to the AM plants in wheat (Al-Karaki *et al*., 2004), citrus (Wu & Xia, 2006) and marigold (Asrar & Elhindi, 2011). Despite water stress, increased phosphorus uptakes in both the roots and shoots of mycorrhizal tomato plants have been noticed by Subramanian *et al*. (2006). Host water balance is also influenced by phosphorus concentration as phosphorus starvation affects stomatal conductance. It has been suggested that enhancement in photosynthetic capacity due to phosphorus leads to high stomatal

conductance and transpiration (Koide, 2000). The concentration of phosphorus in the leaves may have impact on the response of stomata to environmental disturbances possibly by affecting energy involved in osmotic potential of guard cells leading to opening of stomata (Weyers & Meidner, 1990). Under drought stress, different inorganic ions and organic solutes are accumulated by higher plants, whose accumulation in AM seedlings results in higher biomass due to better accumulation of carbohydrates and better osmotic adjustment (Wu & Xia, 2006).

Nitrogen Nutrition

Although the role of mycorrhiza in phosphorus nutrition of plants has been well documented, only a few studies on the importance of mycorrhizae in nitrogen nutrition under drought stress conditions have been carried out. An increase in the utilisation and absorption of nitrogen in plant shoots due to mycorrhizal inoculation has been confirmed by Zhao and Yan (2006). It has been well recognised that ammonium is taken by external hyphae (Rains & Bledsoe, 2007), while nitrate is made available to mycorrhizal roots directly by external mycelium under water stress (Subramanian & Charest, 1999). Improved uptake of nitrogen causes key nitrogen assimilating enzymes to increase their activities (Subramanian & Charest, 1998), which in turn cause increases in amino acid and protein concentrations (Subramanian & Charest, 1995). It has been recommended that the increased activities of enzymes like nitrate reductase (NR) and glutamine synthetase (GS) (Subramanian & Charest, 1999) can be associated with enhanced nutrition of phosphorus. In mycorrhizal plants, increased root hydraulic conductivity, as a result of enhanced phosphorus nutrition, results in improved tolerance to water stress (Bryla & Duniway, 1997). On the other hand, no variation in root hydraulic conductivity has been noticed in non-mycorrhizal phosphorus fertilised and mycorrhizal plants having similar size as well as phosphorus status (Davies *et al*., 1993). Other studies on AM plants have shown that despite the phosphorus content, the activities of GS (Azcon & Tobar, 1998) and NR (Ruiz-Lozano & Azcon, 1996) have increased. Studies on the role of N uptake and assimilation in perennial rye grass have shown that AM symbiosis improves uptake of nitrogen, water and also activities of N-assimilating enzymes, leading to higher amounts of amino acids and proteins (Subramanian & Charest, 1999).

EXPRESSION OF AQUAPORIN GENES AND GENES ENCODING DEHYDRIN PROTEINS

Mycorrhizae improve drought tolerance by enhancing plant activities like proline or ABA synthesis (Ruiz-Sanchez *et al*., 2011) and altering the flow of water through networks of hyphae or higher nutrient uptake and photosynthesis. The regulation of the physiological response of mycorrhizal plants to drought stress is brought about by expression of drought related plant genes

like *p5cs* genes encoding rate-limiting enzyme in proline biosynthesis (Porcel *et al*., 2004), aquaporin genes (Porcel *et al*., 2006), as well as *nced* genes encoding key enzyme in ABA biosynthesis (Aroca *et al*., 2008).

Furthermore, in order to improve water deficiency, water may be absorbed and transported directly from the soil in vicinity or even distant places from the root tip to the host plants with the help of fungal extra radical mycelium (Egerton-Warburton *et al*., 2007). In plants, water balance is maintained by diffusion or through water channels across the biomembranes known as aquaporins (Ruiz-Lozano *et al*., 2009). Aquaporins are the proteins encoded by aquaporin genes, which are an important component of cellular transport system. It is considered that aquaporins are generally concerned with the process of symbiotic exchange at fungus-plant interface determining the transport properties of plant and fungus (Maurel & Plassard, 2011). In comparison to diffusion, water movement through aquaporins is 10-100 times higher. Seven classes of aquaporins have been found in plants which include plasma membrane intrinsic proteins (PIPs), NOD 26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), tonoplast intrinsic proteins (TIPs), GlpF-like intrinsic proteins (GIPs), X-intrinsic proteins (XIPs) and hybrid intrinsic proteins (HIPs) (Danielson & Johanson, 2008). To decrease transpiration rate or to increase leaf water potential and root hydraulic conductivity, AMF may downregulate or upregulate aquaporin genes in leaves or roots (Aroca *et al*., 2008; Ruiz-Lozano *et al*., 2009). The regulation of expression of aquaporin genes by AMF improves water stress tolerance by improving plant water status (Li *et al*., 2012). Gene silencing and overexpression have verified the functions of aquaporins (Yu *et al*., 2005). Meanwhile, expression of aquaporin encoding genes has also been demonstrated by Uehlein *et al*. (2007). In AM fungal structures, including extraradical mycelia and periarbuscular membrane (Li *et al*., 2012), an aquaporin has also been identified. Drought stress affects both fungal and plant aquaporins (Uehlein *et al*., 2007; Li *et al*., 2012). Compared to non-AM plants, mycorrhizal plants have shown reduced expression of aquaporin genes during water stress but overall plant water relations in AM plants are influenced by other properties of aquaporins during drought stress (Aroca *et al*., 2007).

When maize plants were subjected to drought stress, enhanced expression of two functional genes encoding aquaporins were identified in fungi and maize roots. As this pattern is related to enhanced root water content and accumulation of proteins, it is evident that water status has been improved by regulation of expression of aquaporins by AM fungi (Li *et al*., 2012). In mycorrhizal plants, enhanced apoplastic flow of water in roots competitive to cell to cell pathway has been reported during water stress by using an inhibitor of aquaporin activity and apoplastic tracer dye (Barzana *et al*., 2012).

Arbuscular mycorrhizal (AM) symbiosis can help plants to cope with the detrimental effects of soil water deficit

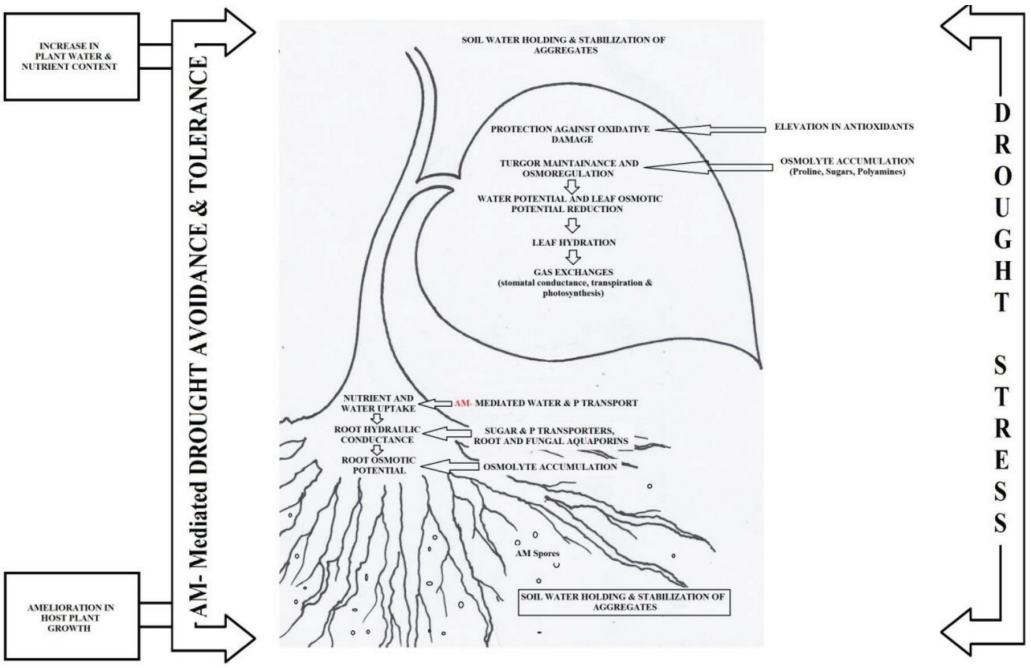

Fig.1 Arbuscular mycorrhizal (AM) symbiosis can help plants to cope with the detrimental effects of soil water deficit.

acting, directly or indirectly, on plant functionality both above and below ground (Fig.1). At the levels of both leaves and roots, the osmotic stress which is usually caused by drought is counteracted by mycorrhizal plants through biochemical changes that mostly include increased biosynthesis of metabolites (mainly proline and sugars) that act as osmolytes. These compounds contribute to the lowering of osmotic potential, and in turn, of the leaf water potential. These lower potentials allow the plants to maintain high organ hydration and turgor that sustain overall cell physiological activity that is mainly related to the photosynthetic machinery. The AM plants withstand drought-induced oxidative stress by increasing their production of antioxidant compounds that scavenge ROS and enhancing the activities of antioxidant enzymes. The AM root colonisation can enhance root growth, architecture and hydraulic properties and thus induce the formation of a highly functional root system for nutrient/water uptake. At the same time, AM fungal hyphae in the soil provides an efficient pathway for nutrient/water uptake and transport, allowing a more efficient exploitation of the water and nutrient reservoirs in the soil where only fungal hyphae can grow, thereby bypassing the zones of water and nutrient depletion around the roots. Molecular mechanisms activated by AM symbiosis to counteract drought include gene activation of functional proteins such as the membrane

transporter aquaporins, and potentially, ion and sugar transporters, in both roots and fungi. Improved nutrient/water uptake and transport in roots translate into enhanced hydration of the aboveground organs that in turn affects physiological and biochemical processes. In addition, AM symbiosis can increase the resistance of plants to drought through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water.

AMF AND ECOSYSTEM SERVICES

Global environment is being changed by human at an unprecedented rate. Thus, to predict the path of future ecosystems and communities in a changing world, a sound understanding of mycorrhizal reaction to anthropogenic environmental changes is helpful. Due to the higher interference of landscape by humans, it is important to understand the effects of land use changes on AMF activity, their abundance and influence on ecosystem services. Meanwhile, loss of AMF propagules due to tilling, liberal fertilisation and use of pesticides (Helgason *et al*., 1998) may threaten ecosystem stability by lowering the soil fertility and nutrient uptake capacity of plants (Jeffries *et al*., 2003). Among major impacts of AMF are elaborated in the subsequent sections.

Reduction in the Use of Phosphorus Fertilisers

Diffusion capacity of inorganic phosphate (Pi) is limited in soil and Pi depletion zones are generated at root surface due to its rapid absorption from the soil solution leading to a decline in concentration of Pi (Marschner & Dell, 1994). In a given cropland, phosphate requirements are difficult to predict, whereas recommendations of phosphorus fertilisers are often vague as these recommendations are based on only soil tests (Olsen, 1954). Organic fraction of soil phosphorus pool is ignored and this results in application of phosphorus in a wasteful manner (Gilbert, 2009). Considering the different adverse effects of phosphates like algal blooms, eutrophication, etc., a main step towards sustainable agriculture is to reduce huge amounts of phosphate applied to croplands. Excess fertilisation causes phosphorus build up in the soils, runoff losses, pollution hazards and increased dependence of crops on fertilisers (Vance *et al*., 2002). Moreover, phosphorus fertilisers have negative impacts on AM fungi even though AMF strains have shown tolerance to high phosphorus levels (Ndiaye *et al*., 2009).

Benefits derived from mycorrhizae are reduced due to excess of P fertilisation (Plenchette *et al*., 2005). Also in soil, phosphate ions get rapidly bound to cations leading to the formation of insoluble complexes which can not be used by plants, whereas existence of mycorrhizae in soil increases solubility of phosphate (Vance *et al*., 2002; Smith & Read, 2008). It is still unknown whether or not the fungus releases the enzymes itself for degrading insoluble P complexes. Yet, it is definite that mycorrhizal fungi cooperate with rhizosphere microorganisms and increase the establishment of bacteria which in turn release enzymes for phosphate

solubilization (Barea *et al*., 2002). The processes of nutrient mobilisation explained above are significant in nutrition of plants and provide a reason for high soil fertility requirements of non-mycorrhizal plants for their maintenance.

AMF Enhances Soil Stability

During mycorrhizal development, a complex ramifying network of mycelium grows from the mycorrhizal roots into surrounding soil reaching up to 30 meters per gram of soil (Cavagnaro *et al*., 2005). The network forms up to 50% of mycelium in soil and contributes significantly to soil aggregation and formation of macroporous structure in soil that allow air and water to penetrate (Rillig *et al*., 2002). This enhances soil quality and stability and prevents soil erosion (Jeffries *et al*., 2003). The AMF retains soil aggregates physically with the mycelium and also secretes a hydrophobic, non-proteinaceous glue-like fungal substance called glomalin which binds the soil particles to other soil particles and hyphae (Rillig *et al*., 2002). Even after the death of their host, AMF are known to stabilise the soils up to 5 months (Tisdall & Oades, 1980). Agronomic practices including ploughing, monoculture cropping or fertilisation affect the diversity and quantity of AMF adversely (Helgason *et al*., 1998). The decrease in fungal biomass reduces soil stability, and thus the risk of soil erosion also increases. As soil is a non-renewable resource, the effect of soil erosion is increasing and it is irreversible in most cases.

CONCLUSION

The utmost importance of mycorrhizal symbiosis lies in establishing a connection between heterogeneously distributed nutrients especially nitrogen and phosphorus to plant systems. Mycorrhizae are valuable for sustainability and ecosystem services as they help in shaping plant communities and terrestrial ecosystems. Their appropriate management in nutrient deficient soils will allow a greater sustainable management of production without productivity loss. A variety of protective mechanisms are employed by mycorrhizal plants to neutralise the adverse effects of drought. In all aspects, it is obvious that AM symbiosis causes altered water movement rates into, through and out of plants affecting physiology and morphology of plants. Unlike mycorrhizal effect on phosphate uptake and growth, the influence of mycorrhizae on plant water relations is not dramatic and reliable. The effect of mycorrhizae on gas exchange and tissue hydration is often subtle, temporary and specific to symbiont. The mechanisms increasing drought resistance in plants due to mycorrhizae are still a matter of debate. Two characteristics are important for mycorrhizal symbiosis to increase drought resistance, one being activation of defense system rapidly and the other is the synthesis of some biochemicals which are able to resist water stress. Significant progress has been made in understanding the role of AM symbiosis in conferring drought resistance to plants; however, more attention is required to unravel hidden metabolic pathways and metabolites.

REFERENCES

Abbott, L. K., Robson, A. D., & De Boer, G. (1984). The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *New Phytologist, 97,* 437-446.

Abdel-Fattah, G. M. (1997). Functional activity of VA-mycorrhiza (*Glomus mosseae*) in the growth and productivity of soybean plants grown in sterilized soil. *Folia Microbiologica, 42,* 495-502.

Abdel-Fattah, G. M. (2001). Measurement of the viability of arbuscular mycorrhizal fungi using three different stains; relation to growth and metabolic activities of soybean plants. *Microbiological Research, 156,* 359-367.

Abdelmoneim, T. S., Tarek, A. A. Moussa, Almaghrabi, O. A., Hassan, S. Alzahrani, & Ismail, A. (2014). Increasing plant tolerance to drought stress by inoculation with arbuscular mycorrhizal fungi. *Life Science Journal, 11,* 10-17.

Al-Amri, S. M., Al-Whaibi, M. H., Abdel-Fattah, G. M., & Siddiqui, M. H. (2013). Role of mycorrhizal fungi in tolerance of wheat genotypes to salt stress. *African Journal Microbiology Research, 7,* 1286-1295.

Al-Karaki, G., McMichael, B., & Zak, J. (2004). Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza, 14,* 263-269.

Al-Karaki, G.N., & Clark, R.B. (1998). Growth, mineral acquisition and water use by mycorrhizal wheat grown under water stress. *Journal of Plant Nutrition, 21,* 263-276.

Allen, M. F., Smith, W. K., Moore, T. S., & Christensen, M. (1981). Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis*. *New Phytologist, 88,* 683-693.

Almagrabi, O. A., & Abdelmoneim, T. S. (2012). Use of Arbuscular mycorrhizal fungi to reduce the deficiency effect of phosphorous fertilization on maize plants (*Zea mays* L.). *Life Science Journal, 9,* 1648-1654.

Amijee, F., Tinker, P. B., & Stribley, D. P. (1989). The development of endomycorrhizal root systems. VII. A detailed study of effects of soil phosphorus on colonization. *New Phytologist, 111,* 435-446.

Arntz, A., & Delph, L. (2001). Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia, 127,* 455-467.

Aroca, R. (2006). Exogenous catalase and ascorbate modify the effects of abscisic acid (ABA) on root hydraulic properties in *Phaseolus vulgaris* L. plants. *Plant Growth Regulation, 25,* 10-17.

Aroca, R., Bago, A., Sutka, M., Antonio, P. J., Cano, C., Amodeo, G., & Ruiz-Lozano J. M. (2009). Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. *Plant Microbe Interactions, 22,* 1169-1178.

Aroca, R., Ferrante, A., Vernieri, P., & Chrispeels, M. J. (2006). Drought, abscisc acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Annals of Botany, 98,* 1301-1310.

Aroca, R., Porcel, R., & Ruiz-Lozano, J. M. (2007). How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist, 173*, 808-816.

Aroca, R., Vernieri, P., & Ruiz Lozano, J. M. (2008). Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. *Journal of Experimental Botany, 59,* 2029-2041.

Asar, A., & Elhindi, K. M. (2011). Alleviation of drought stress of marigold (Tagetes erecta) plants by using arbuscular mycorrhizal fungi. *Saudi Journal of Biological Sciences, 18,* 93-98.

Asensio, D., Rapparini, F., & Penuelas, J. (2012). AM fungi root colonization increases the production of essential isoprenoids vs nonessential isoprenoids especially under drought stress conditions or after jasmonic acid application. *Phytochemistry, 77,* 149-161.

Auge, R. M. (2001). Water relations, drought and vesicular arbuscular mycorrhizal symbiosis. *Mycorrhiza, 11,* 3-42.

Auge, R. M. (2004). Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science, 84,* 373-381.

Auge, R. M., & Moore, J. L. (2005). Arbuscular mycorrhizal symbiosis and plant drought resistance. In V. S. Mehrotra (Ed). *Mycorrhiza: Role and Applications* (pp. 136–162). Nagpur: Allied Publishers.

Azcon, R., & Tobar, R. (1998). Activity of nitrate reductase and glutaminesynthetase in shoot and root of mycorrhizal *Allium cepa*- Effect of drought stress. *Plant Science, 133,* 1-8.

Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annual Review of Plant Biology, 59,* 89-113.

Barzana, G., Aroca, R., Paz, J. A., Chaumont, F., Martinez-Ballest, M. C., & Carvajal, M. (2012). Arbuscular mycorhhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. *Annals of Botany, 109,* 1009-1017.

Bethlenfalvay, G. J., Brown, M. S., Ames, & Thomas, R. N. (1988). Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Physiologia Plantarum, 72,* 565-571.

Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research, 56,* 1159-1168.

Bok-Rye, L., Sowbiya, M., Jean-Christophe, A., Woo-Jin J., & Kim T. H. (2012). Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation inperennial ryegrass under well-watered and drought-stressed conditions. *Mycorrhiza, 22*(7)*,* 525-534.

Borde, M., Dudhane, M., & Jite, P. (2012). Growth, water use efficiency and antioxidant defense responses of mycorrhizal and non mycorrhizal *Allium sativum* L. under drought stress condition. *Annals of Plant Sciences, 1*, 6–11.

Borkowska, B. (2002). Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. *Acta Physiologiae Plantarum, 24,* 365-370.

Bouwmeester, H. J., Roux, C., Lopez-Rae A., & Becard, G. (2007). Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends Plant Science, 12,* 224-230.

Bray, E. A. (1993). Molecular responses to water deficits. *Plant Physiology, 103,* 1035-1040.

Bray, E. A. (2002). Abscisic acid regulation of gene expression during water-deficit stress in the era of the Arabidopsis genome. *Plant Cell and Environment, 25,* 153-161.

Brundrett, M. (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews, 79,* 473–495.

Brundrett, M. (2009). Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and develop ingreliable means of diagnosis. *Plant and Soil, 320,* 37-77.

Cavagnaro, T. R., Smith, F. A., Smith, S. E., & Jakobsen, I. (2005). Functional diversity in arbuscular mycorrhizas: Exploitation of soil patches with different phosphate enrichment differs among fungal species. *Plant Cell and Environment, 28,* 642-650.

Chaudhry, M. S., Batool, Z., & Khan A. G. (2005). Preliminary assessment of plant community structure and arbuscular mycorrhizas in rangeland habitats of Cholistan desert, Pakistan. *Mycorrhiza, 15,* 606-611.

Chaves, M., Maroco, J., & Pereira, J. (2003). Understanding plant responses to drought- from genes to the whole plant. *Functional Plant Biology, 30,* 239-264.

Danielson, J., & Johanson, U. (2008). Unexpected complexity of the aquaporin gene family in the moss *Physcomitrella patens*. *BMC Plant Biology, 8,* 45.

Davies, F. T., Potter, J. R., & R. G. Linderman (1993). Drought resistance of mycorrhizal pepper plants independent of leaf P-concentration-response in gas exchange and water relations. *Physiolgia Plantarum, 87,* 45-53.

Demming-Adams, B., & Adams, W. W. (2006). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist, 172,* 11-21.

Dixon, R. K., Rao, M. V., & Garg, V. K. (1994). Water relations and gas exchange of mycorrhizal *Leucaena leucocephala* seedlings. *Journal of Tropical Forest Science, 6,* 542-552.

Doubkova, P., Vlasakova, E., & Sudova, R. (2013). Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on *Knautia arvensis* plants in serpentine soil. *Plant and Soil, 370,* 149-161.

Duan, X., Neuman, D. S., Reiber, J. M., Green, C. D., Saxton, A. M., & Auge R. M. (1996). Mycorrhizal influence on hydraulic and hormonal factors implicated in control of stomatal conductance during drought. *Journal of Experimental Botany, 47,* 1541-1550.

Egerton-Warburton, L. M., Querejeta, J. I., & Allen, M. F. (2007). Common mycorrhizal networks provide a potential pathway for the transfer of hydraulically lifted water between plants. *Journal of Experimental Biology, 58,* 1473-1483.

Faber, B. A., Zasoki, R. J., Munns, D. N., & Shackel, K. (1991). A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. *Canadian Journal of Botany, 69,* 87-94.

Fernandez-Marin, B., Balaquer, L., Esteban, R., Becerril, J. M., & Garcia-Plazaola J. I. (2009). Dark induction of the photoprotective xanthophyll cycle in response to dehydration. *Journal of Plant Physiology, 166,* 1734-1744.

Foyer, C. H., & Harbinson, J. (1994). Oxygen metabolism and the regulation f photosynthetic electron transport, In C. H. Foyer & P. M. Mullineauex, (Eds). *Causes of Photooxidative Stress and Amelioration of Defense System in Plants* (pp. 1-4). Boca Raton: CRS Press.

Foyer, C. H., & Noctor, G. (2005). Redox homeostis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. *Plant Cell, 17*, 1866-1875.

Garriock, M. L., Peterson, R. L., & Ackerley C. A. (1989). Early stages in colonization of *Allium porrum* (leek) roots by the vesicular arbuscular mycorrhizal fungus, *Glomus versiforme*. *New Phytologist, 112,* 85-92.

Gemma, J. N., Koske, R. E., Roberts, E. M., Jackson, N., & De Antonis, K. (1997). Mycorrhizal fungi improve drought resistance in creeping bentgrass. *Journal of Turfgrass Science, 73,* 15-29.

George, E., Marschner, H., & Jakobsen, I. (1995). Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology, 15,* 257-270.

Gianinazzi, S., Gianinazzi- Pearson, V., & Dexheimer, J. (1979). Enzymatic studies on metabolism of vesicular arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected with *Glomus mosseae*. *New Phytologist, 82,* 127-132.

Gianinazzi, S., & Vosatka, M. (2004). Inoculum of arbuscular mycorrhizal fungi for production systems: science meets business. *Canadian Journal of Botany, 82,* 1264-1271.

Gianinazzi-Pearson, V., & Gianinazzi, S. (1978). Enzymatic studies on the metabolism of vesicular arbuscular mycorrhiza. II. Soluble alkaline phosphatase specific to mycorrhizal infection in onion roots. *Physiology Plant Pathology, 12,* 46-53.

Gilbert, N. (2009). Environment: The disappearing nutrient. *Nature, 461,* 716-718.

Gong, M., Tang, M., Chen, H., Zhang, Q., & Feng, X. (2013). Effects of two *Glomus* species on the growth and physiological performance of *Sophora davidii* seedlings under water stress. *New Forest, 44,* 399-408.

Gong, M., You, X., & Zhang, Q. (2014). Effects of *Glomus intraradices* on growth and reactive oxygen metabolism of foxtail millet under drought. *Annals of Microbiology.* DOI 10.1007/s13213-014-0895-y

Gong, Q., Xu, D., & Zhong, C. (2000). *Study on biodiversity of mycorrhizae and its application* (pp. 51–61). Beijing: Chinese Forest Press.

Gunasekera, D., & Berkowitz, G.A. (1992). Evaluation of contrasting cellular-level acclimation responses to leaf water deficits in three wheat genotypes. *Plant Science, 86,* 1-12.

Habte, M., & Osorio, N. W. (2001). *Arbuscular mycorrhizas: producing and appliying arbuscular mycorrhizal inoculums*.University of Hawai, Honolulu. Retrieved from www.ctahr.hawaii.edu./oc/freepubs/pdf/amf_manual.pdf

Hamel, C. (1996). Prospects and problems pertaining to the managementof arbuscular mycorrhizae in agriculture. *Agriculture Ecosystem and Environment 60,* 197-210.

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H., & Young, J. P. W. (1998). Ploughing up the wood-wide web. *Nature, 394,* 431.

Huang, L. L., Yang, C., Zhao, Y., Xu, X., Xu, Q., Li, G. Z., Cao, J., & Herbert, S. J. (2008) Antioxidant defenses of mycorrhizal fungus infection against SO_2- induced oxidative stress in *Avena nuda* seedlings. *Bulletin of Environmental Contamination and Toxicology, l81,* 440-444.

Huang, Z., Zou, Z. R., He, C. X., He, Z. Q., Zhang, Z. B., & Li, J. M. (2011). Physiological and photosynthetic responses of melon (*Cucumis melo* L.) seedlings to three *Glomus* species under water deficit. *Plant and Soil, 339,* 391-39.

Ingram, J., & Bartels, D. (1996). The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant molecular Biology, 47,* 377-403.

Jacobson, K. M. (1997). Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland. *Journal of Arid Environments, 35,* 59-75.

Jeffries, P., Gianinazzi, S., Peretto, S., Turnau, K., & Barea, J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils, 37,* 1-16.

Jones, H. G., & Corlett, J. E. (1992). Current topics in drought physiology. *Journal of Agricultural Sciences, 119,* 291-296.

Kala, S., & Godara, A. K. (2011). Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of Ziziphus. *Journal of Scientometric Research, 55,* 65-69.

Khalvati, M. A., Hu, Y., Mozafar, A., & Schmidthalter, U. (2005). Quantification of water uptake by Arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations and gas exchange of barley subjected to drought stress. *Plant Biology, 7,* 706 -712.

Koide, R. T. (2000). Mycorrhizal symbiosis and plant reproduction. In Y. Kapulnik & D. D. Douds (Eds), *Arbuscular Mycorrhizas: Physiology and Function* (pp. 19–46). Dordrecht: Kluwer Academic Publishers.

Kramer, P. J., & Boyer, J. S. (1995). Water relations of plants and soils. San Diego: Academic Press Inc.

Krause, G. H., & Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology, 42,* 313-349.

Kubikova, E., Moore, J. L., Ownlew, B. H., Mullen, M. D., & Auge, R. M. (2001). Mycorrhizal impact on osmotic adjustment in *Ocimum basilicum* during a lethal drying episode. *Plant Physiology, 158,* 1227-1230.

Kuhn, K. D. (1991). Distribution of vesicular-arbuscular mycorrhizal fungi on a fallow agriculture site. II. Wet Habitat. *Angewandte Botanik, 65,* 187-203.

Kuhn, D. K., Weber, H. C., Dehne, H. W., & Gworgwor, N. A. (1991). Distribution of vesicular-arbuscular mycorrhizal fungi on a fallow agriculture site. I. Dry habitat. *Angewandte Botanik, 65,* 169-185.

Li, T., Hu, Y. J., Hao, Z. P., Li, H., Wang, Y. S., & Chen, B. D. (2012). First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist, 197,* 617-6.

Li, X., Marschner, H., & George, H. (1991). Acquisition of phosphorus and copper by VA mycorrhizal hyphae and root-to-shoot transport in white clover. *Plant and Soil, 136,* 49–57.

Linderman, R. G. (1994). Role of VAM fungi in biocontrol. In F. L. Pfleger & R. G. Linderman (Eds.), *Mycorrhizae and Plant Health* (pp.1–26). St. Paul: APS Press.

Ludlow, M. M., & Muchow, R. C. (1990). A critical evaluation of traits for improving crop yields in water-limited environment. *Advances in Agronomy, 43,* 107-153.

Ludwig-Muller, J., (2010). Hormonal responses in host plants triggered by arbuscular mycorrhizal fungi. In H. Koltai & Y. Kapulnik (Eds.), *Arbuscular mycorrhizas: physiology and function* (pp. 169–190). New York: Springer.

Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil, 159,* 89-102.

Mathur, N., & Vyas, A. (1995). Influence of VA mycorrhiza on net photosynthesis and transpiration of *Ziziphus mauritiana*. *Journal of Plant Physiology, 147,* 328-330.

Maurel, C., & Plassard, C. (2011). Aquaporins: for more than water at the plant-fungus interface? *New Phytologist, 190,* 815–817.

Meixner, C., Ludwig-Muller, J., Miersch, O., Gresshoff, P., Staehelin, C., & Vierheilig, H. (2005). Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutantnts1007. *Planta, 222,* 709-715.

Miransari, M. (2010). Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biology, 12,* 563-569.

Mohammadi, A., Habibi, D., Rohami, M., & Mafakheri, S. (2011). Effect of drought stress on antioxidant enzymes activity of some chickpea cultivars. *Amer-Eurasian Journal of Agricultural and Environmental Sciences, 11,* 782-785.

Morton, J. B. (2000). *International culture collection of arbuscular and vesicular arbuscular mycorrhizal fungi.* West Virginia University.

Morton, J. B., & Benny, G. L. (1990). Revised classification of Arbuscular Mycorrhizal (Zygomycetes): A new order, Glomales, two new sub-orders Glomineae, Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon, 37,* 471-491.

Morton, J. B., Bentivenga, S. P., & Bever, J. D. (1995). Discovery, measurement and interpretation of diversity in symbiotic endomycorrhizal fungi (Glomales, Zygomycetes). *Canadian Journal of Botany,* S25-S32.

Munns, R. (1988). Why measure osmotic adjustment? *Australian Journal of Plant Physiology, 15,* 717-726.

National Drought Mitigation Center (n.d). *Causes of Drought*. Retrieved from http://drought.unl.edu/DroughtforKids/WhatisDrought/CausesofDrought.asp

Ndiaye, F., Manga, A., Diagne-Leye, G., Ndiaye Samba, S. A., & Diop, T. A. (2009). Effects of rockphosphate and arbuscular mycorrhizal fungi on growth and nutrition of *Sesbania sesban* and *Gliricidia sepium*. *African Journal of Microbiology Research, 3,* 305-309.

Nelsen, C. E., & Safir, G. R. (1982). The water relations of well-watered, mycorrhizal and non-mycorrhizal onion plants. *Journal of American Society for Horticultural Science, 107,* 271-274.

Olsen, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* In Agriculture UDo, US Department of Agriculture.

Palmer, W. C. (1964). *Climatic variability and crop production. In Weather and Our Food Supply*. Centre for Agriculture and Economic Development, Report 20, pp.173 -187.

Palmer, W. C. (1965). *Meteorological drought*. Research Paper No.45. US Department of Commerce.

Panozzo, J., & Eagles, H. (1999). Rate and duration of grain filling and grain nitrogen accumulation of wheat cultivars grown in different environments. *Australian Journal of Agricultural Research, 50,* 1007-1015.

Penuelas, J., & Munne-Bosch, S. (2005). Isoprenoids: an evolutionary pool for photoprotection. *Trends in Plant Science, 10,* 166-169.

Pfleger, F. L., & Linderman R. G. (1994). *Mycorrhizae and plant health.* The American Phytopathololological Society. St. Paul, MN: APS Press.

Plenchette, C., Clermont-Dauphin, C., Meynard, J. M., & Fortin, J. A. (2005). Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Sciences, 85,* 31-40.

Pond, E. C., Menge, J. A., & Jarrell, W. M. (1984). Improved growth of tomato in salinized soil by vesicular-arbuscular mycorrhizal fungi collected from saline soils. *Mycologia, 76,* 74-84.

Porcel, R., Aroca, R., Azcon, R., & Ruiz-Lozano, J. M. (2006). PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Molecular Biology, 60,* 389-404.

Porcel, R., Azcon, R., & Ruiz-Lozano, J. M. (2004). Evaluation of the role of genes encoding for D1-pyrroline-5-carboxlyate synthetase (P5CS) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. *Physiological and Molecular Plant Pathology, 65,* 211-221.

Power, M. E., & Mills, L. S. (1995). The keystone cops meet in Hilo. *Trends in Ecology and Evolution, 10,* 182-184.

Powles, S. (1984). Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology, 35,* 15-44.

Qiangsheng, W. U., & Renxue, X. I. A. (2006). Effects of arbuscular mycorrhizal fungi on leaf solutes

and root absorption areas of trifoliate orange seedlings under water stress conditions. *Frontier for China, 3,* 312-317.

Rains, K. C., & Bledsoe, C. S. (2007). Rapid uptake of ^{15}N-ammonium and glycine-^{13}C, ^{15}N by arbuscular and ericoid mycorrhizal plants native to a Northern California coastal pygmy forest. *Soil Biology and Biochemistry, 39,* 1078-1086.

Rillig, M. C., Wright, S. F., Nichols, K. A., Schmid, W. F., & Torn, M. S. (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. *Plant and Soil, 238,* 325-333.

Rincon, A., Priha, O., Lelu-Walter, M. A., Bonnet, M., Sotta, B., & LeTacon, F. (2005) Shoot water status and ABA responses of transgenic hybrid larch *Larix kaempferi* X L. decidua to ectomycorrhizal fungi and osmotic stress. *Tree Physiology, 25,* 1101-1108.

Ruiz-Lozano, J. M., & Azcon, R. (1996). Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants. *Agricultural Ecosystems and Environment, 60,* 175-181.

Ruiz-Lozano, J. M., Mar Alguacil, M., Barzana, G., Vernieri, P., & Aroca, R. (2009). Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. *Plant Molecular Biology, 70,* 565-579.

Ruiz-Lozano, J. M., & Aroca, R. (2010). Host response to osmotic stresses: stomatal behaviour and water use efficiency of arbuscular mycorrhizal plants. In H. Koltai & Y. Kapulnik (Eds.), *Arbuscular mycorrhizas: Physiology and Function* (pp. 239–256). Berlin: Springer.

Schellembaum, L., Muller, J., Boller, T., Wienken, A., & Schuepp, H. (1998). Effects of drought on non-mycorrhizal and mycorrhizal maize: changes in the pools of non-structural carbohydrates in the activities of invertase and trehalase, and in the pools of amino acids and imino acids. *New Phytologist, 138,* 59-66.

Sekhon, H. S., Singh, G., Sharma, P., & Bains, T. S. (2010). Water use efficiency under stress Environments. In S.S. Yadav, L.C. Mc Neil, R. Redden, & S.A. Patil (Eds.), *Climate change and management of cool season grain legume crops*. Dordrecht-Heidelberg London-New York: Springer Press.

Shao, H. B., Chu, L. Y., Wu, G., Zhang, J. H., Lu, Z. H., & Hu, Y. C. (2007). Changes of some antioxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. *Colloids and Surfaces B: Biointerfaces, 54,* 143-149.

Shinozaki, K., & Yamaguchi-Shinozaki, K. (2007). Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany, 58,* 221-227.

Singh, S., Gupta, A. K., & Kaur, N. (2012). Differential responses of antioxidative defence system to long-term field drought in wheat (*Triticum aestivum* L.) genotypes differing in drought tolerance. *Journal of Agronomy and Crop Sciences, 198,* 185-189.

Smith, S. E., & Read, D. J. (1997). *Mycorrhizal symbiosis* (2nd edn). New York: Academic Press.

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal Symbiosis* (3rd edn.). New York: Academic Press.

Smith, S. E., & Smith, F. A. (2011). Roles of Arbuscular Mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology, 62,* 227-250.

Smith, S. E., St. John, B. J., Smith, F. M., & Bromley, J. L. (1986). Effects of mycorrhizal infection on plant growth, nitrogen and phosphorus nutrition of glasshouse grown *Allium cepa* L. *New Phytologist, 103,* 369-373.

Song, F., Song, G., Dong, A., & Kong, X. (2011). Regulatory mechanisms of host plant defense responses to arbuscular mycorrhiza. *Acta Ecologia Sinica, 31*, 322-327.

Subramanian, K. S., & Charest, C. (1995). Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress. *Mycorrhiza, 5,* 273-278.

Subramanian, K. S., & Charest, C. (1998). Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. *Physiologia Plantarum, 102,* 285-296.

Subramanian, K. S., & Charest, C. (1999). Acquisition of N by external hyphae of arbuscular mycorrhizal fungus and its impact on physiological response in maize under drought-stressed and wellwatered conditions. *Mycorrhiza, 9,* 69-75.

Subramanian, K. S., Charest, C., Dwyer, L. M., & Hamilton, R. I. (1997). Effects of arbuscular mycorrhizae on leaf water potential, sugar content and P content during drought and recovery of maize. *Canadian Journal of Botany, 75,* 1582-1591.

Subramanian, K. S., Santhanakrishnan, P., & Balasubramanian, P. (2006). Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. *Scientia Horticulturae, 107,* 245-253.

Szabados, L., & Savoure, A. (2009). Proline: a multifunctional amino acid. *Trends in Plant Sciences, 15,* 89-97.

Tawaraya, K., Turjaman, M., & Ekamawanti, H. A. (2007). Effect of arbuscular mycorrhiza colonization on Nitrogen and Phosphorus uptake and growth of *Aloe vera* L. *Horticultural Science, 42*(7)*,* 1737-1739.

Tian, Y. H., Lei, Y. B., Zheng, Y. L., & Cai, Z. Q. (2013). Synergistic effect of colonization with arbuscular mycorrhizal fungi improves growth and drought tolerance of *Plukenetia volubilis* seedlings. *Acta Physiolgia Plantarum, 35,* 687-696.

Tisdall, J. M., & Oades, J. M. (1980). The effect of crop rotation on aggregation in a red-brown earth. *Australian Journal of Soil Research, 18,* 423-433.

Trenberth, K. E. (2011). Changes in precipitation with climate change. *Climate Research, 47,* 123-138.

Uehlein, N., Fileschi, K., Eckert, M., Bienert, G., Bertl, A., & Kaldenhoff, R. (2007). Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry, 68,* 122-129.

Vadez, V., Berger, J. D., Warkentin, T., Asseng, Ratnakumar, P., Rao, K. P. C., Gaur, P. M., Munier-Jolain, N., Larmure, A., Voisin, A.S., Sharma, H. C., Pande, S., Sharma, M., Krishnamurthy, L., & Zaman, M. A. (2012). Adaptation of grain legumes to climate change: a review. *Agronomy for Sustainable Development, 32,* 31-44.

Vance, P. C., Uhde-Stone, C., & Allan, D. L. (2002). Phosphorus acquisition and use: critical adaptations by plants for securing a non renewable resource. *New Phytologist, 157,* 423-447.

Vander-Heijden, M. G. A., Klironomos, J. N., Ursic, M., Mou-Toglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., & Sanders I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature, 396,* 69-72.

Walter, M. H., & Strack, D. (2011). Carotenoids and their cleavage products: biosynthesis and functions. *National Product Reports, 28,* 663-692.

Weyers, J. D. B., & Meidner, H. (1990). Methods in Research, pp. 9–12. London: Longman.

Wu, C. A., Lowry, D. B., Nutter, L. I., & Willis, J. H. (2010). Natural variation for drought-response

traits in the *Mimulus guttatus* species complex. *Oecologia, 162,* 23-33.

Wu, Q. S., Xia, R. X., & Zou, Y. N. (2006). Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. *Journal of Plant Physiology, 163,* 1101-1110.

Wu, Q. S., Zon, Y. N., Liu, W., Ye, X. E., Zai, H. E., & Zhao, L. J. (2010). Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: Changes in leaf antioxidant defense systems. *Plant Soil and Environment, 56,* 470-475.

Xavier, L. J. C., & Germida, J. J. (2003). Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viceae* enhance pea yield and nutrition. *Biology and Fertility of Soils, 37,* 261-267.

Yu, Q., Hu, Y., Li, J., Wu, Q., & Lin, Z. (2005). Sense and antisense expression of plasma membrane aquaporin BnPIP1 from *Brassica napus* in tobacco and its effects on plant drought resistance. *Plant Science, 169,* 647-656.

Zhang, Z., Zhang, J., & Huang, Y. (2014). Effects of arbuscular mycorrhizal fungi on drought tolerance of *Cyclobalanopsis glauca* seedlings under greenhouse conditions. *New Forests, 45,* 545-556.

Zhang, J., Jia, W., Yang, J., & Ismail, A. M. (2006). Role of ABA in integrating plant responses to drought and salt stresses. *Field Crop Research, 97,* 111-119.

Zhang, Y., Zhong, C. L., Chen, Y., Chen, Z., Jiang, Q. B., & Wu, C. (2010). Improving drought tolerance of *Casuarina equisetifolia* seedlings by arbuscular mycorrhizas under glasshouse conditions. *New Forest, 40,* 261-271.

Zhao, X., & Yan, X. F (2006). Effects of arbuscular mycorrhizal fungi on the growth and absorption of nitrogen and phosphorus in *Camptotheca acuminata* seedlings. *Journal of Plant Ecology, 30,* 947-953.

Zhu, X. C., Song, F. B., Liu, S. Q., Liu, T. D & Zhou, X. (2012). Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. *Plant Soil and Environment, 58*(4), 186-191.

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Meat Characteristics of Red Jungle Fowl *(Gallus gallus Spadiceus)*, Malaysian Domestic Chickens *(Gallus gallus Domesticus)* and Commercial Broiler

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ABSTRACT

The meat characteristics of Red jungle fowl (*Gallus gallus Spadiceus*) and Malaysian Domestic chicken (*Gallus gallus Domesticus*), which are known as slow growing birds, were studied. Results were compared with those of the commercial broilers (ROSS) which are fast growing birds. The objective of the study is to determine the meat characteristics (pH, muscle fibre diameter and collagen content) of the breeds and the correlation to their meat quality. For this purpose, a total of 90 chickens (30 chickens for each breed) were used in this study. The chickens in each group were sacrificed at 20, 56 and 120 days post-hatching. Findings indicated that collagen content, pH, cooking loss and shear force values in Red jungle fowl and Malaysian Domestic chicken were significantly higher ($P<0.05$) than the commercial broilers. The smaller muscle fibre diameters and lower glycogen reserved contributed to higher pH. Meanwhile, the collagen content showed significantly ($P<0.05$) positive correlation to shear force and more prominent factors than the size of muscle fibre that determines tenderness of the meat. The commercial broilers' meat is much tender than that of the Malaysia Domestic chicken and Red jungle fowl.

Keywords: Red jungle fowl, Malaysian Domestic chicken, Commercial broilers, meat characteristic, correlation, meat quality.

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INTRODUCTION

The quality of meat can be described as the attractiveness of the meat to the consumers

(Wood *et al.*, 2003), encompassing diverse issues such as nutritional, hygienic, technological and sensory quality (Hofman, 1994). The sensory and physical quality of poultry product varies with growth rate and body composition (Duclos *et al*., 2007). Although it remains inconclusive, the correlation between pH, size of muscle fibre and collagen content attribute to the meat quality has been discussed by most researchers (Liu *et al.,* 1996). Skeletal muscles rich in collagen are less tender (Dransfield, 1977; Light *et al.,* 1985); however, other research has shown a weak relationship between collagen content and meat tenderness (Hunsley *et al.*, 1971).

In relation to meat quality, there are differing opinions regarding the diameter of muscle fibre that influences meat tenderness. In commercial broilers, Smith and Fletcher (1988) reported that an increase in myofiber diameter potentially leads to less tender meat. Furthermore, Rehfeidt *et al.* (2000) suggested that animal with greater fibre number and moderate size produce higher quality and quantity of meat.

The Red jungle fowl (Wall & Anthony, 1995) and the present Malaysian domestic chicken (Azahan & Zahari, 1983; Peterson *et al.,* 1991) are known as slow growth birds. The Red jungle fowl breeds are protected by the Malaysian government and there are potential niche market in the future and Malaysian domestic chickens are among the growing industries (Engku Elini Engku Arif, 2010). Nevertheless, scientifically based information on those breeds is extremely limited. On the other hand, commercial broiler chickens have gone tremendous progress in the selection to increased growth, feed conversion and carcass quality (Steven, 1991; Schreiweis *et al*., 2005).

However, there is no correlation study conducted on the meat characteristic and meat quality of Red jungle fowl and Malaysia domestic chickens in the literature. Thus, the objective of the study is to determine the meat characteristics (pH, muscle fibre diameter and collagen content) of the Red jungle fowl (RJ) and domestic chickens (DC) and their correlation with meat quality, as well as to determine which factors that significantly determine the tenderness of meat.

MATERIALS AND METHODS

Animals and Experimental Design

Red jungle fowls (RJ), Malaysian domestic chickens (DC) and commercial broilers (CB), comprising of 30 birds for each breed with mixed sexes, were used in this study. The eggs of RJ were supplied by a farmer who has RJ in his farm at Jenderam Hulu, Sepang in Selangor, Malaysia. The RJ was identified and confirmed through phenotypic characteristics which include colour, head, comb and lappet, ear lobes, tail, body size, leg and vocal (Amin Babjee, 2009). The eggs of DC were also supplied by the same farmer and reared in different cages. The DC was also identified and confirmed through their phenotypic characteristics which include colour, head, comb and lappet, ear lobes, tail, body size, leg and vocal which differ from those of the RJ (Aini, 1990;

Roberts, 2008; Amin Babjee, 2009). The eggs of both the RJ and DC were incubated and hatched in a laboratory in Universiti Putra Malaysia (UPM).

Day-old chicks (DOC) of commercial line (Ross) were supplied by a private hatchery (Linggi Poultry farm Sdn. Bhd., CP lot 1354, Mukim LubukTebrau, 33010 Kuala Kangsar, Perak, Malaysia). All the three breeds of chickens were reared in an experimental house (located at N 03. 00551º, E 101. 70501º in UPM) in different cages according to their age and breed. Feed and water were provided *ad libitum* consisting of standard commercial starter (201-P, Malayan Federal Flour Mill Sdn. Bhd) given from Day 1 to Day 21 post hatch, while finisher (203-P, Malayan Federal Flour Mill Sdn. Bhd) was given from Day 22 to Day 120 post hatch. All the birds were euthanized serially at Days 20, 56 and 120 post hatch through intravenous (cutaneous ulnar vein) administration of 80mg/kg of pentobarbitone sodium (Mitchell & Smith, 1991). As a standard for comparison, the comparisons were based on chronological time to examine differences in growth rather than physiological time (Chambers, 1990).

Sampling and Measurement

Ten birds were selected randomly from each breed and the *pectoralis major* muscle was selected for the analysis. The measurement of cross sectioning muscle fiber diameter was performed using a computerised image analyser (Leica DM LB2, Germany) after staining it with Haematoxylin and Eosin and six muscle bundles were selected randomly from each section and the diameter of muscle fibres was consistently evaluated. An attempt was made not to include the longitudinal and oblique muscle fibres so as to avoid the tendency of wrong measurement interpretation, and thus obtaining the most accurate results. The results were expressed as mean fibre diameter in a muscle bundle.

Meanwhile, pH measurements were carried out by using combined glass electrode pH meter (Mettler Toledo, USA) as described by Wattanachant *et al.* (2004). The samples were subjected to moist cooking at 80°C in a pre-heated water bath as described by Sazili (2003) for cooking loss determination. Measurement of meat tenderness was carried out by using Volodkovich shear force method. The total collagen analyses were determined by direct measurement of hydroxyproline after acid hydrolysis, as described by Reddy and Chukuka (1996). Hydroxyproline was converted to total collagen by using the factor of 8.0 (Kolar, 1990; Salakova *et al*., 2009).

Statistical Analysis

One-way ANOVA and Duncan's multiple range tests were used to elucidate differing means by using SPSS (17.0) programme.

RESULTS AND DISCUSSION

Fibers Diameter, pH and Collagen Content

The diameter of muscle fibres, pH and collagen content (mg/g) of the breast muscles in RJ, DC and CB at different ages are shown in Table 1. Within all

the breeds, the diameter of the muscle fibres significantly increased (P<0.05) as the age increased. Both RJ and DC showed significantly (P<0.05) smaller fibre diameters as compared to the CB, whereas the diameter of RJ muscle fibre was the smallest (P<0.05) among the three breeds. Meanwhile, the diameter of the RJ muscle fibres was approximately 3 times smaller than CB at days 56 and 120 post hatch. As compared to DC, the diameter of RJ muscle fibres was significantly smaller (P<0.05) at days 56 and 120 post hatch. Small increase in the myofibres diameter (hypertrophy) in RF and DC were the results of slow growth of RJ and DC as compared to CB.

The muscle pH of RJ and DC was significantly higher (P<0.05) than the CB, as shown Table 1. High accumulations of lactic acids (Aberle *et al*., 2001; Duclos *et al*., 2007) due to anaerobic glycolytic pathway caused pH to decline. In this study, RJ and DC were found to have much smaller muscle fibres and less glycolytic muscle fibres than CB, and thus, the muscle pH of RJ and DC was higher than that of CB.

The significant reduction of the muscle pH in RJ and DC as the age increased (days 20 to 56 post hatch) in Table 1 might be due to the increased glycogen storage resulting from the increase in the muscle fibre diameters (Klosowska *et al*., 1993; Remignon *et al*., 1993). Meanwhile, higher glycolytic process might produce higher lactic acids accumulation after bleeding and this led to lower pH (Aberle *et al*., 2001; Duclos *et al*., 2007). Total collagen contents of the breast muscles in RJ and DC were significantly higher (P<0.05) than CB (Table 1). The total collagen of breast muscle for all the three breeds increased as the age increased and this finding agrees well with most previous studies (Dawson *et al*., 1991; Lee & Lin, 1993; Nakamura *et al*., 2004; Watanachant *et al*., 2004). Up to date, there have been no reported data on the collagen composition in RJ muscle. Smaller fibre diameters in RJ and DC might be the reason for the higher composition of collagen in the slow growing birds (Nakamura *et al*., 2004).

Shear Force Value and Cooking Loss

The shear force values in RJ and DC were significantly (P<0.05) higher than the CB at days 56 and 120 post hatch (Table 2). Among all the three breeds, the shear force values were found to increase with age of the chicken. Significantly higher compositions of collagen content in the breast muscles of RJ, followed by DC and the least in the CB (Table 1), were the reasons for the different values of the shear force between the breeds. High collagen contents associated with high shear force value and high toughness of the meat reduce the tenderness of meat (Sims & Bailey, 1981; Liu *et al.,* 1996; Fletcher, 2002; Lawrie, 2006). In this study, the CB meat was found to have better quality in term of meat tenderness than the meat of DC and RJ due to the lower collagen contents which result in lower shear force value.

In general, the mean percentages of cooking loss of breast muscles in RJ and DC were significantly higher (P<0.05) than CB (Table 2). The percentage of cooking loss of the breast muscle showed a similar

TABLE 1
Mean diameter (μm) of muscle fiber, pH and collagen content (mg/g) of breast muscle in RJ, DC and CB at different ages (mean ± SE)

	RJ			DC			CB		
	Diameter (μm)	pH	Collagen (mg/g)	Diameter (μm)	pH	Collagen (mg/g)	Diameter (μm)	pH	Collagen (mg/g)
Day20	21.71 ±0.20 $^{a, x}$	6.18 ± 0.05 $^{a, y}$	2.27± 0.26 $^{a, x}$	22.52 ± 0.47 $^{a, x}$	6.09 ± 0.06 $^{a, y}$	2.48 ± 0.21 ^{a,x}	31.76 ± 1.18 $^{b, x}$	5.89 ± 0.06 $^{b, x}$	0.72 ±0.16 ^{b,y}
Day56	27.54 ±0.79 $^{a, y}$	5.89 ± 0.05 $^{a, z}$	2.94 ± 0.25 $^{a, y}$	31.27 ± 0.55 $^{b, y}$	5.85 ± 0.04 $^{a, z}$	3.58± 0.10 $^{b, y}$	61.29 ± 1.39 $^{c, y}$	5.76 ± 0.06 $^{a, x}$	1.07 ±0.14 ^{c,y}
Day120	36.68 ±1.60 $^{a, z}$	5.91 ± 0.04 $^{a, z}$	5.21± 0.32 ^{a,z}	59.40 ±0.7 $^{b, z}$	5.70 ± 0.04 $^{b, z}$	4.52± 0.21 $^{a, z}$	84.51 ± 2.55 $^{c, z}$	5.74 ± 0.07 $^{b, x}$	2.54 ±0.43 ^{b,z}

^{abc} Mean values within a row with different superscript were significantly different (P<0.05).
^{xyz} Mean values within a column with different superscript were significantly different (P<0.05).

TABLE 2
Mean shear force value (kg) and percentage of cooking loss of breast muscles in RJ, DC and CB at different ages (mean ± SE)

	RJ		DC		CB	
	Shear Force (kg)	Cook loss(%)	Shear Force(kg)	Cook loss(%)	S.Force(kg)	Cook loss(%)
Day20	0.91 ±0.07 $^{a, x}$	14.59 ±0.50 $^{a, x}$	0.89 ±0.05 $^{a, x}$	16.35 ±0.78 $^{b, y}$	0.76 ±0.03 ^{b,x}	12.65 ±0.34 $^{c, y}$
Day56	1.49 ±0.11 $^{a, y}$	18.02 ±0.53 $^{a, y}$	1.42 ±0.04 $^{a, y}$	17.09 ±0.29 $^{a, y}$	1.20 ±0.06 $^{b, y}$	16.03 ±0.98 $^{b, z}$
Day120	1.71 ±0.07 $^{a, z}$	19.98 ±0.34 $^{a, z}$	1.74 ±0.11 $^{a, z}$	19.16 ±0.56 $^{a, z}$	1.41 ±0.09 ^{b,z}	17.05 ±0.63 $^{b, z}$

^{abc} Mean values within a row with different superscripts were significantly different (P<0.05).
^{xyz} Mean values within a column with different superscripts were significantly different (P<0.05).

pattern at days 56 and 120 post hatch, where it was the highest in RJ, followed by DC and the least in CB. The percentage of cooking loss of the breast muscle showed an increasing pattern as the age increased in all the breeds evaluated. The low pH of the breast muscle in CB (as shown in Table 1) causes higher protein denaturation (Van Laack *et al*., 2000; Barbut, 1997) and thus loss of water binding ability and functionality of many proteins (Fujii *et al*., 1991), which break the attraction of water to the protein. The denaturation of myosin head at low pH is also responsible for higher shrinkage within the myofiber that causes water to be squeezed out from the muscle (Offer, 1991); the current results however showed that percentage of cooking loss of breast muscle in RJ and DC was higher with higher pH values. Among the breeds, the percentages of cooking loss of the breast muscles increased with age although with no significant difference ($P>0.05$) for DC at days 20 to 56 post hatch and at days 56 to 120 post hatch for CB (Table 2).

The increase in the content of collagen within the muscles with age (as shown in Table 1) was found to be directly associated with the increase in the percentage of cooking loss. The results showed similar pattern for all the three breeds evaluated, suggesting that collagen content is a prominent factor than the pH of the muscles in determining the cooking loss of muscles. In term of meat quality, slow growing birds like RJ and DC have high content of collagen in the meat which causes high water loss during cooking as compared to CB meat. Further study should be conducted to evaluate the nutrient contents of meat after cooking.

Correlation Study

The relationship between muscle tenderness and collagen quality remains still inconclusive (Liu *et al.*, 1996). The result shows a positive correlation between collagen composition and shear force value at all age evaluated, with more significant positive association shown in RJ and DC at older age (Table 3). The increase in the stability of thermal and mechanical collagen with the increase in age (Bailey & Light, 1989) may also be the reason for the difference in the shear force values between the breeds.

There was also an increasing pattern of positive correlation between cooking loss and muscle fibre diameter of the shear force value as the age increased in all the three breeds evaluated (Table 3). During cooking, the shrinkage of collagen, especially thermal stable collagen, squeezes the water out and also toughens the collagen which leads to higher shear force value. Cooking contributes to toughness (Kopp & Bonnet, 1987) and the contribution of myofiber is even more prominent at the temperature above 60°C, where the shrinkage also reduces muscle fibre volume to increase their toughness (Lepetit *et al*., 2000).

In this study, the diameter of muscle fibre also shows a positive correlation to the shear force value. The increase of diameter as the age of the birds increases (Table 1) leads to increased toughness or less tender meat (Table 2). This may due to

TABLE 3
Correlation between shear force, collagen, cooking loss and muscle fibre diameter of breast muscles in RJ, DC and CB at different ages

	Shear Force								
	Day20			Day56			Day120		
	RJ	DC	CB	RJ	DC	CB	RJ	DC	CB
Collagen	0.41	0.36	0.25	0.74*	0.57	0.69	0.81*	0.82*	0.43
Cooking loss	0.19	0.71	0.36	0.82*	0.40	0.24	0.72*	0.73*	0.64
Diameters	0.37	0.56	0.43	0.47	0.73*	0.49	0.61	0.71	0.71

* shows significant difference at $P<0.05$

the increased content of collagen in the meat as the age increased (Table 1).

CONCLUSION

The collagen composition, pH, cooking loss and shear force values in RJ and DC were found to be higher than the CB. Thus, the meat of Red jungle fowl and Malaysian Domestic chicken was less tender than that of the Commercial broiler. The muscle fibre diameters and glycogen reserved are the main factors contributing to muscle pH. The increasing pattern of the collagen contents within the muscle as the age increased was the reason for the increase in the percentage of cooking loss, suggesting collagen content as a more prominent factor than pH in determining the cooking loss of meat. There was also a positive correlation between the collagen composition and shear force value, as well as the increasing pattern in the positive correlation between cooking loss and muscle fibre diameter to shear force as the age increased in all the three breeds evaluated. This led to less tender meat. Thus, the diameter of muscle fibre could potentially lead to less tender meat. In this study, however, the collagen composition was the more prominent factor influencing meat tenderness than the diameter of the muscle fibre. The higher collagen content in the muscle results in tougher meat or less tender meat. Good quality meat should consist less collagen to produce less cooking loss and much tender meat. CB meat is much tender and has better quality than DC and RJ at the same age of evaluation. The age of the breeds is a very important factor influencing collagen content, and thus a further research on Malaysian Domestic chicken and Red jungle fowl should emphasise on chronological age in order to determine a suitable time to market the birds and fulfil customers' need for quality meat.

REFERENCES

Aberle, E. D., & Stewart, T. S. (1983). Growth of fiber types and apparent fiber number in skeletal muscle of broiler-and layer type chicken. *Growth*, *47*, 135-144.

Aberle, E. D., Forrest, J. C., Gerrard, D. E., & Mills, E. W. (2001). Principles of Meat Science (4th Edition). Dubuque, IA: Kendall and Hunt Publication Co. pp. 165-185.

Aini, I. (1990). Indigenous chicken production in South-east Asia. *World Poultry Science Journal, 46*, 51- 54.

Alvarado, C. Z., Wenger, E., & O'Keefe, S. F. (2005). Consumer perception of meat quality and shelf-life in commercially raised broilers compared to organic free range broilers. *Poultry Science, 84*(1), 129.

Amin Babjee, S. M. (2009). *The Red Jungle Fowl of Peninsular Malaysia*. Perpustakaan Negara Malaysia. pp. 20-35.

An, J. Y., Zheng, J. X., Li, J. Y., Zeng, D., Qu, L. J., Xu, G. Y., & Yang, N. (2010). Effect of myofiber characteristics and thickness of perimysium and endomysium on meat tenderness of chickens. *Poultry Science, 89*, 1750-1754.

Azahan, E. A., & Zahari, M. W. (1983).Observations on some characteristics of the carcass and meat of Malaysian 'kampung' chicken. *MARDI Research Bulletin, 11*, 225-232.

Barbut, S. (1997). Problem of pale soft exudative meat in broiler chickens. *British Poultry Science, 38*, 355–358.

Baily, A. J., & Light, N. D. (1989). Connective Tissue in Meat and Meat Product. *Meat Science, 2*, 1-28.

Bouton, P. E., Harris, P. V., Shorthose, W. R., & Baxter, R. I. (1973).A comparison of the effects of aging, conditioning and skeletal restraint on the tenderness of mutton. *Journal of Food Science*, *38*, 932-937.

Castellini, C., Mugnai, C., & Dal Bosco, A. (2002a). Effect of organic production system on broiler carcass and meat quality. *Italy Journal Food Science, 14*, 401–412.

Chambers, J. R. (1990). Genetic of growth and meat production in chicken. *Poultry Breeding and Genetics,* 559-643

Culioli, J., Touraille, C., Bordes, P., & Giraud, J. P. (1990). Carcass and meat quality in fowls given the "farm production label". *ArchivGeflugelkunde, 53*, 237–245.

Dawson, P. L., Sheldon, B. W., & Miles, J. J. (1991). Effect of aseptic processing on the texture of chicken meat. *Poultry Science, 70*, 2359–2367.

Dransfield, E. (1977). Intramuscular composition and texture of beef muscles. *Journal of the Science of Food and Agriculture, 28*, 833.

Dransfield, E., & Sosnicki, A. A. (1999). Relationship between Muscle Growth and Poultry Meat Quality. *Poultry Science, 78*, 743–746.

Duclos, M. J., Berri, C., & Le Bihan-Duval, E. (2007). Muscle Growth and Meat Quality. *Journal of Applied Poultry Research, 16*, 107–112.

Elini E. A., Tapsir, S., Sarmin, S., & Abu Kasim, A. (2010). Cost and return for local fowl rearing. *Economic Technology Management Review*, *5*, 41-49.

Farmer, L. J., Perry, G. C., Lewis, P. D., Nute, G. R., Piggot, J. R., & Patterson, R. L. S. (1997). Responses of two genotypes of chicken to the diets and stocking densities of conventional UK and Label Rouge production systems–II. Sensory attributes. *Meat Science, 47*, 77–93.

Fletcher, D. L. (2002). Poultry meat quality. *Journal of World's Poultry Science, 58*, 131–145.

Forrest, J. C., Aberle, E. D., Hedrick, H. B., Judge, M. D., & Merkel, R. B. (1975). Principles of Meat Science. San Francisco: W. H. Freeman and Company, pp. 42-54.

Fujii, J., Otsu, K., Zorzato, F., Deleon, S., Khanna, V. K., & Weiler, J. E. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science 253*, 448–451.

Hunsley, R. E., Vetter, R. L., Kline, E. A., & Burroughs, W. (1971). Effects of Age and Sex on Quality, Tenderness and Collagen Content of Bovine Longissimus Muscle. *Journal of Animal Science, 3*, 33-938.

Hofman, K. (1994). What is quality? Definition, measurement and evaluation of meat quality. *Meat Focus International, 3*, 73-82.

Klosowska, D. B., Rosinski, A., & Elminowska-Wenda, G. (1993). Microstructural characteristics of the pectoralis muscle of white Italian geese. 144–148. In *Proceedings of the XI European Symposium on the Quality of Poultry Meat*. Vol. 1. Tours, France, 144–148.

Kolar, K. (1990). Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *Journal of AOAC, 71*, 54-57.

Kopp, J., & Bonnet, M. (1987). Stress–strain and isometric tension measurements in collagen. In A. M. Pearson, T. M. Dutson, & A. J. Bailey (Eds.), *Advances in Meat Research*, *4*, 163–185.

Lawrie, R. A., & Ledward, D. A. (2006). Lawrie's Meat Science (7th Edition). Cambridge, England: Woodhead Publishing Limited.

Lee, H. F., & Lin. L. C. (1993). Studies on the general composition and characteristics of meat quality of the Taiwan country chicken and broiler. *Journal of Food Science (Taiwan) 20*, 103—111.

Lepetit, J., Grajales, A., & Favier, R. (2000). Modelling the effect of sarcomere length on collagen thermal shortening in cooked meat: consequence on meat toughness. *Meat Science, 54*, 239–250.

Liu, A., Nishimura, T., & Takahashi, K. (1996). Relationship between Structural Properties of Intramuscular Connective Tissue and Toughness of Various Chicken Skeletal Muscles. *Meat Science, 43*(1), 43-49.

Light, N. D., Champion, A. E., Voyle, C. A., & Bailey, A. J. (1985). The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscle. *Meat Science, 13*, 137.

Mitchell, M. A., & Smith, M. W. (1991). The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the Domestic Fowl (*Gallus domesticus*). *Comparative Biochemistry and Physiology*, *99*, 251-258.

Nakamura, Y. N., Iwamoto, H., Shiba, N., Mitachi, H., Tabata, S., & Nishimura, S. (2004). Developmental states of the collagen content, distribution and architecture in *pectoralis, iliotibialislateralis* and *puboischiofemoralis* muscles of male Red Cornish, New Hampshire and normal broilers. *British Poultry Science, 45*, 31-40.

Offer, G. (1991). Modeling of the formation of pale, soft and exudative meat effects of chilling regime and rate and extent of glycolysis. *Meat Science*, *30*, 157–184.

Petersen, J. B., Guzman Jr., M. R. D., and Wu, M. C. (1991). *Catalogue of the native poultry of Southeast Asia*. Food and Fertilizer Technology Center for Asian Pacific Region, Taiwan. Taiwan Livestock Research Institute. Taiwan, pp.35-45.

Rehfeldt, C., Feidler, I., Dietl, G., & Ender, K. (2000). Myogenesis and postnatal skeletal muscle cell growth as influence by selection. *Livestock Production Science, 66*(2), 177-188.

Reddy, G. K., & Chukuka, S. E. (1996). Simplified Method for analysis of Hydroxyproline in Biological Tissues. *Clinical Biochemistry, 29*(3), 225-229.

Remignon, H., Lefaucheur, L., Blum, J. C., & Ricard, F. F. (1993). Effect of divergent selection for body weight on three skeletal muscle characteristic in chicken. *British Poultry Science, 35*, 65-76.

Roberts, V. (2008). *British Poultry Standards* (6th edition). Oxford, United Kingdom: Blackwell Publishing, pp. 169-171.

Sazili, A. Q., Lee, G. K., Parr, T., Sensky, P. L., Bardsley, R. G., & Buttery, P. J. (2003). The effect of altered growth rates on the calpain proteolytic system and meat tenderness in cattle. *Journal of Meat Science, 66,* 195-201.

Salakova, A., Strakova, E., Valkova., V., Buchtova, H., & Steinhauserova, I. (2009). Quality Indicators of Chicken Broiler Raw and Cooked Meat Depending on Their Sex. *ActaVeterinaria Brno, 78*, 497-504.

Schreiweis, M. A., Hester, P. Y., Settar, P., & Moody, D. E. (2005). Identification of quantitative trait loci associated with egg quality, egg production and body weight in an F2 resource population of chicken. *Journal of Animal Genetics*, *37*, 106 – 112.

Sims, T. J., & Bailey, A.J. (1981). Relationship between structural properties of intramuscular tissue and toughness of various chicken skeletal muscle. *Meat Science, 2*, 29.

Smith, D. P., & Fletcher, D. L. (1988). Chicken breast muscle fibre type and diameter as influenced by age and intramuscular location. *Poultry Science*, *67*, 908–913.

Stevens, L. (1991). *Genetics and Evolution of the Domestic Fowl*. New York, USA: Cambridge University Press. pp. 125-131.

Van Laack, R. L., Liu, C. H., Smith, M. O., & Loveday, H. D. (2000).Characteristics of pale, soft, exudative breast meat. *Poultry Science, 79*, 1057–1061.

Wall, C. W., & Anthony, N. B. (1995). Inheritance of carcass variables when Giant Jungle Fowl and Broilers achieve a common physiological body weight. *Poultry Science*, *74*, 231-236.

Wattanachant, S., Benjakul, S., & Ledward, D. A. (2004). Composition, Colour and Texture of Thai Indigenous and Broiler Chicken Muscle. *Poultry Science*, *83*, 123-128.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., & Kasapidou, E. (2003). Effects of Fatty acids on meat quality. *Meat Science, 66*, 21-32.

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Influence of Nut Size, Hydro Priming Duration and Storage Period on Seedling Emergence and Early Seedling Vigour Characters in Cashew (*Anacardium occidentale* L.)

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ABSTRACT

The influence of seed nut size, storage period and hydro priming duration on seedling emergence and early seedling vigour in Brazilian cashew biotype was investigated. Seed nuts were hand graded into three sizes - large, medium and small - and dried under ambient conditions (29^{0}C, RH70%) for 30 days before storing them under ambient conditions for 210 days. Stored nuts were evaluated at 0, 30, 90, 120, 150 and 210 days under three different pre-sowing hydro priming hours (0, 12 and 24 hrs) for seedling emergence and vigour characters. Data collected were statistically analysed. Significant differences were found to have occurred among storage periods, hydro priming durations and nut sizes for the four seed quality characters examined. In particular, large seed nuts had the highest seedling emergence of 79% above the medium and small nuts with a marginal increase of 5 to 6%, respectively. Meanwhile, small nuts emerged earlier than the other seed nut fractions but large nuts had the longest days to emergence. Seed nuts hydro primed for 24 hrs had the best seedling emergence (79 %), reduced days to emergence (15 days) and greater seedling vigour and shoot growth. Highest seedling emergence of 80 to 81% was observed at early storage (30-90 days) and which thereafter declined to 72% at the end of 210 days, with 10% reduction. Hydro primed small nuts had significant reduction in days to emergence (12-16 days) at each storage time investigated. The beneficial effect of priming was observed in large nuts hydro primed for 24 hrs with the highest storage performance. Thus, in order to obtain good seed nut quality parameters,

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storage period of large nuts can be extended up to 210 days or thereafter, while medium nuts can be stored for 150-210 days but the storage duration of small nuts should not exceed 150 days under ambient conditions. In conclusion, hydro priming of cashew nuts before sowing is a possible way of enhancing seedling emergence and early vigour characters.

Keywords: Germination, seed deterioration, seed enhancement, seed grading, water treatment, seedling growth.

INTRODUCTION

The cashew plant, *Anacardium occidentale,* is a native to Central and South America with its main centre of variation in eastern Brazil. However, cashew is now grown in many parts of the world including Nigeria (Ohler, 1979; FAO, 2001; FAO, 2007). The annual world production of cashew nuts - the main commercial product of the cashew plant – is over 1.2 million tonnes, with India topping the production, followed by Mozambique and Tanzania (FAO, 2004). Nigeria was a foremost producer before it was overtaken by Tanzania, Cote D'Ivorie and Guinea Bissau in 2006 (FAO, 2007). Kogi State, Nigeria, is one of the highest producers of cashew in the category of Kwara, Anambra, Oyo, Imo, Edo and Lagos States, Nigeria. It is grown to a lesser extent in Niger, Ondo, Delta, Ogun and Osun States (Udoh *et al.,* 2005). It is found all the way from the coast to the north but mostly as windbreaks and soil erosion prevention in the northern parts of Nigeria.

Seed nuts are vital to the propagation of the cashew plant (Udoh *et al.,* 2005; FAO, 2007). In Nigeria, these are usually obtained from current season harvests, sun dried and stored under ambient temperature before sowing. The germination of viable seed nuts is a product of many variables, but of significant importance is water imbibition, which depends among other factors on seed size (Turner, 1956; Auckland, 1961; Ibikunle & Komolafe, 1973), the level of water available in the seed as this determines the 'thirst' for water and lastly the permeability of the seed nut coat.

Cashew nuts possess thick seed coat thus requiring considerable time for water imbibition to prompt nut germination. Slow imbibition of dry intact seed nuts is reported to be the main cause of delayed germination in cashew (Subbaiah, 1982), a problem which is greatest in larger seeds (Turner, 1956; Auckland, 1961; Ibikunle & Komolafe, 1973). Previous observations revealed that slow imbibition of dry intact seed is the main cause of delayed germination in cashew (Nmadzhanova *et al.,* 1977; Subbaiah, 1982), with the nut covering structures and epicarp presenting a formidable barrier to embryo growth and germination (Joley, 1960). Similarly, the endocarp nature has been identified to be responsible for reducing the rate of imbibition (Crane & Forde, 1974).

However, early germination can be induced by the following means: cracking the seed nut coat: a delicate operation which must be carried out with care to avoid damage to the embryo; treatment with dilute

sulphuric acid (H_2SO_4); and soaking in water for 24 to 36 hours (Turner, 1956; Auckland, 1961; Ibikunle & Komolafe, 1973). Pre-soaking for 1 or 2 days (Rao *et al.,* 1957a, 1957b; Ibikunle & Komolafe, 1973) or removing the waxy layer of the pericarp by treating with chloroform or acetone (Subbaiah, 1982) have been observed to promote imbibition and reduce the time taken to germinate and increase the proportions of seed nuts germinating. Light (Rocchetti & Panerai, 1968; Rocchetti & Panerai, 1970; Adams, 1975) and gibberellins (Ayfer & Serr, 1961; Shanmugavelu, 1970; Dahab *et al.,* 1975) are also reported to promote germination in cashew seed nuts.

Large seed nuts are more vigorous than small ones and thus, they are more desirable to farmers; however, these are likely to sprout last due to the presence of thicker seed coats (Maggs, 1973; Crane & Forde, 1974; Casini & Conticini, 1979). Cashew orchards are majorly established through nut seeds which are harvested in the dry season of between January and April of every year and thereafter stored for sowing in the rainy season (May – September/ October). The length of storage and nut size affect the viability of cashew seeds (Akinyemi *et al.,* 2011; Aliyu & Akintaro, 2007). Similarly, Hammed (2012) reported significant effect of nut sizes of cashew on seedling performance in the nursery. Meanwhile, highly significant correlations between nut sizes, seedling emergence and physiological characters in cashew were reported by Aliyu and Akintaro (2007).

The length of hydro priming affects the emergence and other seedling vigour parameters of cashew (Ibikunle & Komolafe, 1973) and other crop species (Adebisi *et al.,* 2014; Oyedele, 2014 & Ogunbayo, 2014). High emergence and seedling vigour are major factors in the establishment of good and productive cashew orchard. However, information on the combined effects of length of storage, nut size and hydro priming duration for optimum seed emergence and seedling vigour performance under ambient humid tropical conditions is desirable. Hence, the study was initiated to investigate the response of seed emergence and early seedling vigour traits to length of storage, seed nut size and hydro priming duration in Brazilian cashew biotype.

MATERIALS AND METHODS

Collection of Nuts

Seed nuts obtained from the 2010 dry season, with no sign of damage, insect pest attack or disease symptoms, from the cashew plantation (Brazilian type) of Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State (Latitude 7.1^0N and longitude 3.2^0E) in Nigeria were used in the study. The nuts were collected from five healthy cashew plants with good history of robust fruit production and then air dried for 30 days after collection under ambient laboratory conditions (average temp. 29^0C, RH70 %).

Experimental Treatment

The treatments investigated were:

a. Seed nut sizes: Cashew seed nuts were visually graded according to size into large, medium and small. The seed nuts were weighed and classified into categories depending on the size, with nut sizes ranging between 10.0 and 11.9 g/nut for large nuts, while medium nuts ranged between 6.5 and 7.4 g/nut and small nuts between 2.0 and 3.9 g/nut.

b. Storage duration: Five storage times (0, 30, 90, 150, 210 days) were investigated in the study. Pure seed nuts were packed into baskets and then covered with polythene sheet and stored under ambient laboratory conditions for 210 days.

c. Hydro priming hours: Three hydro priming hours were investigated (0, 12 and 24 hrs). Hundred seeds from each nut size were placed in individual net bag and then immersed in water for 0, 12 and 24 hours at each storage duration investigated. All the seeds were then removed from the priming solution and then surface dried lightly for 5 hrs at room temperature (29^0C).

Experimental design: The experiment was arranged in a factorial fitted into completely randomised design. There were 3 factors [nut size (3), storage time (5) and hydro priming hours (3)], which gave a total of 45 treatment units which were replicated three times.

Soil Collection and Poly Bags Filling: Top soil (5–9 cm depth) was collected from fallow farm land of the Teaching and Research Farm Unit, FUNAAB, Nigeria. Soil was freed of extraneous materials: plant roots, weed seeds and pebbles. The soil samples were then filled into polythene bags of 25 x 15 cm to 2.5 cm from the brim to allow for watering and placed inside the screen house at the College of Plant Science and Crop Production, FUNAAB.

Seed nut sowing: A seed nut was sown at 4–5 cm depth with concave end upward into polythene bags and observed daily for seedling emergence parameters.

Seed quality assessment: Data were collected on the following seedling emergence and seedling vigour parameters at each of the storage times and priming hours were investigated.

Seedling emergence: The numbers of emerged seedlings and the percentage of seedling emergence were determined as follows:

$$\frac{\text{Number of emerged seedlings at 30 days}}{\text{Seed sown}} \times 100$$

Days to 50 % seedling emergence: Number of days taken for seeds to emerge up to 50%.

Seedling shoot length: Average lengths of 10 randomly selected seedlings were measured 30 days after emergence in centimetre.

Seedling vigour index: It was calculated using the following formula (Kim *et al.,* 2002; Adebisi, 2004):

$$\frac{\text{Seedling emergence (\%) at 30 days} \times \text{seedling shoot length at 30 days}}{100}$$

Data Analysis

Data on seedling emergence involving percentages were transformed using angular transformation (arcsin√sin-¹). Analysis of variance was carried out on the data obtained on the four seed quality parameters examined. Tukey's HSD test at 5% probability level was used to separate significant treatment means. All the analyses were carried out using SPSS version 16 statistical software package.

RESULTS

The mean square values for the characters evaluated in the seed nut sizes of Brazilian cashew biotype after storage and hydro priming treatments are presented in Table 1. From the ANOVA results, the main effects of storage period, hydro priming and nut size were highly significant ($p \leq 0.01$) on seedling emergence, days to 50% seedling emergence, seedling vigour index and seedling shoot length. All the two-way interaction effects (nut size x hydro priming treatment, nut size x storage, storage x hydro priming treatment) were highly significant ($p \leq 0.01$) on seedling emergence, days to 50% seedling emergence, seedling vigour index and seedling shoot length. The three-way interaction (nut size x storage time x hydro priming treatment) also had significant effect ($p \leq 0.05$) on seedling emergence, days to 50% seedling emergence, seedling vigour index and seedling shoot length.

In Fig.1, the results indicate different responses in seed quality traits among various storage durations investigated. For seedling emergence, the values declined with the increase in storage, durations from 0 day to 210 days. Maximum seedling emergence (81%) was found in the seed nuts stored for 0 day, followed by 80% at 30 days, while the lowest value occurred at the end of storage. In terms of days to 50%

TABLE 1
Summary of analysis of variance showing mean square values for the characters evaluated in nut sizes of Brazilian cashew after storage and water soaking treatment.

Source of Variation	Degree of freedom	Seedling emergence (%)	Days to 50% emergence	Seedling vigour index	Seedling shoot length (cm)
Replicate	2	45.87	1.410	51.257	60.267
Storage Period (S)	4	418.252**	91.220**	56.773**	186.70**
Hydro priming (H)	2	220.089**	106.615**	53.836**	85.031**
Nut size (N)	2	241.422**	155.033**	56.502**	188.034**
S*N	8	115.941**	4.576**	42.979**	145.130**
H*N	8	111.911*	3.445*	35.350*	129.60**
S*H	4	94.385**	1.815**	34.477**	126.77**
S* H*N	8	112.207**	1.181**	42.105**	142.103**
Error	88	13.139	0.330	3.158	6.88

**Significant at 1% probability level
*Significant at 5% probability level

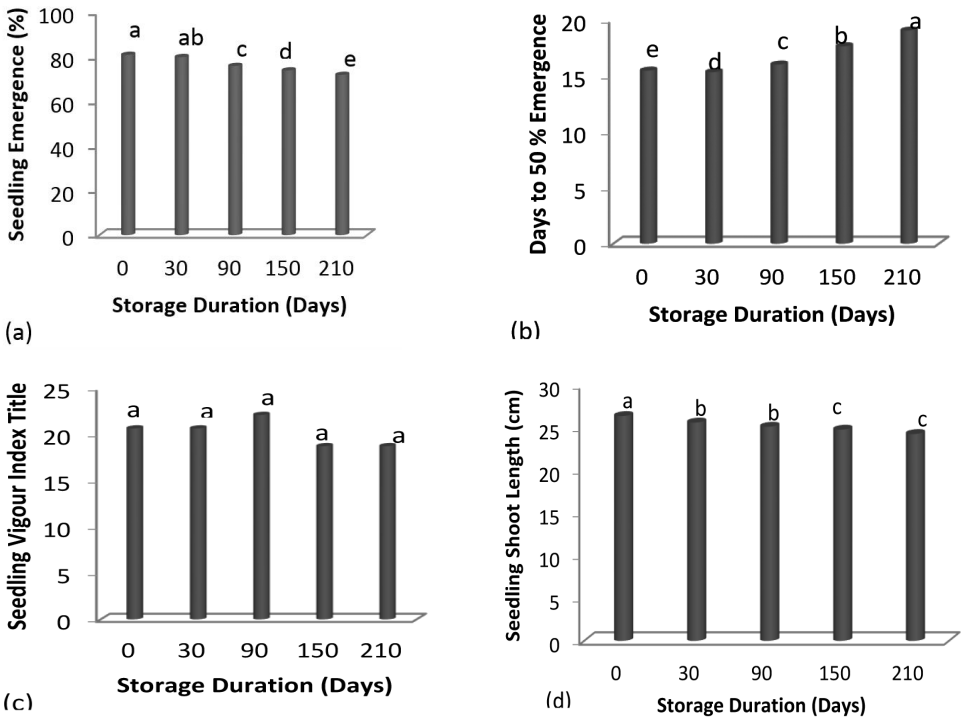

Fig.1 (a-d). Effect of storage period on seedling emergence, days to 50% seedling emergence, seedling vigour and seedling shoot length across hydro priming duration and nut size in Brazilian cashew biotype.

seedling emergence, the values increased with advancement in storage duration, with the highest days to seedling emergence (19 days) obtained at 210 days of storage while the lowest days to emergence occurred at 0 and 30 days of storage. As for seedling vigour index, storage duration did not have any effect on this character at all. Data on seedling growth, as indicated by seedling shoot length, progressively declined with length of storage. The greatest seedling shoot length was obtained at 0 day of storage whereas seedling shoot length values at 30 and 90 days were statistically similar. Thereafter, there was a steady and significant decline up till 210 days.

Data in Fig.2 show that seed nuts hydro primed for 24 hrs had the maximum seedling emergence of 79%, whereas 0 and 12 hrs of hydro priming recorded statistically similar values. Data on days to 50% seedling emergence show that seed nuts hydro primed for 24 hrs recorded the lowest value of 15 days, while longest day to emergence of 18 days was obtained at 0 hr of hydro priming. In terms of seedling vigour index, seed nuts hydro primed for 12 and 24 hrs had higher values of 21.26 and 21.30, respectively, while lower value of 19.69 was recorded at 0 hr of hydro priming. For seedling shoot length, cashew seed nuts soaked for 12 and 24 hrs had significantly

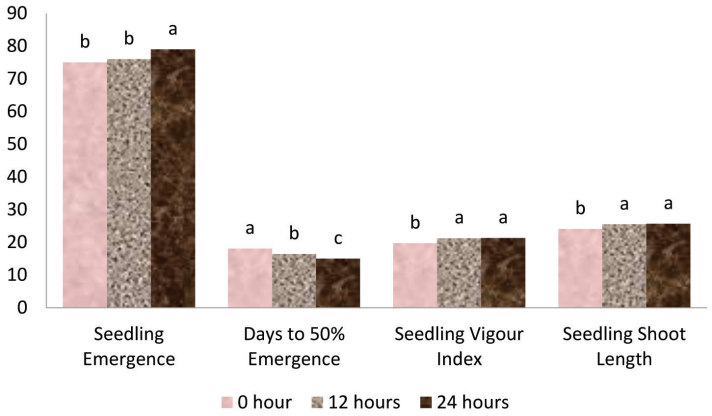

Fig.2 Effects of hydro priming duration on seedling emergence, days to 50% seedling emergence, seedling vigour and seedling shoot length across storage and nut size in Brazilian cashew biotype.

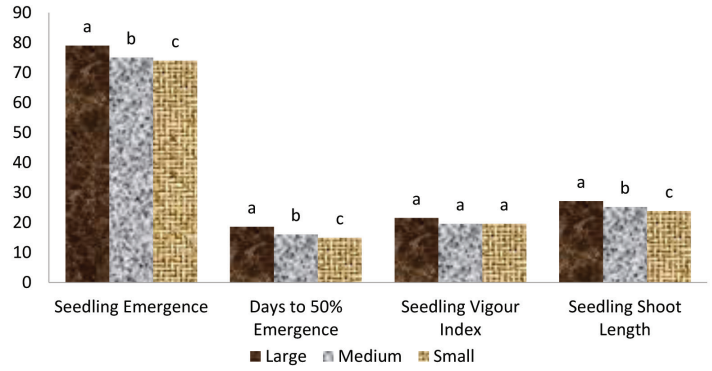

Fig.3 Effects of seed nut sizes on seedling emergence, days to 50% emergence, seedling vigour and seedling shoot length across storage duration and hydro priming hours in Brazilian cashew biotype.

higher seedling shoot length values above unsoaked nuts. Statistically similar values of seedling shoot length were observed after 12 and 24 hrs of priming.

Data represented in Fig.3 show that large seed nuts had the highest emergence of 79%, followed by medium seed nuts, while small seed nuts retained the lowest (74%). For days to 50% seedling emergence trait, small seed nuts had the lowest value of 15 days, followed by medium seed nuts, while longest day (19 days) was obtained for the large nut size. For seedling vigour index, seed nut sizes did not have any significant effect as the three nut sizes had comparable seedling vigour values. Data on seedling shoot length revealed that large seed nut size of cashew recorded the highest value (27.12 cm), closely followed by medium nut sizes, while the small seed nut sizes had the lowest value of seedling shoot length of 23.84 cm.

Data on the influence of nut size, hydro priming duration and storage periods on seedling emergence are displayed in Table

2. The results indicate different responses in the seed nut emergence among nut sizes after hydro priming duration at each storage time investigated. At 0 day storage time, large seed nuts hydro primed for 24 hrs had the highest emergence of 87%, followed by small nuts while other hydro primed seeds of the three nut sizes had emergence values of between 80 to 81% but un-primed seed had the lowest emergence of less than 80%. At 30 days of storage, large seed nuts hydro primed for 24 hrs retained the highest emergence of 85%, whereas the lowest emergence value of 70% was recorded with small seed nuts at 0 hr of hydro priming. With the increase in storage time to 90 days, large seed nuts that were hydro primed for 24 hrs had the highest emergence of 81%, though not significantly different from the value of 79% obtained for the medium seed nuts that were hydro primed for 24 hrs. At 150 days of storage, large seed nuts still maintained the maximum emergence of 80%, while other treatment combinations had statistically similar emergence except small seed nuts hydro primed for 0 and 12 hrs, which had the lowest values of 60 to 69% emergence. At the end of storage (210 days), large seed nuts hydro primed for 24 hrs recorded the highest seedling emergence (77%), while other treatment combinations had the emergence of between 71 and 74%. However, medium seed nuts hydro primed for 0 hr, as well as small seed nuts hydro primed for 0 and 12 hrs, had the lowest emergence of 65-69%.

A perusal of the data along the row indicates that large seed nuts hydroprimed for 24 hrs consistently showed the highest emergence values of between 77 and 87% at each storage period. Consistently, un-primed small nuts had the lowest emergence values under each storage period.

Data on the influence of seed nut size, hydro priming duration and storage period on days to 50% seedling emergence in Brazilian cashew biotype are presented in Table 3. The results indicate significant differences in days to 50% seedling emergence in hydro primed cashew nut sizes at each storage period examined. However, the lowest the days to 50% seedling emergence, the better it is for the seed lot. At 0 day of storage, small nut size primed for 24 hrs recorded the lowest days to 50% seedling emergence of 12 days, while medium and small nut sizes hydro primed for 24 and 12 hrs, respectively, had 13 days to seedling emergence. The highest days to 50% seedling emergence of 18 days was recorded for un-primed large seed nut sizes. At 30 days of storage, small nut size hydro primed for 12 and 24 hrs recorded the lowest days to seedling emergence of 13 days whereas large, medium and small seed nuts hydro primed for 24, 12 and 0 hrs, respectively, had 15 days to seedling emergence. However, un-primed large seed nuts recorded the longest days (19 days) to seedling emergence after 30 days of storage. After 90 days of storage, large seed nuts hydro primed for 0 hr had the highest days to seedling emergence of 19 days, followed by the medium nuts hydro primed for 0 hr with 18 days seedling emergence whereas the medium seed nuts hydro primed for 24

TABLE 2
Responses of seed nut emergence (%) to nut size, hydro priming duration and storage period of Brazilian cashew biotype.

Nut size (g)	Hydro priming treatment (hrs)	Storage period (days)				
		0	30	90	150	210
Large	0	81c	77bc	77b	75b	73b
	12	81c	80b	77b	76b	74b
	24	87a	85a	81a	80a	77a
Medium	0	79cd	77bc	77b	77b	69c
	12	80c	79b	76b	75b	71b
	24	81c	80b	79ab	75b	74b
Small	0	77d	75c	73cd	68c	65d
	12	80c	79b	75c	69c	69c
	24	84b	81b	71b	77b	71b

Means followed by the same alphabet along the column are not significantly different from one another according to Tukey's HSD test at 5% probability level.

TABLE 3
Effects of nut size, hydro priming duration and storage period on days to 50% seedling emergence of Brazilian cashew biotype.

Nut size (g)	Hydro priming duration (hrs)	Storage Period (days)				
		0	30	90	150	210
Large	0	18a	19a	19a	24a	30a
	12	16b	17b	17c	20b	21b
	24	14d	15c	16d	18c	21b
Medium	0	16b	17b	18b	18c	19c
	12	14d	15c	16d	17d	19c
	24	13e	14d	14e	16e	18d
Small	0	15c	15c	17c	18c	17e
	12	13e	13e	15d	16e	17e
	24	12f	13e	13f	14f	16f

Means followed by the same alphabet along the column are not significantly different from one another according to Tukey's HSD test at 5% probability level.

hrs had 14 days to emergence and small seed nuts hydro primed for 24 hrs recorded the lowest seedling emergence of 13 days. Data generated at 150 days storage time showed that large nuts hydro primed for 0 hr recorded the longest days to emergence (24 days), followed by large seed nuts hydro primed for 12 hrs with 20 days. However, small seed nuts hydro primed for 24 hrs had the lowest days to emergence (14 days), while medium and small nuts primed for 24 and 12 hrs respectively recorded 16 days

to 50% seedling emergence. At the end of storage (210 days), large seed nuts hydro primed for 0 hr recorded the highest days to seedling emergence (30 days), followed by large seed nuts hydro primed for 12 and 24 hrs with 21 days, while small seed nuts hydro primed for 24 hrs had the lowest days to emergence of 16 days.

Analysis of the data along the row showed that un-primed large nuts had a consistent highest day to emergence at each storage period. Days to emergence progressively increased with the increase in storage time, irrespective of the treatment combination. The highest value of 30 days to emergence was recorded with large nuts stored for 120 days, while the lowest days to emergence (16 days) was observed with small nuts primed for 24 hrs.

Data in Table 4 show the effects of nut size, hydro priming duration and storage period on seedling vigour in Brazilian cashew biotype. The results indicate that at 0 and 30 days of storage, large seed nuts hydro primed for 0, 12 and 24 hrs had statistically similar and greater seedling vigour index of between 21.14 and 23.48 units, whereas other treatment lots had statistically similar and lower seedling vigour. At 90 days of storage, large seed nuts hydro primed for 24 hrs recorded the maximum seedling vigour of 21.97 unit, although were not significantly from other treatment lots except small seed nuts hydro primed for 0, 12 and 24 hrs which had the lowest seedling vigour. A similar trend in seedling vigour was obtained at 150 and 210 days of storage, with large seed nuts hydro primed for 24 hrs having the highest seedling vigour, which was not significantly different from other treatment lots except for the small seed nuts hydro primed for 0, 12 and 24 hrs with the lowest seedling vigour values.

A cursory analysis of the data along the row indicates that large nuts hydro-primed for 24 hrs gave a consistent highest seedling vigour under each storage period. Seedling vigour values slightly declined with the increase in storage time from 23.84 at 0 day to 19.89 at 210 days of storage. However, seed nuts primed for 0, 12 and 24 hrs generally recorded the lowest seedling vigour values during the storage periods.

Table 5 presents data on the influence of seed nut size, hydro priming duration and storage period on seedling shoot length in Brazilian cashew biotype. The results reveal that at 0 day of storage, large seed nuts hydro primed for 0, 12 and 24 hrs recorded the highest seedling shoot length values of 28.01 to 28.51cm. The influence of medium seed nuts reveals statistically similar values under the three hydro priming durations while small seed nuts hydro primed for 0 and 12 hrs had the lowest seedling shoot length values. At 30 days of storage, large seed nuts hydro primed for 24 hrs had the maximum seedling vigour of 28.43cm, followed by large seed nuts hydro primed for 0 and 12 hrs. Similar seedling shoot length values were obtained for the medium seed nuts primed for 0, 12 and 24 hrs, while small seed nuts hydro primed for 0 and 12 hrs had the lowest seedling shoot length values. With the increase in storage days to 90 days, large nuts hydro primed for 0, 12 and 24

TABLE 4
Effects of nut size, hydro priming duration and storage period on seedling vigour of Brazilian cashew biotype.

Nut size (g)	Hydro priming treatment (hrs)	Storage period (days)				
		0	30	90	150	210
Large	0	21.14a	21.12a	20.90ab	19.87ab	18.83ab
	12	23.32a	23.13a	20.70ab	19.47ab	19.10a
	24	23.84a	23.30a	21.97a	20.10a	19.89a
Medium	0	19.95bc	19.73b	19.23ab	18.90ab	18.10ab
	12	20.10b	19.97b	19.03ab	18.33ab	18.32ab
	24	20.87b	20.47b	19.83ab	19.90ab	18.88ab
Small	0	18.00bc	17.53b	17.23b	17.13b	16.01c
	12	18.25bc	18.60b	17.33b	17.10b	16.08c
	24	19.95bc	19.80b	18.60b	17.73b	16.54c

Means followed by the same alphabet along the column are not significantly different from one another according to Tukey's HSD test at 5% probability level.

TABLE 5
Effects of nut size, hydro priming duration and storage period on seedling shoot length (cm) of Brazilian cashew biotype.

Nut size (g)	Hydro priming treatment (hrs)	Storage period (days)				
		0	30	90	150	210
Large	0	28.01a	27.67b	27.03a	26.40b	25.13b
	12	28.21a	27.07b	27.20a	26.93b	25.22b
	24	28.51a	28.43a	27.97a	27.03a	26.23a
Medium	0	25.83b	25.50c	24.90c	24.93d	24.33c
	12	25.87b	25.57c	25.07b	24.94d	24.43c
	24	25.93b	25.60c	25.20ab	25.03c	24.81c
Small	0	23.62d	23.50e	23.33d	23.10e	22.90e
	12	23.81d	23.63e	23.37d	23.23e	22.91e
	24	24.89c	24.37d	23.47d	23.41e	23.10d

Means followed by the same alphabet along the column are not significantly different from one another according to Tukey's HSD test at 5% probability level.

hrs had statistically similar and maximum seedling shoot length, followed by medium nuts hydro primed for 12 and 24 hrs with 25 cm seedling shoot length, while small seed nuts hydro primed for 0, 12 and 24 hrs had the lowest values of approximately 23cm. After 150 days of storage, large seed nuts hydro primed for 24 hrs showed the highest seedling shoot length value of 27.03 cm. This was closely followed by large seed nuts primed for 0 and 12 hrs whereas small nuts hydro primed for 0, 12 and 24 hrs had the lowest value of 24 cm. At the end of storage (210 days), large seed nuts still maintained

the highest seedling shoot length value of 26.23 cm, closely followed by large seed nuts hydro primed for 0 and 12 hrs with the value of 25 cm, while small seed nuts hydro primed for 0 and 12 hrs showed the lowest seedling shoot length value.

A perusal of data on seedling shoot length along the row reveals that large nuts hydroprimed for 24 hrs had consistent highest values of between 26.23 and 28.51 during the storage periods. Conversely, small nuts primed for 0, 12 and 24 hrs had the lowest values of below 25.00 unit during the storage period evaluated.

DISCUSSION

Many factors can influence the imbibition and germination process, among them integument composition and water availability in the environment, seed physiological condition in storage (Vertucci, 1989; Adebisi *et al.,* 2008; Adebisi*,* 2012). In this study, significant differences were observed among the storage durations, seed nut sizes and hydro priming durations in respect of seedling emergence, days to 50% seedling emergence, seedling vigour index and seedling shoot length of Brazilian cashew biotype. Seed nut sizes significantly influenced seedling emergence with large nuts having the highest emergence of 79%, which was 5 to 6% higher than the values obtained for medium and small seed nuts. Small nuts were the first to emerge while large nuts were usually the last to emerge. Also, large nuts had the maximum seedling vigour level with greater seedling shoot length. In addition, hydro priming hours significantly influenced seedling emergence, seedling vigour index, days to 50% seedling emergence and seedling shoot length with hydro priming of 24 hrs having the best performance with reduced days to 50% seedling emergence.

Significant and progressive seed deterioration was noticeable during storage in seedling emergence, irrespective of nut size grading. As the storage time advanced, the seed nuts deteriorated progressively. Meanwhile, higher seedling emergence values of 80-81% were obtained in the early storage time of 30 days, which then declined to 72% at the end of storage (210 days) with 10% reduction in seedling emergence. Days to 50% seedling emergence increased with the increase in storage duration but reduced days to emergence of 15-16 days were observed at 30 to 90 days of storage with greater seedling vigour and seedling shoot length.

The results presented help to understand some earlier observations in the literature. Priming stimulates many of the metabolic processes involved with the early phases of germination (Copeland & McDonald 1995; Dessai *et al.,* 1997). Given that part of germination processes have been initiated, seedlings from primed seeds grow much faster, more vigorously and perform better in adverse conditions (Basker & Halton, 1987; Dessai *et al.,* 1997). According to Simon (1984), water uptake in seeds follows a triphasic pattern with an initial rapid uptake phase known as imbibition (Phase I), followed by lag period (Phase II) and then a second increase in water uptake

associated with seedling growth (Phase III). At each phase, water uptake is controlled by the availability of water to the seed. In seed priming regime, seed water potential is at a level sufficient enough to initiate metabolic events in phase II of germination process but it prevents radical emergence (Simon, 1984).

In this study, the duration of the emergence period decreased, leading to more uniform plant stand (Mikkelsen, 1981; Basker & Halton, 1987). Nulawadi *et al*. (1973) reported that pre-soaking of soybean seeds in water for 24 hrs had significant effects on germination and seedling growth (fresh weight). The increase in germination percentage was from 21.20 to 54.00, while seedling fresh weights were from 1.735 to 3.445g.

The results of the present work corroborated the previous reports of Ibikunle and Komolafe (1973), Faluyi (1986) and Adebola *et al.* (1999) that large cashew nuts had superior germination than small nuts. Medium and small sized nuts also showed high level of emergence but their percentage emergence values were both less than the least emergence (Aliyu & Akintaro, 2007). In this study, small cashew seed nuts had the least days to emergence (earliest emergence), whereas large seed nut had the largest days to emergence (longest emergence). The problem of slow imbibition of dry intact seed is more pronounced in large seed nuts (Casini & Contiani, 1979; Crane & Forde, 1973; Nmadzhanova *et al.,* 1977).

According to Oyewumi (2014), thick shells in large seed nuts pose greater obstacle to water uptake than in medium and small nuts. Moreso, the inability of large seed nuts to readily take up water as a result of thicker seed coats, could have accounted for the observed latest emergence. Pre-soaking cashew nuts for 24 to 48 hrs was reported to promote imbibition and reduce the time for seeds to emerge but increase the proportion of seeds emerging (Turner, 1956; Auckland, 1961; Hartman, 1967; Joley & Opitz, 1971; Ibikunle & Komolafe 1973; Crane & Forde, 1974). The outcome of this study is, therefore, in line with previous findings. Increased storage duration significantly reduces percentage seedling emergence and increases days taken for seeds to emerge.

Large seed nuts hydro primed for 24hrs were found with consistent significant highest seedling emergence of between 87 and 77% at each of the storage period above control and other treatment lots. Small seed lots without hydro priming were generally found with the lowest seedling emergence, which ranged from 65 to 77% at the end of 210 storage days. Higher seedling emergence of between 73 and 77% was found with large seed nuts hydro primed for 0, 12 and 24 hrs, whereas medium seed nuts hydro primed for 0, 12 and 24 hrs retained 69 to 74% emergence. However, small seed nuts that were hydro primed for 0, 12 and 24 hrs had 65-71% seedling emergence at the end of storage period (210 days). In order to obtain good seedling emergence, large seed nuts storage period could be extended

to 210 days, while medium seed nuts can be stored for up to between 150 and 210 days but small nut storage duration should be between 90 and 150 days under ambient humid conditions (28-30^{o}C).

Pre-sowing hydro priming of large seed nuts for 0, 12 and 24 hrs significantly enhanced seedling vigour index up to 18.83 and 23.84 units above other treatment lots at each of the storage times investigated. In addition, small nuts hydro primed for 0, 12 and 24 hrs had significant lowest seedling vigour at the end of storage time of 210 days. In a similar vein, large seed nuts hydro primed for 24 hrs had increase seedling shoot length above other treatment lots at each storage time. Furthermore, large nuts hydro primed for 0 and 12 hrs had comparable values that were higher than the values from the medium and small nuts at these soaking hours. Also, small seed nuts hydro primed between 0 and 12 hrs retained lower seedling shoot length at each storage time investigated.

CONCLUSION

Within the purview of this study, the beneficial effects of hydro priming were found in large seed nuts hydro primed for 24 hrs with the highest storage performance. Therefore, in order to obtain high emergence after storage, cashew nuts should be hydroprimed for 24 hrs prior to sowing. In addition, the seeds should be separated into various sizes, as large seed can be stored for 210 days, while those of medium size only for between 150 and 210 days and small seed nuts for only 150 days.

REFERENCES

Adams, B. R. (1975). Container production of cashew seedling rootstocks. Seed germination in beds as an alternative to direct sowing. *Acta Horticulturae*, 49, 99-108.

Adebisi, M. A. (2004). Variation, stability and correlation studies in seed quality and yield of sesame *(Sesamum Indicum L.)*. PhD thesis. *Department of Plant Breeding and Seed Technology*, Federal *University of Agriculture, Abeokuta, Ogun State*, Nigeria. 132 pp.

Adebisi, M. A., Adeniyi, T. A, Ajala, M. O., & Akintobi, D. C. (2008). Varietal differences in seed physiological quality of West African rice varieties after dry heat treatment. *Nigerian Agricultural Journal, 39*(2), 159-169.

Adebisi, M. A. (2012). Efficacy of plant and chemical materials on quality of okra seed stored under natural ageing conditions. *Nigerian Journal of Horticultural Science, 17,* 157- 168.

Adebisi, M. A., Kehinde, T. O., Esuruoso, A. O., Ajala, M. O., Oni, O. D., & Ajagbe, K. O. (2014). Improvement of seed germination and seedling vigour of garden egg (*Solanum melongena*) genotypes using hydropriming and dehydration treatments. *Journal of Agriculture and Environment, 1,* 29-35.

Adebola, P. O., Esan, E .B., & Famaye, A.O. (1999). Effect of nut weight on germination and seedling vigour in cashew (*Anarcadium occidentale* L.). *Nigerian. Journal of Tree Crop Research, 3*, 6-11.

Akinyemi, E. A., Tijani-Eniola, H., & Famaye, O. (2011). Nut storage periods and nut sizes effects on cashew (*Anarcadium occidentale* L.) seedling emergence and growth. *ARPN Journal of Agricultural and Biological Science, 7*(7), 33-38.

Aliyu, O. M., & Akintaro, G. O. (2007). Studies on the effect of storage period and nut size on the seedling vigour of cashew. *American-Eurasian Journal of Scientific Research, 2*(1), 68-74.

Auckland, A.K. (1961). The influence of seed quality on the early growth of cashew. *Tropical Agriculture, Trinidad*, *38*, 57-67.

Ayfer, M., & Serr, E. F. (1961). Effects of gibberellin and other factors on seed germination and early growth in *Pistacia* species. *Proceedings of the American Society for Horticultural Science*, *77*, 308-315.

Basker, A. M., & Halton, W. (1987). Calcium peroxide as a seed coating materials for paddy rice III. Glasshouse trials. *Plant Soil, 99*, 379-387.

Casini, E., & Conticini, L. (1979). The germinability of seeds of *Pistacia vera* and *Pistacia terebinthus*. *Rivista di Agricoltura Subtropicale e Tropicale, 73,* 233-240.

Copeland, L. D., & McDonald, M. B. (1995). *Principles of seed science and technology* (3rd Edn). New York: Chapman and Hull.

Crane, J. C., & Forde, H. I. (1974). Improved *Pistacia* seed germination. *California Agriculture*, *28*, 8-9.

Dahab, A. M. A, Shafiq, Y., & Al-Kinany, A. (1975). Effects of gibberellic acid, B-nine and scarification on the germination of seeds of *Pistacia* khinjuk Stock. *Mesopotamia Journal of Agriculture*, *10*, 13-19.

Dessai, B. B., Kotecha, P. M., & Salvnhe, D. K. (1997). *Seeds handbook*. New York: Marcel Dekker Inc.

Faluyi, M. A. (1986). Investigations on seedling vigour in cashew (*Anacardium occidentale* L.). *Plant Breeding, 97*, 237-245.

FAO. (2001). *Food and Agriculture Organization, Online Production Statistics Division*. Retrieved 30th March, 2014.

FAO. (2004). *Food and Agriculture Organization, Online Production Statistics Division.* Retrieved on 30th March, 2014.

FAO. (2007). *Food and Agriculture Organization, Online Production Statistics Division.* Retrieved 30th March, 2014.

Hammed, L. A. (2012). Growth analysis of cashew seedlings as affected by nut size. *Annals of Biological Research, 3*(2), 755-772.

Hartmann, H. T. (1967). Effects of various treatments on seed germination of several tree species. *Plant Propagator*, *12*, 10-12.

Ibikunle, B. O., & Komolafe, D. A. (1973). Some experiments on the germination of cashew nuts (*Anacardium occidentale* Linn.). *Nigerian Journal of Science, 7*, 19-29.

Joley, L. E. (1960). Experiences with propagation of the genus *Pistacia. Proceedings of the Plant Propagators' Society*, *10*, 287-292.

Joley, L. E., & Opitz, K. W. (1971). Further experiences with propagation of *Pistacia*. *Combined Proceedings of the International Plant Propagators' Society, 21*, 67-76.

Kim, S. H, Choe, Z. R., Kang, J. H., Copeland, L. O., & Elias, S. G. (2002). Multiple seed vigour indices to predict field emergence and performance of barley. *Seed Science and Technology, 22*, 29-38.

Maggs, D. H. (1973). The pistachio as an Australian crop. *Journal of the Australian Institute of Agricultural Science, 39*, 10-17.

Mikkelsen, D. S. (1981). Calcium peroxide seed coating could revolutionarise planting. *Rice Farming*, 16-18.

Nulawadi, U. G., Prithviray, S., & Krishnamurthy, K. (1973). Improvement in seed germination of soybean variety by pre-sowing treatments. *Indian Journal of Agricultural Science, 43*, 546-550.

Nmadzhanova, K. N., Abdurakhmanov, N. A., & Rafieva, M. G. (1977). The effect of seed covers on the germination of some nut crops. *Subtropicheskie Kul'tury* 1/2, 129-132. *From Horticultural Abstracts, 1978*(48), 7017.

Ogunbayo, I. (2014). Effect of hydration and dehydration treatments on seed quality of low land NERICA rice varieties. Project Report. Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, South-West Nigeria. 65pp

Ohler, J. G. (1979). Cashew. Koninklijk Institute voor de Tropen. Amsterdam, The Netherland 260pp.

Oyedele, A. M. (2014). Promotion of seed germination, seedling vigour and growth parameters in tomato (*Lycopersicon esculentum*) using hydropriming technique. Project Report, Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, South West, Nigeria 65pp.

Oyewumi, A. A. (2014). Response of nut quality to storage period and nut size in two cashew (*Anacardium occidentale Linn*.) biotypes. Master of Agriculture dissertation, Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Ogun State, South West, Nigeria 83pp.

Rao, V. N. M., Rao, I. K. S., & Hassan, M. V. (1957a). Studies on certain aspects of germination of seeds in cashew (*Anacardium occidentale Linn*.). *Indian Journal of Agricultural Science*, *27*, 25-34.

Rao, V. N. M., Rao, I. K. S., & Hassan, M. V. (1957b). Studies on seed viability in cashew. *Indian Journal of Agricultural Science*, *27*, 289-294.

Rocchetti, G., & Panerai, L. (1968). The effect of temperature on the germination of cashew nuts. *Rivista di Agricoltura Subtropicale e Tropicale, 62*, 228-235.

Rocchetti, G., & Panerai, L. (1970). Further studies on the germination of the cashew nut. *Rivista di Agricoltura Subtropicale e Tropicale, 64*, 151-160.

Simon, E. W. (1984). Early events in germination. In D. R. Murray (Ed.), *Seed Physiology Vol 2: Germination and reserve mobilization* (pp. 77-115). Orlando, Florida: Academic Press,

Shanmugavelu, K. G. (1970). Effect of gibberellic acid on seed germination and development of seedlings of some tree plant species. *Madras Agricultural Journal 57*, 311-314.

Subbaiah, C. C. (1982). Effect of pre-soaking in organic solvents on seed germination and seedling growth of cashew. *Scientia Horticulturae, 18*, 137-142.

Turner, D. J. (1956). Some observations on the germination and grading of cashew nut. *East African Agricultural Journal, 22*, 35-39.

Udoh, D. J., Ndoh, B. A., Asuquo, P. E., & Ndaeyo, N. U. (2005). Crop production techniques for the tropics. *Concept Publication* (Nig.) Ltd, 464pp.

Vertucci, C. N. (1989). The kinetics of seed imbibition. *Seed Science and Technology, 16*, 601-612.

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Effects of Extended Heating Time and Post-urea Treatment on Formaldehyde Emission and Properties of Phenolic *Compreg* Rubberwood

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ABSTRACT

Effects of post-urea treatment and extended heating time after compregnation on the formaldehyde emission and properties of rubberwood were investigated in this work. Rubberwood strips having nominal dimensions of 150 mm x 50 mm x 5 mm were compregnated with medium molecular weight phenol formaldehyde (MmwPF, mw 2,000) and low molecular weight phenol formaldehyde (LmwPF, mw 600), respectively. Compregnated rubberwood were then soaked in urea solutions in different concentrations of 10%, 20% and 40%, respectively, for 1 minute. Extended heating times of 0, 12, and 24 hours under 100 ± 2°C were applied to another set of rubberwood compregnated with LmwPF. Properties such as formaldehyde emission, mechanical and physical properties were also tested. Results showed that the post-urea treatment and extended heating time reduced the formaldehyde emission of the compregnated rubberwood. However, mechanical strength of compregnated rubberwood was not significantly affected by both the treatments. Improvements in water absorption (WA) and thickness swelling (TS) of compregnated rubberwood were observed when the heating time was lengthened. Nevertheless, the formaldehyde emission obtained is still far beyond the standard threshold limit of 0.16 – 2.0 mg/l. Thus, further study has to be conducted by lengthening the heating time and increasing the concentration of urea solution.

Keywords: Compreg, extended heating time, formaldehyde emission, phenol formaldehyde resin, post-urea treatment, rubberwood

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INTRODUCTION

The potential of rubberwood as a source of timber has long been recognised in Malaysia. As an alternative timber species, the wood of this tree is marketed for a wide variety of end products such as furniture, flooring, wood based panels and indoor building components (Killmann & Hong, 2000; Lee *et al.,* 2014; Lee *et al.,* 2015). However, owing to its high starch content, the major problem in rubberwood utilisation is its low durability against insect and fungal attack (Khurshid, 2005). Rubberwood is naturally high in moisture and has a high tendency to warp when in use. In other words, its dimensional stability is poor. In relation to the matter mentioned, a number of studies called bulking treatment with phenol formaldehyde (PF) resin have been carried out to improve the dimensional stability, mechanical strength and durability of the wood. Impregnation with PF, followed by compressing at high temperatures, has been proven to be effective in enhancing the properties of the treated wood (Rabi'atol Adawiah *et al.,* 2012). The success of this treatment is dependent upon the molecular weight of PF resin, compressing ratio, pre-curing time, as well as the thickness of wood (Zaidon *et al.,* 2010).

Impregnation of rubberwood with low molecular weight phenol formaldehyde (LmwPF) resin through the *compreg* method can practically solve the imperfections of rubberwood. Nevertheless, the treatment of rubberwood with LmwPF leads to emission of considerable amount of formaldehyde from the products (Amarullah *et al.,* 2010). Numerous studies have been done to reduce the emission of formaldehyde from wood-based products. One of the methods that can be used is to mix the treating solution with formaldehyde scavenger, such as urea, ammonium phosphate, potassium sulphite and sodium thiosulphate, to capture the free formaldehyde (Roffael, 1993). Urea, in particular, is preferred due to its low cost (Zaidon, 2009).

Urea has been incorporated into resin by various researchers with the effort to reduce formaldehyde emission of the wood-based products (Nur Izreen *et al.,* 2011; Purba *et al.,* 2014) and the results are encouraging. However, the addition of urea was found to have decreased the curing rate of PF resin. LmwPF has slower curing rate compared to amino-type resins and even medium molecular weight phenol formaldehyde (MmwPF) resin due to the presence of a greater amount of shorter chain oligomers in the system (He & Riedl, 2003). In this case, a post treatment is encouraged to reduce formaldehyde although there are limited literature reviews available on the effects of the post treatment. There are actually many advantages of post treatment. One of the notable advantages is the flexibility of using dosage that does not interfere with the curing system of the resin (Lum *et al.,* 2014). In another study, Amarullah *et al.* (2010) found that formaldehyde emission could be further reduced by extending curing time.

This paper reports on the effects of extended heating and post-urea treatment on formaldehyde emission, and the physical

and mechanical properties of rubberwood treated with low and medium weight phenolic resin.

MATERIALS AND METHODS

Preparation of Materials

Fresh and defect-free rubberwood (*Hevea brasiliensis*) obtained from Forest Research Institute Malaysia (FRIM) located in Kepong was used in this study. Rubberwood was cut into wood strips in a nominal dimension of 150 mm long x 50 mm wide x 5 mm thick. The cut samples were conditioned in a conditioning room at 25 ± 2°C and 65 ± 2% RH prior to impregnation process. Low molecular weight phenol formaldehyde (LmwPF, molecular weight of 600) and medium molecular weight phenol formaldehyde (MmwPF, molecular weight of 2,000) resins with solid content of 45% were used as the treating solutions in this study. Both phenol formaldehyde resins were specially synthesised at Malayan Adhesives and Chemical (MAC) Sdn. Bhd., Shah Alam. Urea in the form of granules, which was obtained from MAC, was incorporated into the resin and soaking agent to act as formaldehyde scavenger.

Impregnation and Compregnation Processes

For the impregnation process, the pre-weighed samples were first vacuumed at 689 kPa for 15 minutes. The samples were then left soaked in the solution for 30 minutes under atmospheric pressure. After the process was completed, the treated samples were taken out and blotted with paper towel to remove any excessive resin from the surface. The treated samples were then pre-cured in an oven at 65 ± 2°C for 6 hours. Subsequently after curing, the samples were compressed in a hot press at 150°C for 20 minutes at 80% compression ratio (CR). A set of 4 mm stopper bars were used to control the final thickness. The compression ratio was calculated using Equation 1:

$$CR\ (\%) = 100\ (T_f / T_i) \qquad [1]$$

where T_f = final thickness after compress (mm) and T_i = initial thickness (mm).

The *compreg* samples were then conditioned in a conditioning room at 25 ± 2°C and 65 ± 2% RH until a constant weight was achieved.

Extended Heating Time and Post-urea Treatment

Three treatments were involved in this study. The experimental design of the study is shown in Table 1. Treatment 1 (T1) involved impregnation with admixture of LmwPF resin + 10% urea (based on solid PF) and upon compression, the product (which is also known as *compreg*) was further heated in an oven. The extended heating time study was adopted and modified from the study by Amarullah *et al.* (2010) who heated the samples in an oven at a maintained temperature of 103 ± 2ºC for different periods of 0 hour, 24 hours and 48 hours, respectively. Owing to the concern that prolonged heating at high temperatures may destroy the plasticising effect of the urea and PF (Forest Products Laboratory, 1943), shorter heating times were used in this study, namely 0 hour, 12 hours and 24 hours,

respectively. Ten percent urea admixed with 20%, 25% and 30% LmwPF, respectively, were prepared and used as treating solutions. After the impregnation process, the wood samples were further heated in an oven at a temperature of 103 ± 2ºC for durations of 0 hour, 12 hours and 24 hours, respectively, prior to the compregnation process. The *compreg* samples were then conditioned in a conditioning room at 25 ± 2°C and 65 ± 2% RH until a constant weight was achieved.

For Treatment 2 and Treatment 3 (see Table 1), the samples were impregnated with different concentrations of LmwPF and MmwPF, respectively, and the impregnated wood samples were soaked in the urea solution prior to the compregnation process. LmwPF, with concentrations of 20%, 25% and 30%, were prepared and used as treating solutions. The wood samples were impregnated with the treating solution separately using the vacuum pressure process as described in the above section. After impregnation, the wood samples were soaked in 10%, 20% and 40% urea (based on solid PF), respectively, for 1 minute, followed by hot stacking at 125°C for 20 minutes. The urea concentrations used in this study were modified from an earlier study by Zaidon (2009) in order to reduce the formaldehyde emission of the treated wood. The final weight of each soaked sample was determined and this value was used to calculate urea spread (US) (Equation 2). The untreated samples were used for comparison purposes.

$$US\ (g/m^2) = [(W_f - W_i) \times C] / A \quad [2]$$

where W_f = weight after hot stacking (g), W_i = weight after hot pressing before soaking (g), C= concentration of urea (%) and A= Total surface area of samples (m^2).

The soaked samples were then pre-cured in an oven at 65±2°C for 6 hours prior to compregnation process. The same procedures were repeated for the wood samples treated using MmwPF at the concentrations of 10%, 15% and 20%, respectively. All of the *compreg* samples were conditioned in a conditioning room at 25 ± 2°C and 65 ± 2% RH until a constant weight was achieved.

TABLE 1
Experimental design of the study

Treatment	Treatment process	PF conc. (%)	Urea conc. (%)	heating time (h)
T1	Treatment with admixture of LmwPF and10% urea, followed by extended heating in oven at 100°C	20, 25 and 30	-	0, 12 and 24
T2	Treatment with LmwPF, followed by soaking in urea solution	20, 25 and 30	10, 20 and 40	-
T3	Treatment with MmwPF, followed by soaking in urea solution	10, 15 and 20	10, 20 and 40	-

* PF = phenol formaldehyde; LmwPF = low molecular weight phenol formaldehyde; MmwPF = medium molecular weight phenol formaldehyde

Formaldehyde Emission Test

Formaldehyde emission of the treated samples was conducted in accordance with Malaysian Standards (MS 2005). A calibration curve was first produced from a standard formaldehyde solution by iodometric titration. Wood samples with a total surface area of approximately 1,800 cm^2 were placed in a desiccator having 300 ml of distilled water. The wood samples were kept in the desiccator for 24 hours at ambient temperature. The background formaldehyde was prepared using a desiccator containing no test samples. Formaldehyde absorbance in water was measured photometrically at 412nm wavelength. The concentration of formaldehyde was determined using the following equation:

$$G = f \times (A_d - A_b) \times 1800/S \quad [3]$$

Where G= concentration of formaldehyde due to test samples (mg/L), A_d = absorbance of the solution from the desiccator containing the test samples, A_b = absorbance of the background formaldehyde solution, f = slope of the calibration curve for the standard formaldehyde solution and S = surface area of the test samples (cm^2).

Evaluation of Mechanical Properties

The modulus of rupture (MOR) and modulus of elasticity (MOE) in static bending were tested according to the British Standard BS 373:1957 (BSI 1957), with a modification of the sample size. The test samples (150 mm long x 50 mm wide x 5 mm thickness) were cut from the *compreg* products and untreated rubberwood. Static bending was tested at a loading rate of 0.50 mm/min using universal testing machine (Instron 50 kN). The MOR and MOE were calculated using Equations 4 and 5, respectively.

$$MOE\ (Nmm^{-2}) = 3P_mL^3/2wh^2 \quad [4]$$

$$MOR\ (Nmm^{-2}) = P_1L^3/4Dwh^3 \quad [5]$$

where P_m = maximum breaking load (N), P_1 = load at proportional limit (N), L = span of the test samples (mm), D = deflection at mid-span resulting from P_1 (mm), w = width of the test samples (mm) and h = height of the test samples (mm).

Evaluation of Physical Properties

Five samples with the nominal size of 20 mm wide and 20 mm long and 4 mm thickness were cut from the *compreg* and untreated rubberwood. The thickness and weight of the samples were measured before submerging them in distilled water. The beaker with the content was vacuumed in a vacuum-pressure apparatus for 15 minutes. They were left soaked in the water under atmospheric pressure for 24 hours. Upon completion of the test, the test samples were taken out and blotted with paper, while the final thickness and weight were measured again. Thickness swelling (TS) and water absorption (WA) of the samples were then calculated using Equations 6 and 7, respectively.

$$TS\ (\%) = 100\ [(T_2-T_1) / T_1] \quad [6]$$

$$WA\ (\%) = 100\ [(W_2-W_1) / W_1] \quad [7]$$

where T_1 = thickness of the samples before immersing in water (mm), T_2 = thickness of the samples after immersing in water

(mm), W_1 = weight of the samples before immersing in water (mm) and W_2 = weight of the samples after immersing in water (mm).

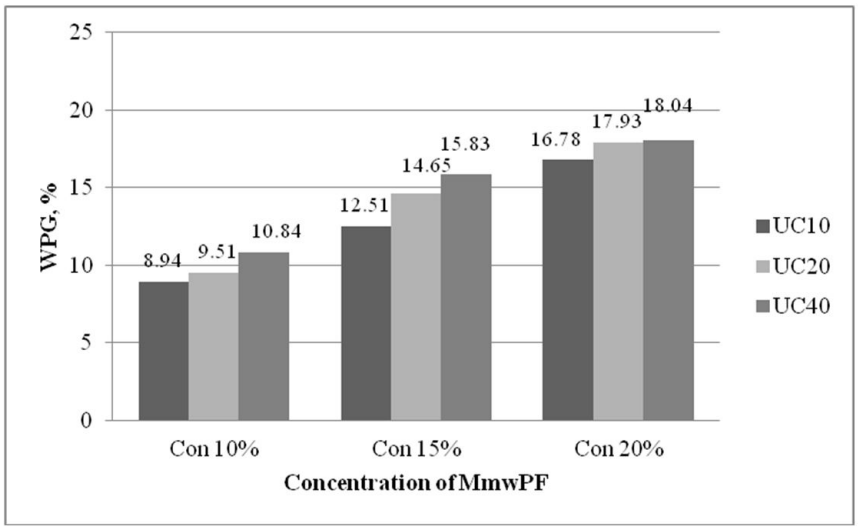
Statistical Analysis

The data were analysed statistically to verify the significance of the variables in this study. The data were analysed using the analysis of variance (ANOVA) at 95% confident level ($P \leq 0.05$). Tukey's honest significant difference tests were then used to further determine the significant level of the average values for each treatment.

RESULTS AND DISCUSSION

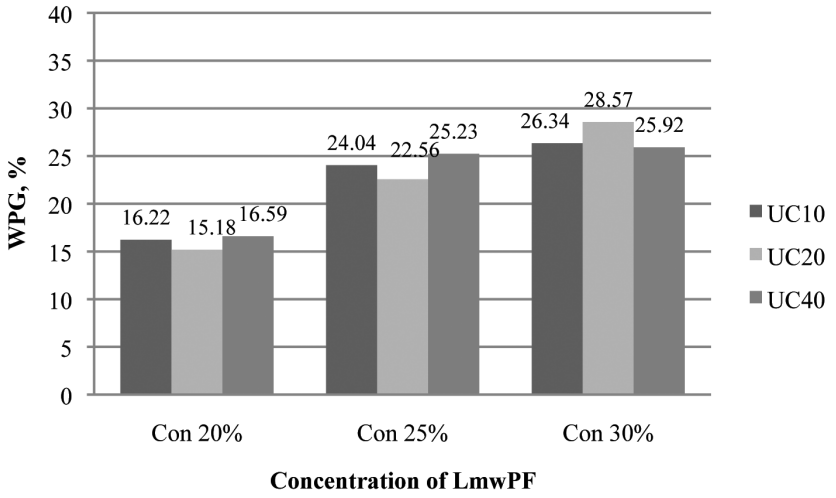
Weight Percent Gain (WPG)

Fig.1 to Fig.3 show the WPG of the rubberwood treated using different treatments. From the results presented in Fig.1 and Fig.2, it was observed that with lower molecular weight, the WPG of the phenolic treated rubberwood increased. Meanwhile, the rubberwood treated with LmwPF showed the highest WPG (28.57%). Shams and Yano (2011) concluded that the short chain and lower viscosity of LmwPF attributed to the achievement of the highest WPG. LmwPF could easily penetrate the parenchyma cells compared to MmwPF. Johnson and Kamke (1994) also reported that the resin penetration behaviour is significantly influenced by the molar masses of polymers present in the resin. Hence, with the same solid content, resin with higher molar masses has higher value of mass per amount of substance and generally higher viscosity but lower wettability. These attributes will lead to poor penetration into wood surface compared to resin with lower molar masses (Nor Hafizah *et al.,* 2012).



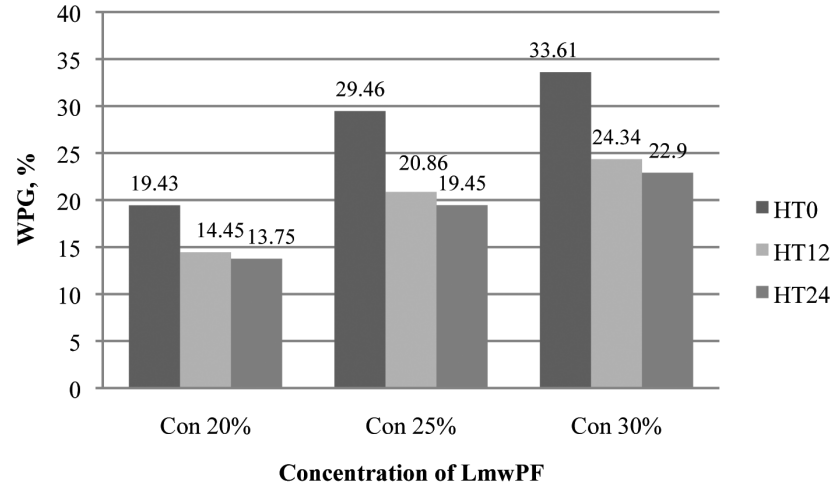
*UC10 = urea concentration of 10%; UC 20 = urea concentration of 20%; UC 40 = urea concentration of 40%

Fig.1 Weight percentage gain (WPG) of the medium molecular weight phenol formaldehyde (MmwPF) *compreg* rubberwood after the post-urea treatments.



*UC10 = urea concentration of 10%; UC 20 = urea concentration of 20%; UC 40 = urea concentration of 40%

Fig.2 Weight percentage gain (WPG) of the low molecular weight phenol formaldehyde (LmwPF) *compreg* rubberwood after the post-urea treatments



*HT0 = heating time of 0 hour; HT12 = heating time of 12 hours; HT24 = heating time of 24 hours

Fig.3 Weight percentage gain (WPG) of the low molecular weight phenol formaldehyde (LmwPF) *compreg* rubberwood at different extended heating times

On the other hand, the rubberwood treated with LmwPF followed by extended heating period was recorded to have slightly lower WPG than the rubberwood treated with LmwPF without extended heating time (Fig.3). This might be due to the loss of the moisture of resin through vaporisation before it was fully cured.

Effects of the Post Treatment on Formaldehyde Emission

Calibration curve from iodometric titration is illustrated in Fig.4. This graph has estimated slope which was used in determining formaldehyde emission of the samples. The slope obtained was 8.351x – 0.126 with r^2 = 0.999. Fig.5 to Fig.7 show the formaldehyde emission of the rubberwood treated using different treatments. The formaldehyde emission of the samples was significantly reduced by incorporating urea in phenol formaldehyde, followed by extended heating of the *compreg* wood (Fig.5). Formaldehyde emission of the samples treated with 30% PF incorporated with urea (10% based on solid PF) was decreased up to only 7.90% as compared to the samples treated with 20% and 25% PF which recorded 30.21% and 30.63% reductions in formaldehyde emission, respectively. This is because of the higher concentration of the 30% PF applied. Urea was used to absorb some of the free formaldehyde in the resin system and to form cross-linked polymer of urea formaldehyde (Zaidon *et al.,* 2010). Formaldehyde emission is free formaldehyde partly released from the impregnated LmwPF resin of the treated rubberwood, which was not completely polymerised. Meanwhile, extended heating time after hot pressing enhanced the curing of the impregnated LmwPF and thus reduced the formaldehyde emission. These results are in agreement with Amarullah *et al.* (2010) who found that the formaldehyde emission of the oil palm wood decreased after 48 hours of extended heating in the oven.

For rubberwood compregnated with LmwPF, the formaldehyde emission was reduced around 40% with the presence of urea (Fig.6). The formaldehyde emission decreased with the increase in the concentration of urea solution. For rubberwood compregnated with MmwPF, the results showed that all *compreg* rubberwood with post-urea treatment

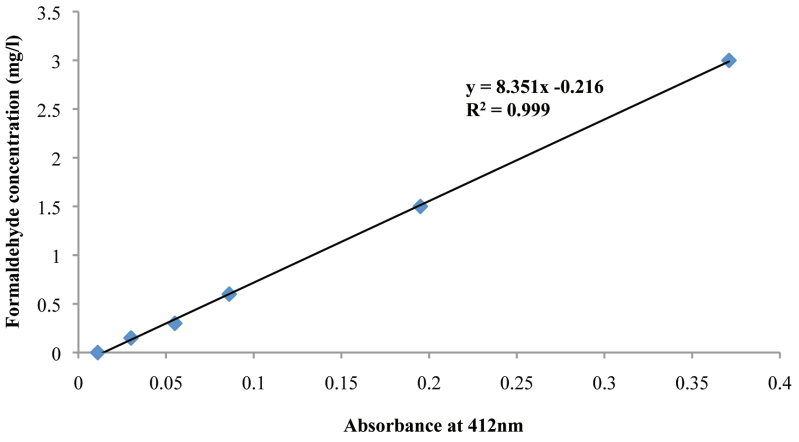
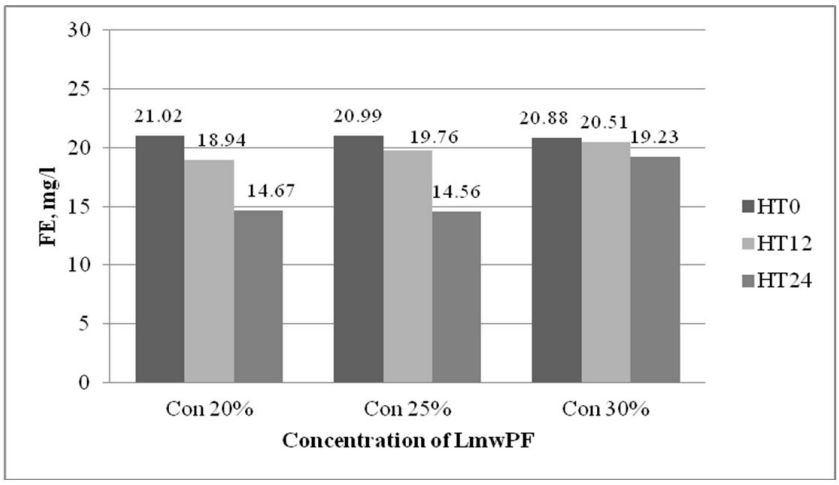
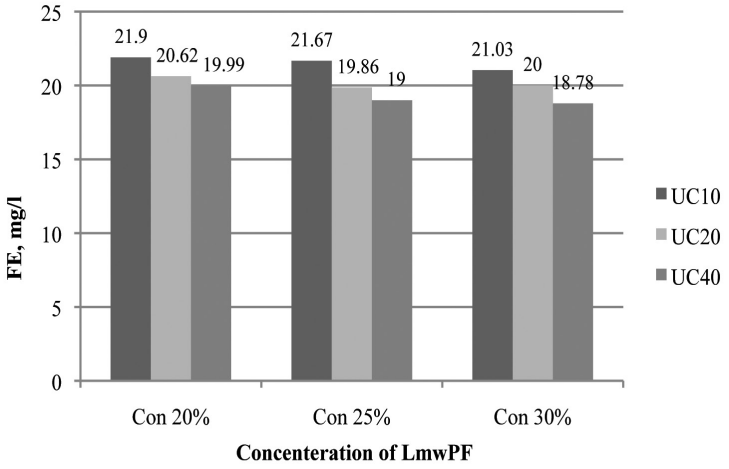


Fig.4 Calibration curve of standard formaldehyde concentration vs. absorbance using spectrophotometer



*HT0 = heating time of 0 hour; HT12 = heating time of 12 hours; HT24 = heating time of 24 hours

Fig.5 Formaldehyde emission (FE) of the low molecular weight phenol formaldehyde (LmwPF) *compreg* rubberwood at different extended heating times



*UC10 = urea concentration of 10%; UC 20 = urea concentration of 20%; UC 40 = urea concentration of 40%

Fig.6 Formaldehyde emission (FE) of the low molecular weight phenol formaldehyde (LmwPF) *compreg* rubberwood after the post-urea treatments

recorded significantly lower formaldehyde emission compared to the untreated rubberwood (Fig.7). Formaldehyde emission was reduced by 92.4% to 0.341 mg/l compared to the untreated rubberwood which recorded formaldehyde emission of 4.46 mg/l. These results are in agreement with Rabi'atol Adawiah *et al*. (2012) who confirmed that the presence of urea had successfully reduced the formaldehyde emission of the treated samples by absorbing some of the free formaldehyde in the PF resin. Earlier work by Zaidon (2009) also showed that urea concentrations in the range of 10% to 30% were able to reduce the formaldehyde emission from impregnated and compregnated sesenduk wood. The results in Table 2 revealed that better urea spread was obtained as the concentration of urea increased. Thus, it is anticipated that urea acts as the formaldehyde catcher that slightly reduces the level of formaldehyde emission. Better urea spread means better uptake of urea by wood, exerting its effect as formaldehyde scavenger which brings down the formaldehyde emission.

TABLE 2
Urea spread of the rubberwood treated with different phenolic resins

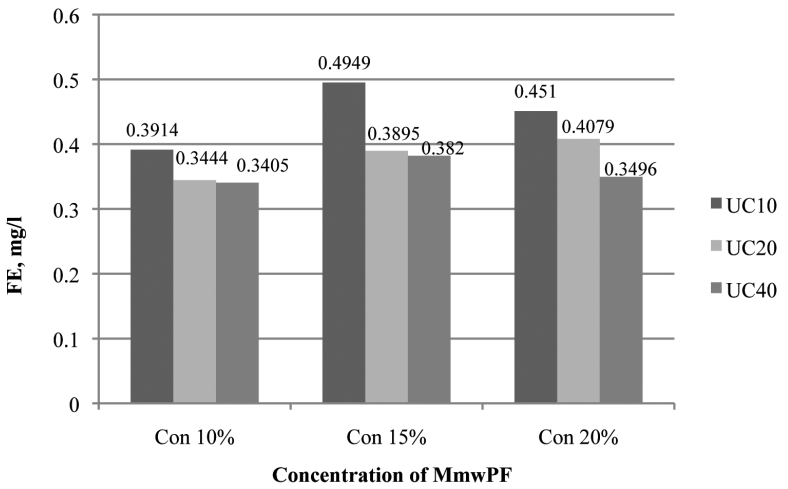
PF Concentration (%)	Urea Concentration (%)	Urea spread (g/m^2)
MmwPF		
10	10	0.35 a ± 0.24
10	20	0.45 ab ± 0.13
10	40	0.59 ab ± 0.14
15	10	0.99 bc ± 0.31
15	20	1.03 bc ± 0.23
15	40	1.38 c ± 0.35
20	10	1.52 c ± 0.65
20	20	3.63 d ± 0.71
20	40	3.68 d ± 0.66
LmwPF		
20	10	0.33 a ± 0.14
20	20	0.41 ab ± 0.09
20	40	0.57 ab ± 0.11
25	10	0.60 ab ± 0.22
25	20	0.62 ab ± 0.31
25	40	0.78 bc ± 0.39
30	10	1.11 bc ± 0.44
30	20	1.52 c ± 0.58
30	40	2.53 c ± 0.50

*Values with different letter in the same row signifies significant difference $p \leq 0.05$
** PF = phenol formaldehyde; LmwPF = low molecular weight phenol formaldehyde; MmwPF = medium molecular weight phenol formaldehyde

TABLE 3
The average values of effects from the extended heating times on density, modulus of rupture (MOR), modulus of elasticity (MOE), water absorption (WA) and thickness swelling (TS) for *compreg* rubberwood

PF Conc. (%)	Extended heating time (h)	Density (kg/m^3)	MOR (N mm^{-2})	MOE (N mm^{-2})	WA (%)	TS (%)
20	0	815.85 ab ± 69.68	91.42 a ± 18.42	7650 a ± 1978.4	9.41 b ± 3.41	2.67 ab ± 1.06
20	12	749.95 ab ± 31.11	86.41 a ± 23.95	7768 a ± 2203.3	6.02 b ± 1.36	1.97 ab ± 0.48
20	24	754.76 ab ± 60.46	97.98 a ± 18.77	8032 a ± 1576.1	7.18 b ± 2.31	1.23 b ± 0.56
25	0	853.52 ab ± 89.75	81.58 a ± 23.88	7861 a ± 1459.2	5.30 b ± 1.98	1.96 ab ± 1.02
25	12	810.55 ab ± 83.50	97.78 a ± 30.07	8784 a ± 2560.7	5.48 b ± 1.50	1.96 ab ± 0.82
25	24	805.29 ab ± 100.15	85.57 a ± 29.46	7861 a ± 2104.9	8.78 b ± 2.18	1.75 ab ± 0.74
30	0	880.59 b ± 77.82	95.54 a ± 19.02	8156 a ± 2109.6	5.48 b ± 2.43	1.31 b ± 1.06
30	12	768.63 ab ± 92.15	81.16 a ± 19.26	7970 a ± 1638.7	7.30 b ± 5.69	1.27 b ± 1.01
30	24	859.02 ab ± 64.93	92.73 a ± 38.11	8888 a ± 2543.4	6.89 b ± 4.29	1.18 b ± 0.57
untreated		598.48 a ± 72.68	69.64 a ± 20.33	7544 a ± 2371.8	72.77 a ± 18.54	4.25 a ± 2.17

*Values with different letters in the same column signify a significant difference $p \leq 0.05$
** PF = phenol formaldehyde; LmwPF = low molecular weight phenol formaldehyde; MmwPF = medium molecular weight phenol formaldehyde



*UC10 = urea concentration of 10%; UC 20 = urea concentration of 20%; UC 40 = urea concentration of 40%

Fig.7 Formaldehyde emission (FE) of the medium molecular weight phenol formaldehyde (MmwPF) *compreg* rubberwood after the post-urea treatments

Effects of Extended Heating Time on Mechanical and Physical Properties

As shown in Table 3, the MOR of the *compreg* rubberwood at different treatment combinations did not differ significantly between all the treatments. In specific, the MOR values ranged from 91.04 Nmm^{-2} to 97.98 Nmm^{-2} for 20% PF, 81.58 Nmm^{-2} to 97.78 Nmm^{-2} for 25% PF, 81.16 Nmm^{-2} to 92.73 Nmm^{-2} for 30% PF and 69.64 Nmm^{-2} for the untreated rubberwood. Compared to the untreated rubberwood, the MOR values of the compreg rubberwood were shown to be slightly higher. The rubberwood treated with 20% PF, followed by heating of 24 hours, recorded the highest MOR value of 97.98 Nmm^{-2} or 40.7% of increment over the untreated rubberwood. This might be due to the resin polymer which bulked perfectly in the cell lumen of the strips and strengthened the structure of wood to withstand the load applied during the testing. Amarullah *et al*. (2010) suggested that the extended heating time had improved the curing behaviour of the PF resin in the wood and subsequently led to better properties. Table 3 shows that the MOE of all different treatment combinations did not differ significantly between each other. However, the MOE values of different treatment combinations were higher than that of the untreated rubberwood. The highest value obtained for the treatment combinations was the treatment of 30% PF and extended heating time of 24 hours, i.e. 8,888 Nmm^{-2} or 18% increment over the untreated rubberwood. This shows that the stiffness of the strips increased the mechanical anchorage of the phenolic resin in the cell lumen, in addition to the extended heating by 24 hours which made the strips to become elastic and helped in the formation of polymer.

TABLE 4
The average values of effects from the urea concentrations on density, modulus of rupture (MOR), modulus of elasticity (MOE), water absorption (WA) and thickness swelling (TS) for *compreg* rubberwood

PF Conc. (%)	Urea Conc. (%)	Density (kg/m^3)	MOR (N mm^{-2})	MOE (N mm^{-2})	WA (%)	TS (%)
MmwPF						
10	10	714.9 ab ± 94.3	107.8 a ± 30.0	10919.5 a ± 3374.5	19.1 b ± 8.4	7.85 ab ± 0.81
10	20	696.9 ab ± 79.9	94.0 ab ± 23.9	7659.7 ab ± 2347.6	14.9 b ± 4.3	6.88 abc ± 0.75
10	40	763.2 ab ± 108.6	115.6 a ± 30.4	11253.6 a ± 4188.8	18.7 b ± 6.2	5.85 abc ± 1.09
15	10	1001.4 a ± 86.4	108.2 a ± 20.0	9712.4 ab ± 1788.6	23.8 b ± 17.8	6.82 abc ± 1.73
15	20	744.9 ab ± 78.8	107.0 a ± 20.7	9507.0 ab ± 2965.8	15.3 b ± 5.0	5.12 bc ± 0.31
15	40	753.1 ab ± 80.8	109.8 a ± 30.4	9994.7 ab ± 2965.8	16.1 b ± 4.1	5.20 bc ± 1.27
20	10	794.2 ab ± 62.7	115.3 a ± 23.3	10259.7 ab ± 2458.9	13.3 b ± 2.7	6.17 abc ± 0.88
20	20	785.7 ab ± 85.5	105.0 ab ± 37.0	10164.8 ab ± 3223.2	12.5 b ± 3.1	5.56 bc ± 0.75
20	40	817.7 ab ± 40.1	116.7 a ± 13.3	10322.0 ab ± 1302.8	11.8 b ± 2.4	4.99 c ± 2.93
untreated LmwPF		572.7 b ± 53.2	69.3 b ± 12.0	6925.3 b ± 1723.4	80.6 a ± 13.6	8.47 a ± 0.73
20	10	942.0 a ± 91.59	123.6 a ± 23.87	14219 a ± 2787.4	8.48 b ± 4.83	14.10 ab ± 13.48
20	20	907.4 a ± 80.68	124.8 a ± 26.10	11969 a ± 3465.7	6.03 b ± 1.64	28.87 a ± 0.52
20	40	879.8 a ± 77.19	109.8 ab ± 20.78	10868 a ± 3128.6	8.33 b ± 5.07	18.87 ab ± 13.54
25	10	933.4 a ± 72.46	109.1 ab ± 32.88	10516 a ± 1950.6	5.63 b ± 2.40	7.45 ab ± 12.33
25	20	899.0 a ± 93.74	105.9 ab ± 26.89	14165 a ± 1314.9	3.98 b ± 1.82	27.83 a ± 0.65
25	40	947.2 a ± 55.06	112.7 ab ± 16.57	11112 a ± 1531.0	3.92 b ± 1.49	28.17 a ± 0.86
30	10	937.7 a ± 81.61	116.5 ab ± 37.02	11461 a ± 2794.2	3.59 b ± 1.34	17.02 ab ± 13.90
30	20	958.1 a ± 87.66	112.0 ab ± 22.29	10929 a ± 2811.5	4.36 b ± 2.26	11.90 ab ± 14.44
30	40	920.9 a ± 105.60	122.1 a ± 39.91	11845 a ± 2768.3	3.44 b ± 1.01	17.22 ab ± 13.40
untreated		607.9 b ± 48.76	80.5 b ± 12.11	7820 a ± 1678.1	54.71 a ± 3.43	4.87 b ± 3.13

*Values with different letter in the same column signifies significant difference $p \leq 0.05$
**PF = phenol formaldehyde; LmwPF = low molecular weight phenol formaldehyde; MmwPF = medium molecular weight phenol formaldehyde

Meanwhile, the treated rubberwood had significantly lower WA value than the untreated strips. Due to its lowest PF concentration, rubberwood strip treated with 20% PF with 0 hour heating time was observed to have the highest WA value among all the treated strips. The results showed that the strips treated with LmwPF lowered the absorption of water into the wood. TS for the treated wood were significantly lower than that of the untreated wood. From Table 3, the TS value was shown to decrease with increasing heating time. As the heating time extended, the cross-linked polymer became hard, infusible and insoluble which could not be softened and melted (Hon, 2003). Furthermore, the extended heating also promotes higher resin polymerisation in the treated strips.

Effects of Post-Urea Treatment on Mechanical and Physical Properties

For the woods treated with MmwPF, no significant difference within the treated groups was found. However, significant difference was observed between the treated and untreated rubberwood (Table 4). In particular, the MOR of the treated groups increased by 34.02% to 40.06% compared to that of the untreated wood. On the other hand, the MOE values of the treated woods increased by 11.77% to 32.91% compared to that of the untreated woods. The wood treated with 20% PF and urea solution of 40% concentration showed the highest MOE value (10,322 Nmm^{-2}). The WA of the treated strips decreased around 70.48% to 85.35% compared to the untreated group (Table 4). The lowest WA was recorded in the strip treated with 20% PF, followed by a post treatment of 40% urea. The TS of the treated rubberwood decreased around 6.66% to 40.5% compared to the untreated rubberwood. The lowest TS value of 5.00% was observed when the rubberwood treated with the highest PF and urea concentration (20% PF + 40% urea). Rubberwood treated with the highest concentration of PF and urea exhibited the best performance mainly because of the cell wall and lumen was filled with phenolic resin and covered with urea coating. Both PF and urea have plasticisation effect on the cell walls by deforming them without rupturing during compression. The compressed wood becomes stronger and more dimensionally stable once the resin is cured (Yano *et al*., 1997).

For the rubberwood treated with LmwPF, the highest MOR value was observed for the treatment of 20% of PF, followed by soaking in 20% urea solution which recorded 124.75 Nmm^{-2}. On the other hand, the highest MOE value of 14,219 Nmm^{-2} was recorded when the rubberwood was treated with 20% of PF, followed by soaking in 10% urea solution. Both the MOR and MOE values of the untreated rubberwood are significantly lower than that of the treated wood. The TS value of the treated rubberwood decreased with the increase in the PF and urea concentrations. All the treated rubberwood showed at least two-fold lower TS than the untreated rubberwood. Meanwhile, WA for the treated rubberwood showed a highly significant difference between the treated and untreated rubberwood. The

untreated rubberwood showed the highest WA value of 54.71%, while the lowest WA value of 3.44% was observed when the rubberwood was treated with 30% PF and soaked in 40% urea solution. Aside from the increasing PF concentration that imparted a better performance onto the treated wood, increasing the urea concentration during post treatment was also shown to have encouraging effect on the physical properties of treated wood. Nevertheless, the findings are dissimilar to the previous study conducted by Rabi'atol Adawiah *et al*. (2012) who reported that the presence of urea increased the viscosity of the resin solution, therefore limiting the penetration into the cell wall and adversely affecting the performance of the treated wood. However, Gabrielli and Kamke (2010) suggested that the higher viscosity of the resin solution might have caused less resin to be squeezed out during compression due to its slower movement through the wood structure. In this study, higher WPG obtained from the samples treated with higher urea concentration confirmed the point.

CONCLUSION

The results revealed that the post-urea treatment on the *compreg* wood rubberwood reduced the formaldehyde emission by 40% compared to the rubberwood without any post-urea treatment. The results also revealed that the post-urea treatment did not significantly affect the mechanical and physical properties of the *compreg* wood. The formaldehyde emission for the *compreg* rubberwood could be successfully reduced by incorporating urea in the treating solution followed by extended heating in an oven. Nevertheless, the formaldehyde emission obtained is still far beyond the standard threshold limit of 0.16 – 2.0 mg/l (Markessini *et al*., 2010). It was also found that the longer the heating time, the higher the reduction of formaldehyde emission would be. Hence, extended heating time did not significantly affect the MOR and MOE values of the wood although it reduced the WA and TS of the treated wood. It is expected that formaldehyde emission can be further reduced by lengthening the curing time of the resin, which in turn will help to increase the rate of polymerization by altering the hot-pressing compression schedule.

REFERENCES

Amarullah, M., Bakar E.S., Zaidon, A., Mohd Hamami, S., & Febrianto F. (2010). Reduction of formaldehyde emission from phenol formaldehyde treated oil palm wood through improvement of resin curing state. *Journal of Tropical Wood Science and Technology*, *8*, 9-14.

BSI British Standard, BS 373:1957. (1957). Method of testing small clear specimens of timber: British Standards Institution, London.

Forest Products Laboratory Report No. R1277. (1943). Urea-plasticized wood (Uralloy). United States Department of Agriculture Forest Service, Forest Products Laboratory, Madison, Wisconsin.

Gabrielli, C. P., & Kamke, F. A. (2010). Phenol–formaldehyde impregnation of densified wood for improved dimensional stability. *Wood Science and Technology*, *44*, 95-104.

He, G., & Riedl, B. (2003). Phenol-urea-formaldehyde resol resins: their synthesis, curing kinetics, and network properties. *Journal of Polymer Science*, *41*, 1929-1938.

Hon, D. N. S. (2003). Analysis of adhesive. In A. Pizzi & K.L. Mittal (Eds.), *Handbook of Adhesives Technology*. New York: Marcel Dekker Inc.

Johnson, S. E., & Kamke, F. A. (1994). Characteristics of phenol-formaldehyde adhesives bonds in steam injection pressed flakeboard. *Wood Fibre Science*, *26*, 259-269.

Khurshid, A. (2005). *Preservative treatment of rubberwood to increase its service life.* Document no. IRG/WP 05-40320. The International Research Group on Wood Protection, Stockholm.

Killman, W., & Hong, L. T. (2000). Rubberwood: The success of an agricultural by-product. *Journal of Forestry*, *51*(2), 66-72.

Lee, S. H., H'ng, P. S., Lum, W. C., Zaidon, A., Bakar, E. S., Nurliyana, M. Y., Chai, E. W., & Chin, K. L. (2014). Mechanical and physical properties of oil palm trunk core particleboard bonded with different UF resins. *Journal of Oil Palm Research*, *26*(2), 163-169.

Lee, S. H., Zaidon, A., Lum, W. C., H'ng P. S., Tan, L. P., Chow, M. J., Chai E. W., & Chin, K. L. (2015). Properties of particleboard with oil palm trunk as core layer in comparison to three-layer rubberwood particleboard. *Journal of Oil Palm Research*, *27*(1), 67-74.

Lum, W. C., Lee, S. H., & H'ng P. S. (2014). Effects of formaldehyde catcher on some properties of particleboard with different ratio of surface to core layer. *Asian Journal of Applied Sciences*, *7*(1), 22-29.

Malaysian Standard, MS 1787: Part 15. (2005). *Determination of formaldehyde emission by desiccator method*. Department of Standards Malaysia, Cyberjaya, Selangor.

Markessini, C., Athanassiadou, E., & Tsiantzi, S. (2010). Producing panels with formaldehyde emission at wood level. In *7th European Wood-based Panel Symposium*. 13-15 October 2010. Hannover.

Nor Hafizah, A. W., Paridah, M. T., Yeoh, B. H., Zaidon, A., Nor Yuziah, M. Y., Mohd Khiarun, A. U., & Mohd Hamami, S. (2012). Adhesion characteristics of phenol formaldehyde pre-preg oil palm stem veneers. *Bioresources*, *7*(4), 4545-4562.

Nur Izreen, F. A., Zaidon, A., Rabi'atol Adawiah, M. A., Bakar, E. S., Paridah, M. T., Mohd Hamami, S., & Anwar, U. M. K. (2011). Enhancing the properties of low density hardwood *Dyera costulata* through impregnation with phenolic resin admixed with formaldehyde scavenger. *Journal of Applied Science*, *11*, 3474-3481.

Purba, T. P., Zaidon, A., Bakar, E. S., & Paridah, M. T. (2014). Effects of processing factors and polymer retention on the performance of phenolic-treated wood. *Journal of Tropical Forest Science*, *26*(3), 320-330.

Rabi'atol Adawiah, M. A., Zaidon, A., Nur Izreen, F. A., Bakar, E. S., Mohd Hamami, S., & Paridah, M. T. (2012). Addition of urea as formaldehyde scavenger of low molecular weight phenol formaldehyde treated compreg wood. *Journal of Tropical Forest Science*, *24*, 265-274.

Roffael, E. (1993). Post-treatment of particleboard to reduce its formaldehyde release potential. In K. C. Choo, M. P. Koh, & C. L. Ong (Eds.), *Formaldehyde Release from Particleboard and Other Wood Based Panels*. Malaysia: Forest Research Institute Malaysia (FRIM), Kepong.

Shams, M. I., & Yano, H. (2011). Compressive deformation of phenol formaldehyde (PF) resin-impregnated wood related to the molecular weight of resin. *Wood Science and Technology*, *45*, 73-81.

Yano, H., Hirose, A., & Inaba, S. (1997). High-strength wood-based materials. *Journal of Material Science*, *16*, 1906-1909.

Zaidon, A. (2009). Improvement of Raw Materials from Underutilized Timber Species Through Chemical and Densification Treatments for Value Added Laminated Products. Report No. 06-01-04-SF0656. Ministry of Science and Technology, Putrajaya.

Zaidon, A., Bakar, E. S., & Paridah, M. T. (2010). Compreg laminates from low density tropical hardwoods. In *Proceedings of the International Convention of Society of Wood Science and Technology and United Nations Economic Commission for Europe – Timber Committee*. 11-14 October 2010. Geneva.

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Life Table of *Cochlochila bullita* Stål (Hemiptera: Tingidae) on *Orthosiphon aristatus* (Blume) Miq. and *Ocimum basilicum* L. in Laboratory Conditions

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ABSTRACT

Ocimum tingid, *Cochlochila bullita* Stål (Hemiptera: Tingidae) is a pest of Lamiaceae plants such as basil, tulsi and coleus. It is now being recorded in Malaysia as a pest of the cat's whiskers plant, *Orthosiphon aristatus* (Blume) Miq. Nevertheless, apart from its brief biological description, no other information is available. The life table of this pest was studied in laboratory conditions. Development time for *C. bullita* feeding on *O. aristatus* was 23.3 ± 0.9 days, which was found to be similar to those feeding on *Ocimum basilicum* (22.8 ± 0.3 days). Although *C. bullita* posts a higher mortality rate on *O. aristatus* than on *O.basilicum* (52% vs. 37%), the adult longevity of the bugs that feed on *O. aristatus* (♀: 33.9; ♂: 38.2 days) was found to be significantly higher than those bugs that feed on *O.basilicum* (♀: 27.2; ♂: 26.0 days). The pre-ovipostion, ovispostion and fecundity of *C. bullita* were also different between the host plants. The net reproductive rates (R_0), finite rate of increase (λ) and intrinsic rate of increase (r) were also higher on *O. aristatus* (10.7504, 1.0690 and 0.0667), although there was an increased in immature survival on *O. basilicum* (6.0287, 1.0556 and 0.0541). Therefore, it is concluded that *O. aristatus* is as good as *O. basilicum*, or the population growth of *C. bullita* is more favoured as compared to *O. basilicum*.

Keywords: Basil, cat's whiskers plant, lace bug, life table parameter, Malaysia, *Ocimum tingid*

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INTRODUCTION

Ocimum tingid, *Cochlochila bullita* Stål (Hemiptera: Tingidae) is a lace bug associated with the plants of the Lamiaceae family. The lace bug has been recorded to attack culinary herbs, sweet basil, *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. in China, India and also some parts of South East Asia (Livingstone & Yacoob, 1987). In Malaysia, the lace bug was first recorded in Selangor, attacking a native plant called *Orthosiphon aristatus* (Blume) Miq., which is a medicinal plant with many health benefits (Sajap & Peng, 2010). In a recent survey, *C. bullita* was found to attack *O. basilicum* planted in Universiti Putra Malaysia. The lace bugs, nymphs and adults fed on the leaves and young shoots of the herb. The infested leaves and shoots became wilted, dried and in many cases, the whole plant died upon heavy or repeated infestations. Meanwhile, pattern of the infestations showed that the lace bugs could pose a serious threat to the herbs and their related industries if they were to be planted in a wider scale. Apart from the life history of *O. basilicum* that has been documented in Thailand (Tigvattnanont, 1989) and related papers by Sajap and Peng (2010) and Peng *et al.* (2013), very little information is available on this particular insect. Even though *C. bullita* appears to be thriving on both plants, its performance in terms of its demographic characteristics on both plants has yet to be known. Demographic parameters of an insect can be elucidated using a life table analysis (Carey, 1993; Southwood & Henderson, 2000). These demographic parameters can be used to estimate the insect's population growth capacity on different regimes such as host plants (Greenberg *et al.*, 2001), prey for predatory insects (Legaspi *et al.*, 2008), temperature and strain (Liu & Meng, 1999; Bong *et al.*, 2012). The parameters in the life table, particularly the intrinsic rate of natural increase (r), can be used to evaluate the magnitude of suitability of a host plant to an insect (Razmjou *et al.*, 2006). Plants that render lower values of r are relatively more resistant or less suitable hosts than those with higher values of r. In this experiment, life table was used to compare the performance of the lace bug *C. bullita* on its hosts, *O. aristatus* and *O. basilicum*. Understanding the growth and development performances of the lace bug on its host plants will lead to development of a better management strategy of the pest.

MATERIALS AND METHODS

The experiments were conducted in the Entomology Laboratory at the Faculty of Forestry in a room at 28 ± 2 ºC, 55 ± 5% RH, and 12h:12h (L:D) photoperiod.

Rearing Method

The lace bugs were reared on potted plants of *O. aristatus* and *O. basilicum*, which were kept in cages (60 × 30.5 × 25.5 cm). Ten pairs of its females and males were introduced into the cages and allowed to breed on the plants. The plants were regularly watered and replaced with new potted plants when wilted. In order to eliminate residual effects of the previous host plant, *O. aristatus* or

the lace bugs intended for the experiment on *O. basilicum* were collected from the F2 generations of the lace bugs that had been reared on *O. basilicum.*

Preparation of the Host Plants

Stems of *O. aristatus* and *O. basilicum* were cut to about 8 cm long and inserted into a plastic cup (3.5 cm diameter × 4 cm height) filled with water through a hole drilled in the lid. The rooted stems (about two weeks old) were used for the experiments.

Life Table Study

Thirty pairs of the females and males (around 1 – 3 days old) were collected from the colonies and placed on a Petri dish (Brandon Disposable Petri dish; 9.0 × 1.5 cm) containing a piece of moistened filter paper (Advantec Grade No. 1 Qualitative Filter Paper; Diameter: 5.5 cm) and two leaves of the respective plants that served for their food and mating arena. The lace bugs were kept in pairs for 7 d and gravid females were taken for oviposition. For oviposition, three rooted stems of *O. aristatus* or *O. basilicum*, as prepared earlier, were placed separately in a plastic container (MS Venture C1-MS30; 11.8 × 10.2 cm) with its lid covered with muslin cloth. Ten gravid females were placed separately in the container for oviposition. After 24 h, the females were removed and transferred into new containers with fresh plants. The eggs were counted, kept on the plants till hatched and their incubation period was also recorded. Once the eggs hatched, the neonates were counted and left on the plants till moulted. The second instars were then transferred individually into plastic cups (MS Venture C5-MS4; 7.5 × 3.9 cm) containing a moistened filter paper and a fresh leaf. The cups were covered with perforated lids. The leaves and moistened filter paper were changed every 2 d. The nymphs were examined daily and their moulting and survival were also recorded. Successful moulting from each stadium was confirmed with the presence of exuviae. A cohort of 100 eggs was used for each of three replicates. Thus, a total of 300 eggs were used for each host plant in the life table experiment. The fecundity, reproductive period and adult longevity of the lace bugs were studied using the adults collected from the development and survivorship experiments. The adults were collected within 24 h after emergence. They were sexed and the sex ratio was recorded. They were then randomly paired and kept in plastic cups. A total of 30 pairs for a cohort were used for this experiment. If the male died earlier, it was replaced with a new male to ensure continuity of mating and oviposition. The pairs were kept until death. Longevity of adults, pre-oviposition, oviposition, post-oviposition periods and fecundity were recorded. Non-laying females were excluded from the data analyses. The possibility of reproduction without mating was also investigated. These virgin females were kept separately in plastic cups and examined every 24 h for oviposition.

Experimental Design and Statistical Analysis

The experimental design for constructing the life table was modified from the design in the life history of *Maconellicoccus hirsutus* (Chong *et al.*, 2008). All life table and fertility table parameters were measured and calculated as described by Birch (1948). Population parameters were calculated as:

x: Age classification

l_x: Probability of surviving to age x

m_x: age-specific fecundity, the number of female eggs born per female in each interval class

GRR: Gross reproductive rate, $GRR = \Sigma m_x$

Intrinsic rate of increase (r) is estimated using the Euler-Lotka equation with age indexed from zero, $\Sigma e^{-r(x+1)} l_x m_x = 1$, $x = 0$ to 40

R_0: Net reproductive rate, $R_o = \Sigma (l_x m_x)$

T_G: Mean generation time,

$T_G = (\ln R_0) / r$.

λ: Finite rate if increase, $\lambda = e^r$

DT: Doubling time, $DT = \ln 2 / r$

RESULTS

Development Time and Survival

Eggs of *C. bullita*, which were held at 28 ± 2 ºC, hatched in 8.8 ± 0.2 d. When fed on *O. aristatus*, the lace bug had a cumulative developmental time from egg laid till adult emergence ranging from 19.8 – 28.3 d, with an average of 23.3 ± 0.9 d. All the nymphs underwent five moults. The lace bugs had a relatively uniform and shorter development time on *O. basilicum* ranging from 21.4 to 24.4 d, with an average of 22.8 ± 0.3 d. Although the development time of the third instars was longer on *O. aristatus* and so did the fifth instars on *O. basilicum*, there was no significant difference on the overall development time of *C. bullita* immatures feeding on both the host plants ($t_{12} = 0.0086$; $P = 0.589$). They lived significantly longer on *O. aristatus* than those on *O. basilicum* (Table 1). The longevity of the males and females on the same host plant, however, was not significantly different. The host plants also influenced the mortality of the immatures. Significant differences in mortality occurred in the early nymphal stages. The mortality of the first instars on *O. aristatus* was about 10% higher compared with that on *O. basilicum* ($t_{18} = 2.626$; $P = 0.017$). Meanwhile, the second instars had a mortality of 7.3% on *O. aristatus* and 3.0% on *O. basilicum* ($t_{18} = 2.847$; $P = 0.010$). These differences contributed to the overall mortality from the egg to adult stages of 52.0% on *O. aristatus*, which was significantly higher compared to 37.0% on *O. basilicum* ($t_{18} = 2.518$; $P = 0.022$) (Table 2). The genders of the pre-adult mortality were assumed using the male to female ratio of *O. aristatus* and *O. basilicum*, which was 2:3 and 1:1, respectively. In general, the survival rate (l_x) on both plants followed almost a similar pattern with a higher mortality occurred during the nymphal stages, especially during the first instar and this decreased gradually as they matured, following a type III survivorship curve, as described by Speight *et al.* (1999) (Figures 1 and 2).

TABLE 1
Development times of *C. bullita* immature stages feeding on either *O. aristatus* or *O. basilicum* leaves under laboratory conditions

Stage	Development time (Days ± SE)					
	n	*O. aristatus*	*n*	*O. basilicum*	*t*	*P*
Egg	300	8.8 ± 0.2 a	300	8.8 ± 0.2 a	0.034	0.973
1st instar	277	2.8 ± 0.3 a	273	2.4 ± 0.1 a	1.486	0.154
2nd instar	220	2.4 ± 0.1 a	244	2.0 ± 0.1 a	1.658	0.115
3rd instar	198	2.4 ± 0.1 a	235	2.1 ± 0.1 b	2.659	**0.015**
4th instar	188	2.8 ± 0.2 a	230	2.7 ± 0.1 a	0.232	0.819
5th instar	176	4.0 ± 0.2 a	217	4.7 ± 0.2 b	2.151	**0.045**
Egg - Adult	144	23.3 ± 0.9 a	189	22.8 ± 0.3 a	0.551	0.589
1st - Adult	144	14.5 ± 0.8 a	189	14.0 ± 0.2 a	1.196	0.247
Longevity (Females)	60	33.9 ± 2.8 a	48	27.2 ± 1.7 b	2.050	**0.049**
Longevity (Males)	78	38.2 ± 3.3 a	69	26.0 ± 2.3 b	3.014	**0.004**

Means followed by different letters in the row are significantly different at $P = 0.05$ (t-test; SPSS)

TABLE 2
Mortality rates of *C. bullita* immature stages feeding on either *O. aristatus* or *O. basilicum* leaves under laboratory conditions

Stage	Mortality (% ± SE)			
	O. aristatus	*O. basilicum*	*t*	*P*
Egg	7.7 ± 3.0a	9.0 ± 2.9a	0.309	0.761
1st instar	19.0 ± 3.1a	9.7 ± 1.9b	2.626	**0.017**
2nd instar	7.3 ± 1.3a	3.0 ± 0.8b	2.874	**0.010**
3rd instar	3.3 ± 1.0a	1.7 ± 0.6a	1.510	0.148
4th instar	4.0 ± 1.6a	4.3 ± 1.0a	0.005	0.960
5th instar	10.7 ± 2.8a	9.3 ± 1.8a	0.377	0.710
Egg - Adult	52.0 ± 4.9a	37.0 ± 2.9b	2.518	**0.022**

Means followed by different letters in the row are significantly different at $P = 0.05$ (t-test; SPSS)

Fecundity

The age-specific fecundity (m_x) and duration of oviposition varied correspondingly with the host plants they fed on (Fig.1 and Fig.2). The lace bugs feeding on *O. aristatus* began to oviposit 1 d earlier and this lasted 7 d later than those feeding on *O. basilicum*. Their fecundity peaked on the 9th and 11th day on *O. basilicum* and *O. aristatus*, respectively, after adult emergence. With a longer oviposition period, the lace bugs feeding on *O. aristatus* had a higher mean of fecundity than on *O. basilicum*. As shown in Table 3, the lace bugs had a significantly shorter pre-oviposition ($t_{38} = 2.404$; $P = 0.021$), longer oviposition period ($t_{38} =$

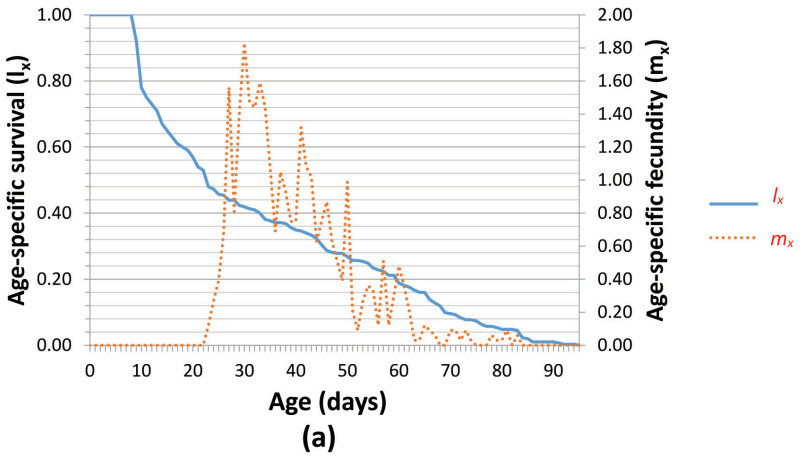

Fig.1 Daily age-specific survival (l_X) and fecundity (m_X) of female *C. bullita* feeding on *O. aristatus*

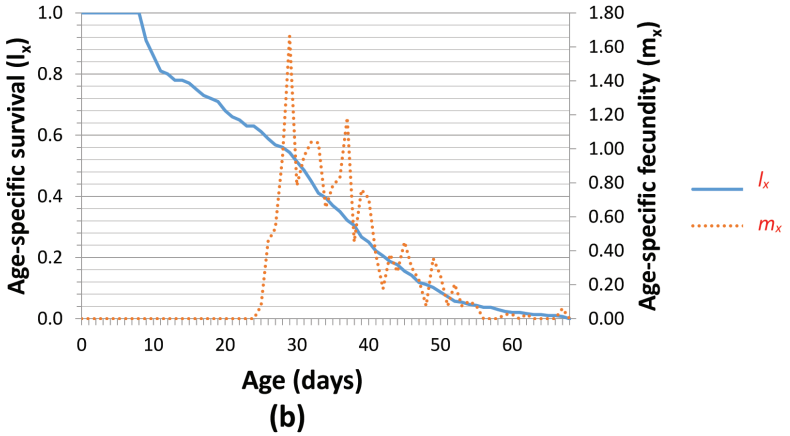

Fig.2: Daily age-specific survival (l_X) and fecundity (m_X) of female *C. bullita* feeding on *O. basilicum*

2.154; P = 0.0138) and higher fecundity (t_{38} = 3.090; P = 0.004) on *O. aristatus* than those on *O. basilicum*. Meanwhile, the male to female ratio on *O. aristatus* and *O. basilicum* was 2: 3 and 1:1, respectively.

Life Table Parameters

The population parameters are summarised in Table 4. The intrinsic rate of natural increase (r) of the lace bug on *O. aristatus* was 0.0667 per female per day and the daily finite rate of increase (λ) was 1.0690 female progenies per female per day, with a mean generation time (T_G) of 35.59 days. The population doubled its number in 10.39 d. The gross (GRR) and net (R_0) reproductive rates were 30.75 and 9.47, respectively. On the other hand, the gross (GRR) and net (R_0) reproductive rates of the bug on *O. basilicum*, were 16.41 and 6.03,

TABLE 3
Reproductive parameters of *C. bullita* feeding on *O. aristatus* and *O. basilicum*

Parameters	*O. aristatus*	*O. basilicum*	t	P
Pre-oviposition (days)	5.1 ± 0.4 a	6.3 ± 0.3 b	2.404	**0.021**
Oviposition (days)	24.3 ± 2.5 a	17.7 ± 1.8 b	2.154	**0.038**
Post-oviposition (days)	3.6 ± 0.8 a	2.5 ± 0.7 a	1.054	0.298
Fecundity (eggs)	51.3 ± 4.5 a	32.4 ± 4.1 b	3.090	**0.004**
Sex ratio (female, %)	59.7 ± 5.4 a	50.3 ± 5.3 a	1.250	0.227

Means followed by different letters in the row are significantly different at $P = 0.05$ (*t*-test; SPSS)

TABLE 4
Population parameters of *C. bullita* feeding on *O. aristatus* or *O. basilicum*

Parameter	*O. aristatus*	*O. basillcum*
Gross reproductive rate (*GRR*)	30.75 ± 2.6910 a	16.41 ± 2.0373 b
Net reproductive rate (R_0)	10.75 ± 6.84 a	6.03 ± 4.45 b
Mean generation time (T_G)	35.59 ± 11.22 a	26.92 ± 10.05 b
Intrinsic rate of increase (r)	0.0667 ± 0.0293 a	0.0541 ± 0.0227 b
Finite rate of increase (λ)	1.0690 ± 0.3208 a	1.0556 ± 0.3171 b
Doubling time (*DT*)	10.39 ± 5.24 a	12.80 ± 5.42 b

Means followed by different letters in a row are significantly different at $P = 0.05$ (*t*-test; SPSS)

respectively. The population doubled its number in 12.80 days. The intrinsic rate of natural increase (r) was 0.0541 per female per day, whereas the daily finite rate of increase (λ) was 1.0556 female progenies per female per day, with a mean generation time (T_G) of 26.92 days.

DISCUSSION

There are many factors influencing growth, longevity and reproduction of an insect in its natural environment. One of the factors that has been shown to greatly influence development of an insect is food quality (Ellers-Kirk & Fleischer, 2006). This factor was also found to have affected *C. bullita* feeding on two different host plants. Even though immatures of *C. bullita* feeding on both plants had a similar development time, they differed significantly in their survival and fecundity. The lace bugs feeding on *O. aristatus* had a lower survival rate in the early stages of development but with a longer longevity with a higher fecundity than those feeding on *O. basilicum*. These differences indicated that *O. aristatus* could be a nutritionally better host than *O. basilicum*. Such an effect has been widely reported to occur on many herbivorous insects (Awmack & Leather, 2002). Even though *O. aristatus* could be nutritionally better, the leaves and stems are phenotypically covered with relatively dense and long trichomes that could have physically hampered young

nymphs from accessing to the leaf surfaces and led to starving individuals. This physical defence mechanism by plants against insect herbivores occurs on wheat against bird cherry oat aphids (Roberts & Foster, 1983) and potatoes against potato leafhopper (Medetros *et al.*, 2005). Like those sap sucking insects, the surviving older lace bug nymphs were able to thrive well on the plant as their longer stylet could have easily reached the leaf surfaces to suck the sap.

Even though, the lace bugs in Malaysia were found to be able to develop and reproduce successfully on *O. basilicum*, their rates of development and reproduction are different from that of the lace bugs feeding on *O. basilicum* in Thailand (Tigvattnanont, 1989). The Malaysian lace bugs have a relatively shorter life span and less fecundity than those lace bugs attacking *O. basilicum* in Thailand. The reasons, apart from the varietal differences of the host plant, could be the climatic differences between the two countries. The warmer temperature of Malaysia, in comparison with the cooler temperature of Thailand, shortens the longevity and reduces the oviposition rate of the lace bugs. These differences are expected as insects usually have a shorter lifespan and less reproductive in the warmer temperature than those insects in the cooler subtropical regions (Chong *et al.*, 2003).

The present work is based on the traditional female age-specific life tables which focus only on the survival and the fecundity of the female population. Problem of stage overlapping throughout the age-specific life table might occur and cause inaccurate estimation. Besides, the improper manipulation of the survival and fecundity curves due to unknown sexes of immature are very likely to occur and lead to error in the derived parameters (Huang & Chi, 2012). Thus, improvisation could be done by performing the age-stage two-sex life tables (Chi 2008) in the future.

CONCLUSION

The lace bug *C. bullita* was found to thrive well on both host plants and has the potential to become a serious pest for the two widely distributed crops. With their shorter lifespan under a relatively warm and constant tropical climate, the insect may have overlapping generations that could contribute to its significance as a pest of *O. aristatus* and *O. basilicum*. The higher rate of increase on *O. aristatus* suggests that *O. aristatus* is as good as *O. basilicum*, or even more, favours the population growth of *C. bullita*. In this study, however, the cohort life table was constructed under a controlled environment that eliminated many natural variables which might have affected the parameters. Thus, the life table data obtained in this study were just an insight into the demographics of *C. bullita* populations to enable us discern patterns and make predictions about the changes of its populations in the future. The insect's ability to adapt into a wide geographical location with different climatic regimes indicates that the insect can be invasive to regions with prevailing host plants. Thus, fundamental knowledge on its biological characteristics is essential to develop a better

integrated pest management strategy against this particular pest.

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REFERENCES

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, *47*, 817 – 844.

Birch, L. C. (1948). The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology*, *17*, 15 – 26.

Bong, L. J., Neoh, K. B., Jaal, Z., & Lee, C. Y. (2012). Life table of *Paederus fuscipes* (Coleoptera: Staphylinidae). *Journal of Medical Entomology*, *49*, 451 – 460.

Carey, J. R. (1993). *Applied Demography for Biologists with Special Emphasis on Insects*. New York: Oxford University Press.

Chi, H. (2008). TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. Retrieved from http:/140.120.197.173/Ecology/.

Chong, J. H., Oettin, R. D., & Van Iersel, M. W. (2003). Temperature effects on the development, survival, and reproduction of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), on chrysanthemum. *Annals of the Entomological Society of America*, *96*, 539 – 543.

Chong, J. H., Roda, A. L., & Mannion, C. M. (2008). Life History of the mealybug, *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) at constant temperatures. *Environmental Entomology*, *37*(2), 323 – 332.

Ellers-Kirk, C., & Fleischer, S. J. (2006). Development and life table *of Acalymma vittatum* (Coleoptera: Chrysomelidae), a vector of *Erwinia tracheiphila* in cucurbits. *Environmental Entomology*, *35*, 875 – 880.

Greenberg, S. M., Sappington, T. W., Legaspi Jr, B. C., Liu, T.-X., & Sétamou, M. (2001). Feeding and life history of *Spodoptera exigua* (Lepidoptera: Noctuidae) on different host plants. *Annals of the Entomological Society of America*, *94*, 566 – 575.

Huang, Y. B., & Chi, H. (2012). Age-stage, two-sex life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. *Insect Science*, *19*, 263 – 273.

Legaspi, J. C., Simmons, A. M., & Legaspi Jr, B. C. (2008). Life history of *Delphastus catalinae*: A predator of *Bemisia tabaci*. *Journal of Insect Science*, *8*(4), 27.

Liu, S. S., & Meng, X. D. (1999). Modelling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures. *Bulletin of Entomological Research*, *89*, 53 – 63.

Livingstone, D., & Yacoob, M. H. S. (1987). A new species of *Parallelaptera* (Hymenoptera: Mymaridae) an egg parasitoid of Tingidae from Southern India. *Journal of Bombay Natural Historical Society*, *84*, 395 – 398.

Medeiros A. H., Delalibera, I., & Tingey, W. M. (2005). Aspects of potato leafhopper (Homoptera: Cicadellidae) biology on *Solanum berthaultii* and other potato genotypes. *Journal of Economic Entomology*, *98*, 1704 – 1709.

Peng, T. L., Sajap, A. S., Jeen, L. H., Lee, S. H., & Lum, W. C. (2013). Morphological re-description of *Cochlochila bullita* (Stål) (Heteroptera: Tingidae), a potential pest of *Orthosiphon aristatus* Blume Miq. (Lamiales: Lamiaceae) in Malaysia. *Pakistan Journal of Biological Sciences*, *16*(23), 1786 – 1790.

Razmjou J., Moharramipour, S., Fathipour, Y., & Mirhoseini, S. Z. (2006). Effect of cotton cultivar on performance of cotton aphid (Homoptera: Aphididae) in Iran. *Journal of Economic Entomology*, *99*, 1820 – 1825.

Roberts, J. J., & Foster, J. E. (1983). Effect of leaf pubescence in wheat on the bird cherry oat aphid (Homoptera: Aphididae). *Journal of Economic Entomology*, *76*, 1320-1322.

Sajap, A. S., & Peng, T. L. (2010). The lace bug *Cochlochila bullita* (Stål) (Heteroptera: Tingidae), a potential pest of *Orthosiphon stamineus* Bentham (Lamiales: Lamiaceae) in Malaysia. *Insecta Mundi*, *0136*, 1 – 5.

Speight, M. R., Hunter, M. D., & Watt, A. D. (1999). *Ecology of Insects*: *concepts and applications*. Oxford: Blackwell Science.

Southwood, T. R. E, & Henderson, P. A. (2000). *Ecological Methods* (3rd edition): Absolute population estimates using capture-recapture experiments. Oxford: Blackwell Science.

Tigvattnanont, S. (1989). Studies on the bionomics and local distribution of some lace bugs in Thailand: I. *Monanthia globulifera* (Heteroptera: Tingidae). *Khon Kaen Agriculture Journal*, *18*, 200 – 212.

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Glutathione Functions on Physiological Characters of Corn Plants to Enhance Mn-induced Corn Production

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ABSTRACT

A non-protein thiol, glutathione (GSH), presents abundantly in plant and affects the growth and development of the plants. In this study, the effects of N-acetyl cysteine (NAC), a precursor of GSH, on manganese (Mn)-induced corn production was evaluated. Different Mn concentrations (0.2, 1.5 and 3.0 ppm of Mn), with or without 100 µM of NAC, were arranged as completely randomised design with 5 replicates. Results show that both NAC and Mn affected plant height and leaf numbers. Treatment of NAC increased Mn-induced relative water content (RWC), photosynthesis (Pn) and photosynthetically active radiation (PAR) in leaves of corn plants. In the Mn-treated plants, chlorophyll (Chl) content, Chl fluorescence (Fm) and quantum yield (Fv/FM) were found significantly higher than the Mn-untreated plants. In addition, corn plants showed improved yield and cob length in NAC-treated plants in the presence of Mn. Thus, this study suggests that NAC might improve some physiological functions of plants to enhance Mn-induced corn production, with 1.5 ppm of Mn showed the best results.

Keywords: Manganese, relative water content, photosynthesis, chlorophyll content and chlorophyll fluorescence

INTRODUCTION

Corn (*Zea mays* L.) is the world's leading cereal grain used as a food source for both humans and animals. Previous studies stated that the application of micronutrient fertilisers to micronutrient-deficient soils improved the yield and crop quality for cereals, corn, beans, forages and oil seeds (Malakouti, 2007). Meanwhile, the

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micronutrients' deficiency reduces plants' performance and profitability (Fisher, 2008) and therefore, micronutrients play an important role in controlling plant growth although plants need a proper balance and low quantity to optimise yield.

Manganese (Mn), which is one of the plant's micronutrients, is involved in plant's metabolic functions such as respiration, photosynthesis, amino acid synthesis and hormone activation through Indole Acetic Acid (IAA)-oxidases (Lido *et al.,* 2004; Ducic & Polle, 2005). It is important that Mn works as a co-factor for most antioxidant enzymes (Cakmak *et al.,* 1999). Excessive Mn concentration in plant tissue affects enzyme activity, absorption, translocation and oxidative stress (Ducic & Polle, 2005; Lei *et al.,* 2007).

On the other hand, glutathione (GSH) is known as the antioxidant that prevents the damage of cell caused by free radicals and peroxides. In plants, GSH is crucial for biotic and abiotic stress management, cellular defence and sulphur metabolism (Noctor *et al.,* 2002). Glutathione functions as antioxidant, stomatal aperture regulator and also direct electron donor to peroxide in reaction that is catalysed by glutathione peroxidase (Murphy & Zarini, 2002; Jahan *et al.,* 2011). These features make glutathione as a multifunctional component in plant (Jahan *et al.,* 2008, 2014a). A recent study stated that NAC improves physiological function and yield of rice plants (Nozulaidi *et al.,* 2015) and seed production in *Arabidopsis* plants (Jahan *et al.,* 2014a).

Mn can change plant growth, chlorophyll content and antioxidant activity, whereby an excess of Mn can reduce root and shoot elongation in *V. faba* plant (Fecht-Christoffers *et al.,* 2003), which are related to photosynthesis production (Henriques, 2003). Rahmati *et al.* (2004) reported that superoxide dismutase and catalase activity increased in Mn-treated cells. In addition, excess of Mn increased reactive oxygen species like superoxide radical, hydrogen peroxide and hydroxyl radical (Demirevska-Kepova *et al.,* 2004; Boojar & Goodarzi, 2008). However, the synthesis of non-enzymatic antioxidants like glutathione (GSH) can protect the plant cell from the harmful effect of ROS (Arora *et al.,* 2002).

N-acetyl cysteine (NAC) improves GSH content in the cells of leaf (Jahan *et al.,* 2014a). To date, no research has been conducted on the effects of NAC on Mn-induced corn production. Therefore, the focus of this study was to justify whether NAC induced physiological parameters of corn plants to enhance Mn-induced corn production. We showed that GSH increased Mn-induced corn production.

METHODOLOGY

Plant Material and Experimental Design

In this study, a hybrid corn variety of L41 was undertaken as the test crop. Two seeds were planted onto seedbed in a hole with a spacing size of 25cm X 75cm. Eight treatments with 5 replicates were arranged as completely randomised design. Four Mn concentrations (0, 0.2, 1.5 and 3.0 ppm of

Mn) were applied with or without NAC (0 and 100 µM) as foliar applications in between 10 am to 12 pm.

Soil and Agronomic Practices

Soil was sandy in texture with 87.2% sand, 7.25% silt and 5.50% clay, soil pH of 5.1, total organic matter of 1.02%. Meanwhile, the Sprinkler irrigation method was used to apply water. Manual weeding method was also applied. However, insecticide was not applied.

Determination of Plant Height and Leaf Number

The plant height was measured from the soil surface to the longest leaf emerged from the whorl by using a measuring tape. In addition, leaf numbers were also counted.

Yield and Yield Parameters

Length and weight of cob were determined after harvest. The length of corn cob was measured with a measuring scale and weigh was also recorded for each treatment.

Determination of Relative Water Content

Fresh weight of leaf (FW) was measured just after collection from the plants before taking turgid weight (TW) of the leaf and after obtaining a full turgidity. Then, the leaves were dried at 60°C for 24 h in an oven, followed by measuring leaf dry weight (DW). Relative water content was determined as previously described by Chelah *et al.* (2011) and Jahan *et al.* (2013). Relative Water Content (%) = [(FW-DW) / (TW-DW)] x 100

FW – Sample fresh weight
TW – Sample turgid weight
DW – Sample dry weight

Determination of Chlorophyll Content in Leaves

A chlorophyll determining meter SPAD-502 (Minolta, Japan) was used to acquire a rapid *in-situ* estimation of chlorophyll (Chl) content from the leaf of corn plants (Khairi *et al.,* 2015a, 2015b). Meanwhile, the second uppermost collared-leaf was used to measure the Chl content. Data were taken in between 11 am to 1 pm to avoid wetness effects on the leaf surface (Jahan *et al.* 2014b). A total of five replicates were implemented.

Determination of Chlorophyll Fluorescence Parameters

A portable Chl fluorescence monitoring meter of Junior-PAM (Walz, Germany) was used to quantify chlorophyll fluorescence in the leaves of corn plants (Jahan *et al.,* 2014a; Khairi *et al.,* 2015b). The second uppermost collared-leaf was selected and data were taken in between 11 am to 1 pm. The maximum fluorescence level (Fm) and quantum yields in PS II photochemistry (Fv/Fm) were recorded.

Determination of Net Photosynthesis Rate and Photosysthetically Active Radiation

A CI-340 portable photosynthesis meter (CID Biosciences, Inc.) was used to measure net photosynthesis rate (Pn) according to Munirah *et al.* (2015). A quantum sensor in the measuring cell was attached to determine photosysthetically active radiation (PAR)

data together with Pn data. Data taking procedures were according to the manual. Five replicates were implemented.

Statistical Analysis

Data were analysed for the differences in the mean value among the treatments by using ANOVA procedure and LSD and T-test using Minitab-16 and MS Excel software. Differences at $P < 0.05$ are considered as significant.

RESULTS

Effects of the Mn concentrations on plant's height and number of leaves in presence of NAC

The effect of NAC on plant height was found to be significant (Fig.1a). Results indicated that under no NAC, the plant height increased with increasing Mn concentration, whereas under NAC, plant height increased until 0.2 ppm Mn, followed by plateau. However, plant height was greater at low concentrations of Mn when Mn was applied under no NAC and at high concentration of Mn, and there was no influence on plant height both with and without NAC. This result suggests that NAC increases plant height. The results further indicated that leaf number per plant increased with the increasing Mn concentration under no NAC, whereas leaf number increased with the increasing Mn concentration till 1.5 ppm, followed by a decline under NAC. However, leaf number was higher in the NAC-treated plants than the NAC-untreated plants. Taken together, these results suggest that NAC might affect plant's morphological parameters.

Effects of NAC on Mn-induced RWC and Net Photosynthesis Rate

The effect of NAC on RWC was significant (Fig.2a). Results showed that RWC was greater in NAC treated plants than those of NAC-untreated plants. Under no NAC, RWC increased with the increasing Mn concentration, whereas with NAC, the RWC was higher in Mn applied plant

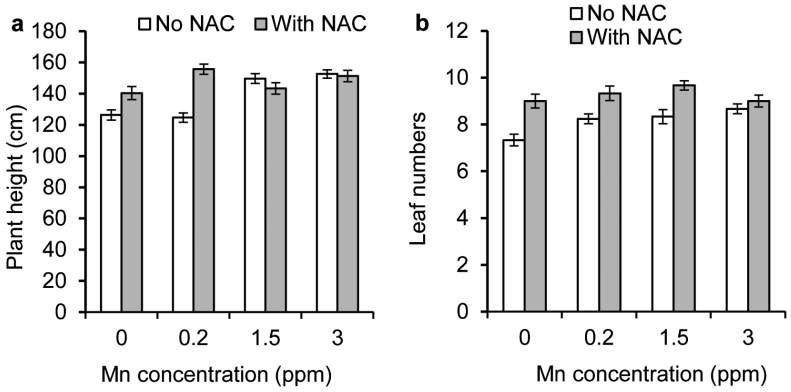

Fig.1: Effects of NAC on Mn-induced plant height (a) and leaf numbers (b) of corn plants.

than the control. In addition, there was no significant difference in RWC amongst the Mn concentrations (Fig.2a; closed bars). This result suggests that NAC might increase RWC in the leaves of corn plants.

Net Pn rate and PAR in the leaves of corn plants were determined under different Mn conditions with or without NAC existence (Fig.2b). Manganese increased Pn rate in the leaves of corn plants than that of Mn-untreated plants (Fig.2b; closed round). In addition, a similar effect of NAC on Pn rate in leaves was observed (Fig.2b; open round). NAC treatment, on the other hand, significantly increased Pn rate, regardless of the Mn treatment. We also measured photosynthetically active radiation which supports Pn data that Pn and PAR might interdependent (Fig.2b; bar graph). This result suggests that NAC enhances Pn rate and PAR in the leaves of corn plants irrespective of the Mn treatment.

Effects of NAC on Mn-induced Chlorophyll Content and Chlorophyll Florescence

The Chl content in the leaves of corn plants was estimated to justify if NAC application affected Mn-induced Chl content in the leaves. Mn-treated plants accumulated higher Chl content than that of the Mn-untreated plants (Fig.3a; open bars). In addition, the Chl contents in the leaves of different Mn-treated plants were similar. In contrast, NAC treatment increased the Chl content in the Mn-untreated plant but not in the Mn-treated plants except 3 ppm of Mn condition (Fig.3a; closed bars), under which the Chl content increased significantly. The chlorophyll fluorescence data (Fig.3b) and quantum yield in photosystem II (Fig.3c) were shown comparable to the chlorophyll content data. This result implies that NAC affects light related reaction in plants which further infers that NAC functions on light-dependent energy production in plants

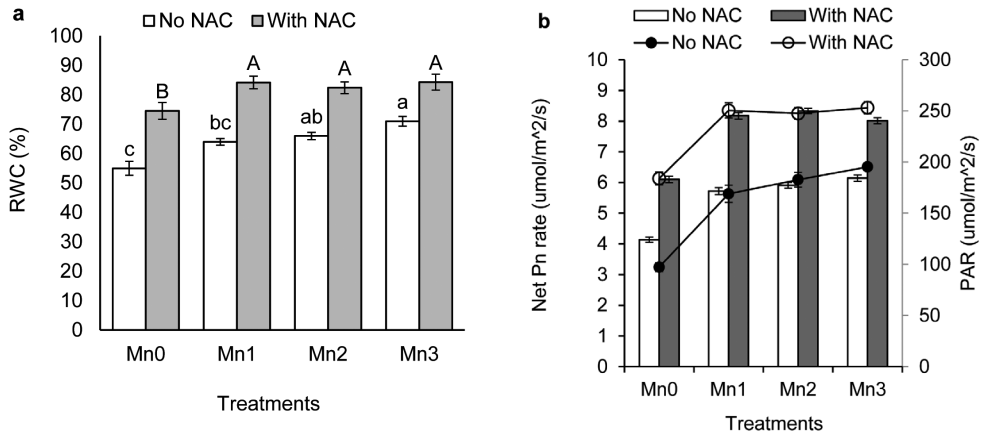

Fig.2: Effects of NAC on different concentrations of Mn-induced RWC (a) and photosynthesis rate (b) in the leaves of corn plants.

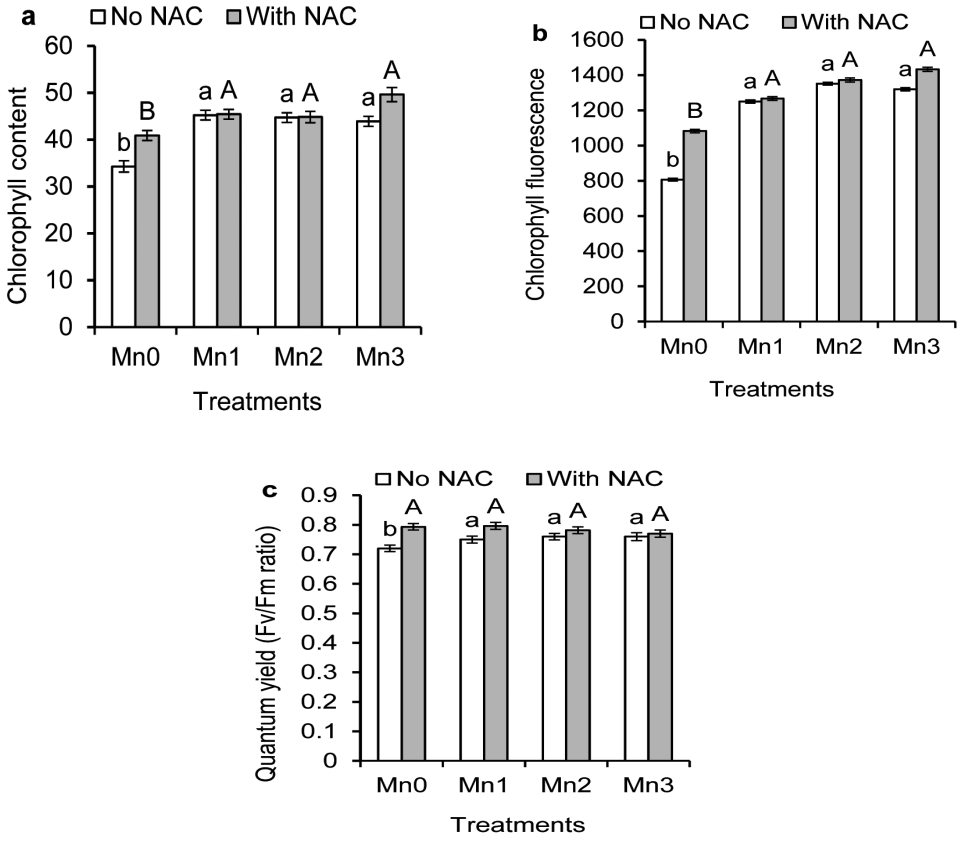

Fig.3:Effects of NAC on Mn-induced Chl content (a), Chl florescence (b), and quantum yield (c) in the leaves of corn plants.

during photosynthesis. Taken together, these results support that NAC might increase Chl content in the leaves of corn plants but the mechanism in this phenomenon is still unknown.

Effects of NAC on Mn-induced Yield of Corn Plants

Results indicated that grain yield was greater in the NAC applied plants than non-NAC applied plant under any doses of Mn (Fig.4a). The results further revealed that under NAC, grain yield increased with the increasing Mn concentration till 1.5 ppm, followed by a decline; under no NAC, however, the grain yield increased with the increasing Mn concentration. In case of the length of corn cob, Mn-treated plants showed longer cob than that of the Mn-untreated plants (Fig.4b; open bars). Additionally, the presence of NAC hastens the size of cob (Fig.4b; closed bars). This result suggests that NAC treatment might increase corn production regardless of Mn application.

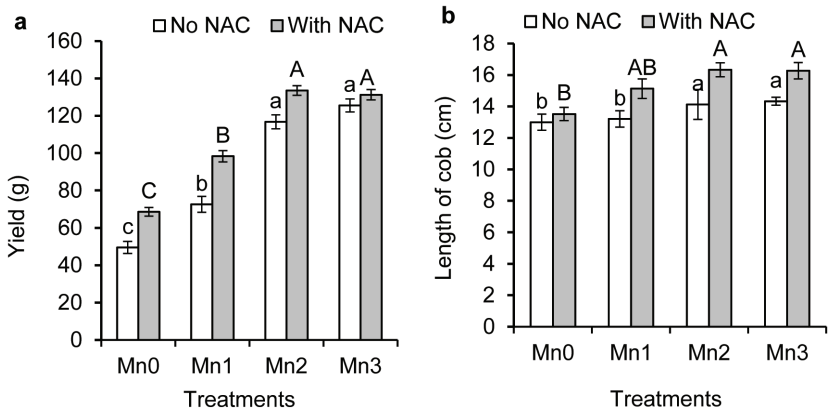

Fig.4: Effects of NAC on Mn-induced yield (a) and cob length (b) of corn plants

DISCUSSION

Glutathione functions on sulphur metabolism, growth, development, cell defense and redox signalling. In specific, GSH scavenges ROS and detoxify toxic chemicals (Noctor & Foyer, 1998; Blum *et al*., 2007). Ascorbate-GSH cycle regulates glutathione level (Halliwell & Asada pathway), and it is likely that alteration of GSH contents may result in adjustment of plant's metabolic function, as well as plant's growth and development (Jahan *et al*., 2008, 2011; Nozulaidi *et al*., 2015). This current study reveals that NAC application increased corn yield through regulation of Mn-induced physiological parameters of corn plants.

Manganese and NAC treatments increased plant height and leaf number (Fig.1), whereby it was observed that plant's height and leaf numbers are regulated by GSH and Mn. A previous study stated that NAC application improved plant physiological parameters of Arabidopsis plants (Jahan *et al*., 2014a) and rice plants (Nozulaidi *et al*., 2015). This may be due to the effects of increasing GSH content in leaves (Jahan *et al*., 2014a) and GSH derivatives synthesised phytochelatins (Rea *et al*., 2004) with micronutrients, including Mn which improves plants' physiological parameters.

In addition, increment of RWC and photosynthetic parameters (Fig.2) implied that Mn might modulate plants' physiological parameters, whereas NAC might enhance Mn-induced enzymatic activity. Chlorophyll, which is a green pigment common to all photosynthetic cells, absorbs light for phosphorylation process and transfers electrons from photosystem II into the photosystem I (Purves *et al*., 1997), suggesting that Chl content increased in the NAC-treated plants (Fig.3a). During the photosynthesis process, light energy uses chloroplastic Chl content and changes it into chemical energy which is stored as sugar bond (Barber, 2006). In this study, Fm values were up in the Mn-treated and

NAC-treated plants than the Mn- and NAC-untreated plants, involving that GSH induced by NAC treatment, might cause energy transfer from PSII to PSI (Jahan *et al*., 2014a) and control physiological parameters (Fig.2). GSH biosynthesis regulates rosette leaves production (Ogawa *et al*., 2004) and flowering time in *ch1-1* Arabidopsis mutants (Jahan *et al*., 2014a). Plant growth and flowering are influenced by nutrient availability, temperature and light intensity (Bernier *et al*., 1993). In addition, these results suggest that NAC treatment might regulate plant physiological parameters, the finding which is supported by Jahan *et al*. (2014a). In this context, this study has shown that NAC controlled the Chl content and Chl fluorescence in the leaves of corn plants (Fig.3). Jiang *et al*. (2010) showed that glutathione modulates the development of Arabidopsis plants, which is similar to the finding of Jahan *et al*. (2014a) that the deficient in GSH content reduced leaf development and plant growth. These results support this study.

Cellular GSH have important consequences in the cell through modification of metabolic functions associated with GSH-regulated functions (Noctor *et al*., 2002). Glutathione can enzymatically and non-enzymatically react in cells (Hwang & Lee, 2006). In more specific, GSH deficient in plants reduces water movement in plants (Jahan *et al*., 2014a), which indicates that NAC may enhance water absorption from soil and induce RWC in leaf (Fig.2a). In addition, Mn application also induces RWC, which may be due to some enzyme activities. Therefore, there may be a positive effect that NAC modulates some extent of physiological functions in plants in the presence of Mn application so that the yield and cob weight increased in Mn- and NAC-treated plants (Fig.4). Taken together, 1.5 ppm of Mn showed the best results in regards to NAC application.

In conclusion, NAC might regulate corn yield through enhancing physiological functions and the functional activity of some enzymes in plants which are to be elucidated in the future research. Application of NAC with Mn as a foliar spray would be benefited the farmers by achieving higher yields.

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REFERENCES

Arora, A., Sairam, R. K., & Srivastava, G. C. (2002). Oxidative stress and antioxidative system in plants. *Current Science, 82*, 1227-1238.

Barber, J. (2006). Photosystem II: an enzyme of global significance. *Biochemical Society Transactions, 34,* 619–631.

Bernier, G., Havelange, A., Houssa, C., Petitjean, A., & Lejeune, P. (1993). Physiological signals that induce flowering. *Plant Cell, 5*, 1147–1155.

Blum, R., Beck, A., Korte, A., Stengel, A., Letzel, T., Lendzian, K., & Grill, E. (2007). Function of phytochelatin synthase in catabolism of glutathione-conjugates. *Plant Journal, 49,* 740-749.

Boojar, M. M. A., & Goodarzi, F. (2008). Comparative evaluation of oxidative stress status and manganese availability in plants growing on manganese mine. *Ecotoxicology and Environmental Safety, 71*, 692-699.

Cakmak, I., & Engels, C. (1999). Role of Mineral Nutrients in Photosynthesis and Yield Formation. In Z. Rengel (Ed.), *Mineral Nutrition of Crops* (pp. 141–168). New York,NY, USA: Haworth Press.

Chelah, M. K. B., Nordin, M. N. B., Musliania, M. I., Khanif, Y. M., & Jahan, M. S. (2011). Composting Increases BRIS Soil Health and Sustains Rice Production on BRIS Soil. *Scienceasia, 37*, 291-295.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R., & Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environmental and Experimental Botany, 52*, 253-266.

Ducic, T., & Polle, A. (2005). Transport and detoxification of manganese and copper in plants. *Brazilian Journal of Plant Physiology, 17*, 103-112.

Fecht-Christoffers, M.M., Maier, P., & Horst, W.J. (2003). Apoplastic peroxidases and ascorbate are involved in manganese toxicity and tolerance of Vigna unguiculata. *Physiologia Plantarum, 117*, 237-244.

Fisher, G. E. J. (2008). Micronutrients and animal nutrition and the link between the application of micronutrients to crops and animal health. *Turkish Journal of Agriculture and Forestry, 32*, 221-233.

Henriques, F. S. (2003). Gas exchange, chlorophyll fluorescence kinetics and lipid peroxidation of pecan leaves with varying manganese concentrations. *Plant Science, 65,* 239-244.

Hwang, E. S., & Lee, H. J. (2006). Induction of quinine reductase by allylisothiocyanate (AITC) and the N – acetylecysteine conjugate of AITC in Hepalc1c7 mouse hepatoma cells. *Biofactors, 26*, 7-15.

Jahan, M. S., Ogawa, K., Nakamura, Y., Shimoishi, Y., Mori, I. C., & Murata, Y. (2008). Deficient glutathione in guard cells facilitates abscisic acid-induced stomatal closure but does not affect light-induced stomatal opening. *Bioscience Biotechnology Biochemistry, 72*, 2795–2798.

Jahan, M.S., Nakamura, Y., & Murata, Y. (2011). Histochemical Quantification of GSH Contents in Guard Cells of Arabidopsis Thaliana. *Scienceasia, 37,* 291–295.

Jahan, M. S., Nozulaidi, M. B. N., Khairi, M. B. C. L., & Khanif, Y. M. (2013). Effects of water stress on rice production: bioavailability of potassium in soil. *Journal of Stress Physiology and Biochemistry, 9*, 97-107.

Jahan, M. S., Nozulaidi, M. B. N., Moneruzzaman, M. K., Ainun, A., & Husna, N. (2014a). Control of plant growth and water loss by a lack of light-harvesting complexes in photosystem-II in Arabidopsis thaliana ch1-1 mutant. *Acta Physiologia Plantarum, 36*, 1627-1635.

Jahan, M. S., Muslianie, I., & Khandaker, M. M. (2014b). Effects of Soil Amendments on BRIS Soil Health, Crop Physiology and Production. *International Journal of Research and Innovations in Earth Science, 1*, 1-4.

Jiang, H. W., Liu, M. J., Chen, I. C., Huang, C. H., Chao, L. Y., & Hsieh, H. L. (2010). A glutathione S-transferase regulated by light and hormones participates in the modulation of Arabidopsis seedling development. *Plant Physiology, 154*, 1646–1658.

Khairi, M., Nozulaidi, M., Afifah A., & Jahan, M.S. (2015a). Effect of various water regimes on rice

production in lowland irrigation. *Australian Journal of Crop Science, 9*,153-59.

Khairi, M., Nozulaidi M., & Jahan, M. S. (2015b). Effects of Different Water Levels on Physiology and Yield of Salinity Rice Variety. *Australian Journal of Basic and Applied Sciences, 9*, 339-345.

Lei, Y., Korpelainen, H., & Li, C. (2007). Physiological and biochemical responses to high Manganese concentrations in two contrasting Populus cathayana populations. *Chemosphere, 68*, 686-694.

Lido, F.C., Barreiro, M., & Ramalho, J. (2004). Manganese accumulation in rice: Implications for photosynthetic functioning. *Journal of Plant Physiology, 161*, 1235-1244.

Malakouti, M. J. (2007). Zinc is a neglected element in the life cycle of plants: A review. Middle East. *Russian Journal of Plant Science and Biotechnology, 1*, 1-12.

Munirah, N., Khairi, M., Nozulaidi, M., Khandaker, M. M., Mat N. & Jahan, M. S. (2015). The Effects of Zinc Application on Physiology and Production of Corn Plants. *Australian Journal of Basic and Applied Sciences, 9*, 339-345.

Murphy, R. C., & Zarini, S. (2002). Glutathione adducts of oxyeicosanoids. *Prostaglandins Other Lipid Mediators, 69*, 471–82.

Noctor, G., & Foyer, C. H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology, 49*, 249-279.

Noctor, G., Gomez, L., Decker, H., & Foyer, C. H. (2002). Interaction between biosynthesis, compartmentation and Transport in the Control of Glutathione Homeostasis and Signalling. *Journal of Experimental Botany, 53*, 1283-1304.

Nozulaidi, N., Jahan, M. S., Khairi, M., Khandaker, M. M., Nashriyah, M., & Khanif, Y. M. (2015). N-acetylcysteine increased rice yield. *Turkish Journal of Agriculture and Forestry, 39*, 204-211.

Ogawa, K., Hatano-Iwasaki, A., Yanagida, M., & Iwabuchi, M. (2004). Level of glutathione is regulated by ATP-dependent ligation of glutamate and cysteine through in Arabidopsis thaliana: Mechanism of strong interaction of light intensity with flowering. *Plant Cell Physiology, 45*, 1–8.

Purves, W. K., Orians, G. H., Heller, H. C., & Sadava, D. (1997). *Life-Tire Science of Biology (5th Edition)*. Sinaur Associates, Inc., pp. 165–188.

Rahmati, M., Yazdani, M., & Ghanati, F. (2004). *Effects of excess amount of Mn on activation of certain enzymes of antioxidant system in suspension-cultured tea cells*. The 2nd Congress on Applied Biology (International Approach). 29-30 Sep. 2004, Mashhad, Iran.

Rea, P. A., Vatamaniuk, O. K., & Rigden, D. J. (2004). Weeds, worms, and more. Papain's long-lost cousin, phytochelatin synthase. *Plant Physiology, 136*, 2463–2474.

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Colonisation of Dung Beetles (Coleoptera: Scarabaeidae) of Smaller Body Size in the Bangi Forest Reserve, Selangor, Malaysia: A Model Sampling Site for a Secondary Forest Area

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ABSTRACT

The diversity of dung beetles (Coleoptera: Scarabaeidae) was measured at the Bangi Forest Reserve in Selangor, Malaysia (Hutan Simpan Bangi, HSB), as a model sampling site for the secondary forest ecosystem. The diversity analysis gave a value of 2.17 for the Shannon diversity index (H'), 1.42 for the richness index (R') and 0.87 for the evenness index (E). A total of 575 individuals belonging to 10 species of dung beetles were collected. They comprised of *Catharsius renaudpauliani*, *Catharsius* sp. 1, *Microcopris* aff. *hidakai*, *Onthophagus* "*obscurior* group", *Onthophagus crassicollis*, *O. recticornutus, O. rutilans*, *O. trituber, Paragymnopleurus maurus* and *Sisyphus thoracicus.* The small dung beetle *Onthophagus crassicollis* had the highest number of individuals (137/575, 23.83%) with a body size range of 4.5 ± 2.5 mm in length. A total of 9/10 species collected in HSB were classified as small-bodied species (8% large body, 92% small body) and the statistical analysis showed a significant body size difference compared with the large-bodied species, *C. renaudpauliani*. *O. crassicollis* showed the highest abundance in the secondary forest, a model site for studying forest disturbance. The abundance of dung beetles could potentially be used as a good bioindicator of habitat disruption in the tropical forest ecosystem. Our study also highlighted that the abundance of species based on body size was affected by the availability of the food sources also from different sizes of mammal dung.

Keywords: Scarab beetles, biodiversity, bioindicator, Shannon diversity index

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INTRODUCTION

Dung beetles (Coleoptera: Scarabaeidae) belong to one of the insect groups that are well-studied because they play multiple

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roles in the ecosystem (Gardener *et al.,* 2008; Nichols *et al.,* 2008). For example, they are involved in nutrient recycling (Yokoyama & Kai, 1993; Vitousek *et al.,* 1997; Bang *et al.,* 2005), secondary dispersal of seeds (Andresen & Feer 2005; D'hondt *et al.,* 2008), biological control of pest fly species (Ridsdill-Smith & Hayles, 1990; Byford *et al.,* 1992; Bishop *et al.,* 2005) and indirectly helping plant growth by providing soil nutrients (Behling *et al.,* 2000; Bang *et al.,* 2005). One of iconic behaviours of dung beetles is food relocating behaviour that belongs to the roller group that makes a ball-shaped sphere from its food and rolls it to other places (others are tunnellers and dwellers) of dung beetles.

Dung beetles are diverse, abundant and widely distributed in the natural ecosystems of tropical rainforests (Hanski & Camberfort, 1991). Several studies have been conducted to measure the species' composition, population structure, as well as dynamics of dung beetles in the humid tropical rainforests (Hanski & Camberfort, 1991; Andresen, 2002; 2003; 2008; Shahabuddin *et al.,* 2005; 2010; Shahabuddin, 2013). In tropical and temperate forests, a 1.5 kg dung pat can attract up to 16,000 dung beetles (Hanski & Camberfort, 1991), which take only about two hours to finish consuming all the dung (Anderson & Coe, 1974). However, tropical rainforest dung beetles are very sensitive to forest changes that could significantly alter the habitat such as vegetation structure, microclimate, soil and food sources (Shahabuddin *et al.,* 2005; Spector, 2006). Furthermore, the abundance of dung beetles could also be influenced by other factors such as temperature, solar radiation and rainfall (Fincher *et al*., 1970). Gardner *et al*. (2008) classified dung beetles as cost-effective to be used in measuring forest condition, which meet both efficiency and low cost sampling consumption. Therefore, dung beetles are well-known as good bioindicators for measuring forest disturbance (Gardner *et al.,* 2008).

Forest disturbance and landscape modification generally reduce the diversity and abundance of most insect taxa (Lawton *et al.,* 1998), except for several species such as solitary bees and wasps, which are able to react and adapt to the extreme conditions or disturbance of an area (Klein *et al.,* 2002). According to Camberfort (1991), a reduction in the number of large mammals occurred parallel to a reduction of dung beetles, influenced by the food source for these scarab species.

A study by Lee *et al*. (2009) indicated that environmental factors influence the abundance, diversity and body mass of the dung beetle, whereas more disturbed area would give less in every aspect. A high number of dung beetles successfully collected from a forest area, however, would not necessarily indicate that it had a greater abundance and diversity compared to other ecosystems; rather, this might be due to and depend generally on food availability. A study conducted by Shahabuddin *et al.* (2005) in Sulawesi confirmed that the abundance and diversity of dung beetles were affected by human-induced activities. Results of their study also showed that the

dung beetle populations in the study forest were significantly different from those in areas that lacked forest mammals. Dung beetles have been the subjects of much study due to their important functions and roles in the ecosystem as great manure decomposers, deterring nitrogen release into the atmosphere (Yokoyama *et al.,* 1991; Yokoyama & Kai, 1993). The presence of scarabs ensures the recycling of carbon and minerals back into the soil to generate humus for plant growth.

Other than measuring the diversity of the dung beetles to evaluate forest disturbance, body size also reflects the same role as diversity. More disturbed forest seems to have smaller sized dung beetles due to less dung amounts produced from the mammals to sustain larger dung beetles (Halffter & Arellano, 2002). Meanwhile, competition among dung beetles also can be one of the factors contributing to small dung beetles because of the competition to get the limited sources. Thus, a disturbed forest may show high diversity but give smaller body size (Filgueiras *et al*., 2011). The main objectives of our study were to measure the diversity and body size of dung beetles in the Bangi Forest Reserve in Selangor, Malaysia, and generate fundamental specific data focusing on the dung beetle as a bioindicator in measuring ecosystem disturbance in the humid tropics by taking into account the body size of dung beetles species.

MATERIALS AND METHODS

Sampling Sites

The study area was Hutan Simpan Bangi (HSB) that is located within the main campus of Universiti Kebangsaan Malaysia (UKM) in Bangi, Selangor, covering an area of approximately 100 ha, with a highest peak at 105 m above sea level (UKM, 2011). The historical significance of this reserved forest is that it is the site of the official launching of the UKM permanent campus developed in the early 1970s. HSB experienced a series of deforestations from 1945 to 1968 (Noraini *et al*., 1990; Mat Salleh, 1999) due to further expansion of the UKM campus, as well as rapid development of the adjacent land mainly for settlement and industrial and urban centres. Thus, the original Bangi Forest Reserves became fragmented into two small forest patches, namely the Hutan Penyelidikan Alam (HPA) and the Bukit Rupa (UKM 2011), which are the two sites selected for this study. Their coordinates are 101° 47.216" E;02° 54.836" N for HPA and 101° 45.969" E; 02° 55.016" N for Bukit Rupa. Although this forest is just reaching its maturity after 30 years since the last major forest clearings, the HSB is still continuously being disturbed either for small-scale development or for research purposes. As a result, there are very few large emergent trees left standing in this forest, which affect forest litter and the associated forest floor and soil fauna composition.

Collection of Dung Beetles

Dung beetles were sampled four times in October and November 2013 by means of six baited pitfall traps. A small plastic pail (20 cm diameter, 17 cm deep) was used as the pitfall trap, buried into the ground up to its upper rim. About 10–15 g of fresh cow dung (less than 6-hrs old) was put into a plastic cup, which was then placed into the pail. A mixture of water and detergent (1000:1 ml) was then poured into the pail to drown any beetles that fell into the trap. All traps were arranged at 4.0 –5.0m intervals in a straight line transect, and they were left for 24 hr before the beetles were collected. The beetles were preserved in 70% alcohol and taken to the laboratory for sorting and taxonomic identification.

Species Identification and Body Size

The beetles were identified to the subfamily level in the laboratory based on morphological characteristics using available keys (Triplehorn & Johnson, 2005; Ek-Amnuay, 2008). They were then further identified into the species (Ochi *et al.,* 1996) and morphospecies levels for diversity analysis. For each species, the length (from tip of clypeus to apex of elytra) of 10 individuals was measured with a pair of Vernier calipers (accuracy 0.05 mm) for body size analysis. Photographic records of each species were taken with a Canon EOS 6D camera mounted on a stereo microscope (model Zeiss Stemi SV11).

Data Analysis

In order to evaluate the sampling efforts for each sampling activity, sample-based rarefaction curves were constructed by using the EcoSim version 7.0 computer software. The Shannon diversity index (H') and the evenness index (E) were determined for the dung beetle community (Hanski, 1983; Klein, 1989) in this study area. The richness index (R') was also calculated to determine the expected number of species that could be found if the sampling efforts were to be increased. However, it is also related to the sample-based rarefaction curve analysis. All of the indices (H', R', and E) were calculated by using the PAST software (Hammer *et al.,* 2001).

RESULTS

Species Composition, Diversity and Body Size

Of the total 575 beetles collected at Hutan SimpanBangi (HSB), they were classified into ten species in five genera, and comprised of *Catharsius renaudpauliani*, *Catharsius* sp. 1, *Microcopris* aff. *hidakai*, *Onthophagus* "*obscurior* group", *Onthophagus crassicollis*, *O. recticornutus, O. ru*tilans, *O. trituber*, *Paragymnopleurus maurus* and *Sisyphus thoracicus*. The genus *Onthophagus* was the most abundant at 63% (362 individuals), followed by *O. crassicollis* about 24% (137 individuals) (Fig.2 – Fig.11). The least frequently collected species was *O. recticornutus*, comprising only 25 individuals (4.3%)

(Table 1). The diversity analyses showed that the Shannon diversity (H'), evenness (E), and richness (R') indices were 2.17, 0.87 and 1.42, respectively (Table 1). The rarefaction curve (sampling effort) showed that the number of individuals from each collection was sufficient to conduct the analysis for diversity estimation of dung beetles in the HSB.

TABLE 1
List of species and number of individuals collected from Bangi Forest Reserve (HSB)

Species	HSB
Catharsius renaudpauliani	46
Catharsius sp. 1	34
Microcopris aff. *hidakai*	46
Onthophagus "*obscurior* group"	96
Onthophagus crassicollis	137
Onthophagus recticornutus	25
Onthophagus rutilans	52
Onthophagus trituber	52
Paragymnopleurus maurus	47
Sisyphus thoracicus	40
Total	575
(t= 5.535, df= 122.58, p=1.7798, P > 0.05)	
Shannon (H')	2.17
Richness (R')	1.42
Evenness (E)	0.87

The richness (R') and evenness (E) indices calculated for HSB were 1.42 and 0.87, respectively (Table 1). The size of the dung beetles that were sampled in the HSB was classified into large-sized and small-sized (Fig.1). Out of the 10 species collected from HSB, only *C. renaudpauliani* is considered a large-sized with an average body length of 27.5 mm (Table 2). *Catharsius* sp., *M.* aff. *hidakai* and *P. maurus*, with an average length of 10.5 mm (i.e., almost half the size of *C. renaudpauliani*) is considered as a group that is close to *C. renaudpauliani* in term of their body-size. The smallest dung beetle from HSB was *O. crassicollis* (average size of 5.5 mm). The average body length of *C. renaudpauliani* does not overlap with other species found in HSB, showing obvious differences between the large and small groups of dung beetles in HSB (Fig.1). With a total of 46 individuals, *C. renaudpauliani* represented 8% of the dung beetles collected in HSB.

TABLE 2
Body size range of each species collected from the Bangi Reserve Forest (HSB)

HSB	Size (mm)
Catharsius renaudpauliani	27.5 ± 6.0
Catharsius sp. 1	10.5 ± 4.0
Microcopris aff. *hidakai*	5.5 ± 2.0
Onthophagus "*obscurior* group"	7.5 ± 3.5
Onthophagus crassicollis	4.5 ± 2.5
Onthophagus recticornutus	5.5 ± 2.0
Onthophagus rutilans	9.0 ± 3.0
Onthophagus trituber	6.0 ± 2.5
Paragymnopleurus maurus	10.5 ± 3.5
Sisyphus thoracicus	6.5 ± 3.0

DISCUSSION

Scheffler (2005) and Gardner *et al*. (2008) reported that the dried body mass and length of dung beetles increase or decrease gradually and are consistent. In this regards, the body length of dung beetles collected in HSB was measured to investigate and

classify the body size. The body size of dung beetles species inhabiting the two different ecosystems has shown significant difference, i.e., logged [body length (mm) 6.9 ± 2.4] and unlogged forests [body length (mm) 8.5 ± 3.5] with p-value = 0.001, < 0.05, whilst the richness and abundance of species at the two ecosystems showed no difference at all (Nichols *et al*., 2008; Hosaka *et al*., 2014). Our result from HSB supported that the high number of the small-sized dung beetle inhabiting a forest area of the secondary forest ecosystem. The body size in dung beetles also showed positive correlation and was sensitive to the effect of forest modification (Gardner *et al*., 2008) and fragmentation. Besides measuring the forest disturbance of forest, body size (large or small) is one of the key factors affecting the magnitude of dung burial and seed dispersal (Slade *et al*., 2011; Nichols *et al*., 2013).

In our study, only one species, that is *C. renaudpauliani*, is considered as a large-sized dung beetle collected in HSB. This might be due to the limitation of the large-sized species to find sufficient food sources to accommodate their needs (Bartholomew & Heinrich, 1978). A similar situation was also reported in Singapore, where a large-sized roller dung beetle species, *P. maurus*, which was sampled at the small area of Lower Pierce. The same finding was also obtained from the small island of Tasik Kenyir, Terengganu, which can be classified as a secondary forest (Lee *et al*., 2009). The presence of a small number large dung beetle species directly reflects on the prevailing status of the disturbed forests, whereby the degraded habitat would not be able to support a high population density of large-sized dung beetles. This is partly because forest disruption dispels the larger animals to other areas, or many would perish because they were unable to adapt and survive in the disturbed area (Horgan, 2005; 2008).

According to Nichols *et al*. (2009), dung beetles species have shown indirect effects with the mammalian faecal resources or for the dung beetles' food sources. Due to that reason, lack of animal wastes would

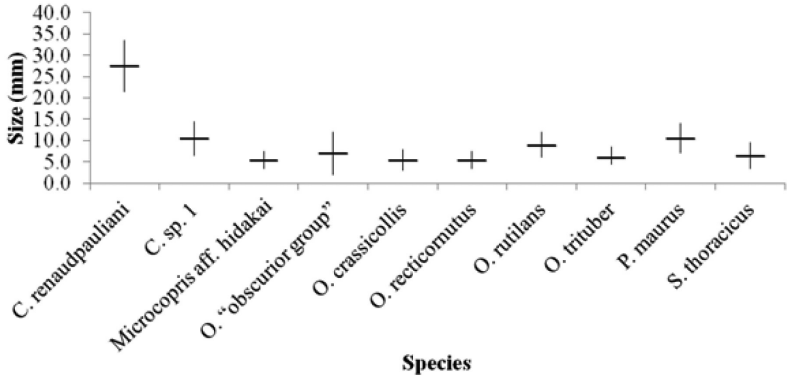

Fig.1: Significant sizes of dung beetles species collected from Bangi Reserve Forest

in turn affect the diversity and population abundance of the dung beetles that depend on them as a food source. Apart from species diversity and population abundance, the body sizes of dung beetles are also useful indicators in evaluating the status of a forest area (Hosaka *et al*., 2014) because these data are indicative of the population structure and diversity of the mammals in the study area (Camberfort, 1991). Unlike

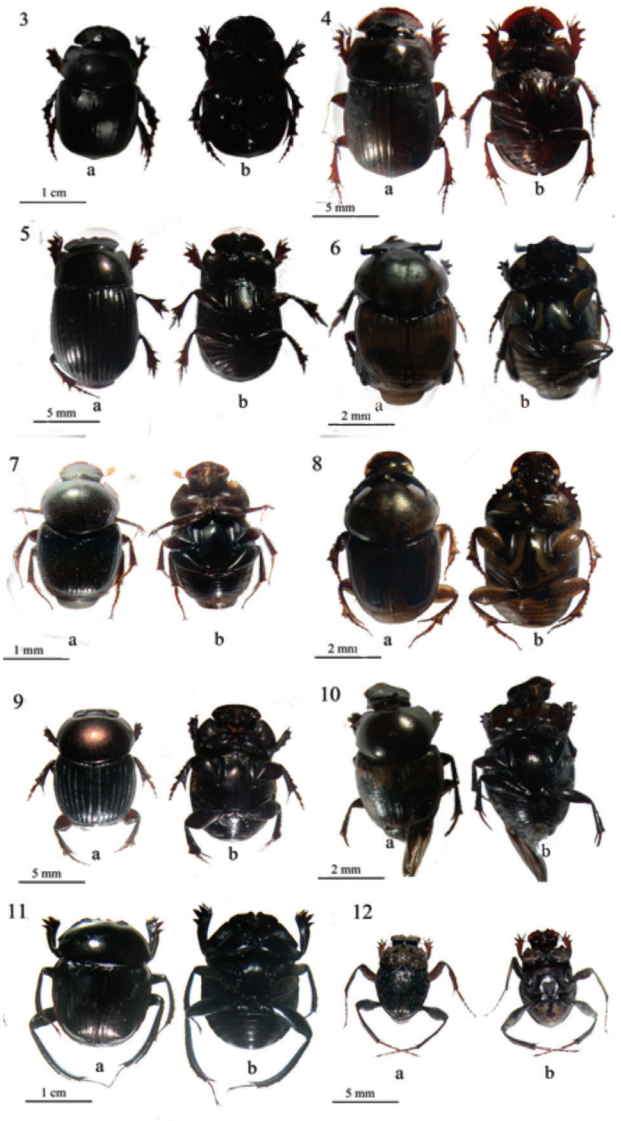

Fig.2 - Fig.11: 2: *Catharsius renaudpauliani*, 3: *Catharsius* sp. 1, 4: *Microcopris* aff. *hidakai*, 5: *Onthophagus* "*obscurior* group", 6: *Onthophagus crassicollis*, 7: *Onthophagus recticornutus*, 8: *Onthophagus rutilans*, 9: *Onthophagus trituber*, 10: *Paragymnopleurus maurus*, 11: *Sisyphus thoracicus*

the natural forest ecosystems, secondary forests have less capacity to support larger mammals and wildlife species, as reflected by the population profile of the dung beetle species. As in Bangi Reserve Forest (HSB), no large mammal has been reported, where it is believed to accommodate only small mammals in the area. Habitat disturbance is one of the factors that reduces the abundance of mid-large-sized mammals (Laidlaw, 2000) that provide food sources for the beetle species (Estrada *et al*., 1993) and it has shown decrement with increasing fragmentation and disturbance of forests such as in the Neartic regions.

A strong relationship can be observed between food source and body mass of beetles because a high demand for food supply is required to maintain the large-sized dung beetles in substantial numbers (Nichols *et al.,* 2007). In Malaysia, it is recorded that the largest dung beetle species is *Heliocopris* sp.; in HSB, however, only *C. renaudpauliani* of the average body length (27.5 mm) was collected. Ong *et al.* (2013) carried out a survey in Singapore and observed the presence of *Catharsius molossus*. This species is of a similar size with *C. renaudpauliani*, and is ecologically preferred herbivorous dung as its food source. Therefore, it was assumed that *C. renaudpauliani* also has similar food preference from larger mammals, although large mammals viz. elephants, Samba deers and rhinos have never been reported in the HSB. A study by Md. Zain *et al.* (2010) noted that omnivorous species such as primate, *Macaca fascicularis* and wild boars are abundant in HSB, as well as Malayan Colugo or flying lemurs and Banded Leaf monkeys (*Galeopterus variegates*) (Yaakop & Aman, 2013). Therefore, it is presumed that somehow, these large-sized beetles could still survive by consuming the excrement of other types of animals, which are usually from the small mammals such as rodents and primates. This is an interesting prospect that merits further investigation. Besides that, Nichols *et al*. (2013) also cited that large tunnellers like *Catharsisus* could persist or continue to exist in the forested agriculture lands, but not in more disturbed areas (non-forested agriculture lands).

Another reason for the obvious lack of large-sized dung beetles may be that these insects are more vulnerable to forest fragmentation (Klein, 1989; Ong *et al.,* 2013). Referring to Ong *et al*. (2013), the largest species (>10mm), *C. molussus* (*Catharsius molussus*) collected from Singapore, is commonly found in old-growth continuous forest or undisturbed forest with elephants, rhinoceros and clouded leopards inhabit the type of forest (Marsh & Greer, 1992). Again, there could be a strong correlation with the abundance of animals that provide dung as a food source. Larger mammals tend to avoid forest fragments because of insufficient food, home range and cover. On the other hand, small-sized dung beetles can survive better in forest fragments because smaller vertebrates can still furnish them with sufficient food supply. In our study area, the small-sized dung beetles of *O. crassicollis* were the most abundant of all species, confirming other reports by

Andresen (2003) and Horgan (2005) and indicating that forest reduction has adverse impacts on the population abundance, species diversity, and distribution of dung beetles.

The results of our study on the diversity of dung beetles are congruent with the findings of other studies, in that dung beetles could serve as useful and representative bioindicator organisms of forest disruption (Andresen, 2002, 2003; Davis *et al.,* 2001; Horgan, 2001, 2005, 2008). Several studies (Klein *et al.,* 2002; Davis & Philips, 2005; Kanda *et al.,* 2005; Shahabuddin, 2013) have concluded that disturbed forests have low to moderate diversity indices ($H' \leq 2.3$) for dung beetles, which are very sensitive to the open forest and forest disturbance, while for more disturbed areas (e.g., plantation and crop areas), the diversity of dung beetles is expected to be even lower when compared to the primary or deep forests. As our study forest had experienced long-term disturbance, it was not expected that there would be a high diversity of species, as reflected by the Shannon diversity index.

On the other hand, continuous and high-intensity forest disturbance could also produce high diversity because many microsites or microclimatic conditions could be created, providing diverse niches for the scarab beetles to exploit, depending on the species (Enari *et al.,* 2011). However, the diversity of dung beetles is also highly related to the population of vertebrates due to their dependency on animal wastes as their primary food source. Among the genera of dung beetles collected in our study area, *Onthophagus* was the most successful genus in terms of survival in a disturbed area, and this genus is also reported to be prevalent in many other types of disturbed habitats (Davis *et al.,* 2001; Prize, 2004; Davis & Philips, 2005; Shahabuddin *et al.,* 2005, 2010; Agoglitta *et al.,* 2012).

CONCLUSION

This study gives an overview of the diversity of the dung beetles of secondary forests. Moderate diversity indexes indicate the environmental conditions of Bangi Forest Reserve (HSB). Apart from that, body size is another factor that serves as the key indicator of forest disturbance. Small-bodied dung beetles survive more successfully in secondary forests compared to larger dung beetles. Small dung beetles are more likely to survive in the forest because the dung of small animals is sufficient for their survival and existence.

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REFERENCES

Agoglitta, R., Moreno, C. E., Zunino, M., Bonsignori, G., & Dellacasa, M. (2012). Cumulative annual dung beetle diversity in Mediterranean seasonal environments. *Ecological Research, 27*, 387-395.

Anderson, J. M., & Coe, M. J. (1974). Decomposition of elephant dung in an arid, tropical environment. *Oecologia*, *14*, 111-125.

Andresen, E. (2002). Dung beetles in a Central Amazonian rainforest & their ecological role as secondary seed dispersals. *Ecological Entomology, 27*, 257-270.

Andresen, E. (2003). Effect of forest fragmentation on dung beetle communities and functional consequences for plant regeneration. *Ecography*, *26*, 87-97.

Andresen, E., & Feer, F. (2005). The role of dung beetles as secondary seed dispersers and their effect on plant regeneration in tropical rainforest. In P. M. Forget, J. E. Lambert, P. E. Hulme, & S. B. Vander Wall, *Seed Fate: Predation, Dispersal and Seedling Establishment* (pp. 331-349). UK: CABI International.

Andresen, E. (2008). Dung beetle assemblages in primary forest and disturbed habitats in a tropical dry forest landscape in western Mexico. *Journal of Insect Conservation, 12*, 639-650.

Bang, H. S., Lee, J. H., Kwon, O. S., Na, Y. E., Jang, Y. S., & Kim, W. H. (2005). Effects of paracoprid dung beetles (Coleoptera: Scarabaeidae) on the growth of pasture herbage and on the underlying soil. *Applied Soil Ecology*, *29*, 165-171.

Bartholomew, G. A. & Heinrich, B. (1978). Endothermy in African dung beetles during flight, ball making, and ball rolling. *Journal of Experimental Biology*, *73*, 65-83.

Behling, M. C. H., Santos, J. C., & Bianchin, I. (2000). The role of *Digionthophagus gazella* on pasture cleaning and production as a result of burial of cattle dung. *Pasturas Tropicales*, *22*, 14-18.

Bishop, A. L., McKenzie, H. J., Spohr, L. J., & Barchia, I. M. (2005). Interactions between dung beetles (Coleoptera: Scarabaeidae) and the arbovirus vector *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). *Australian Journal of Entomology*, *44*, 89-95.

Byford, R. L., Craig, M. E., & Crosby, B.L. (1992). A review of ectoparasites and their effect on cattle production. *Journal of Animal Science*, *70*, 597-602.

Camberfort, Y. (1991). Dung beetles in tropical savannas In I. Hanski & Y. Cambefort (Eds.), *Dung beetle ecology* (pp. 156-178). Princeton, NJ: Princeton University Press.

Davis, A. J., Hooloway, J. D., Huijbregts, H., Krikken, J., Kirk-Springgs, A. H., & Sutton, S. F. (2001). Dung beetle as indicators of change in forest of northern Borneo. *Journal of Applied Ecology, 38*, 593-616.

Davis, A. L., & Philips, T.K. (2005). Effect of deforestation on a southwest Ghana dung beetle assemblage (Coleoptera: Scarabaeidae) at the periphery of Ankasa Conservation Area. *Environmental Entomology*, *34*, 1081-1088.

D'hondt, B., Bossuyt, B., Hoffmann, M., & Bonte, D. (2008). Dung beetle as secondary seed dispersers in a temperate grassland. *Basic and Applied Ecology*, *9*, 542-549.

Ek-Amnuay, P. (2008). *Beetles of Thailand.* Bangkok, Thailand: Amarin Printing and Publishing.

Enari, H., Koike, S., & Sakamaki, H. (2011). Assessing the diversity of dung beetle assemblages utilizing Japanese monkey feces in cool-temperate forests. *Journal of Forest Research, 16*, 456-464.

Estrada, A., Halffter, G., Coates-Estrada, R., & Meritt Jr., D. A. (1993). Dung beetles attracted to mammalian herbivore (*Alouatta palliate*) and

omnivor (*Nasua narica*) dung in the tropical rain forest of Los Tuxtlas, Mexico. *Journal of Tropical Ecology*, *9*, 45-54.

Filgueiras, B. K. C., Iannuzzi, L., & Leal, I.R. (2011). Habitat fragmentation alters the structure of dung beetle communities in the Atlantic Forest. *Biological Conservation*, *144*, 362-369.

Fincher, G. T., Stewart, T.B., & Davis, R. (1970). Attraction of coprophagus beetles to feces of various animals. *Journal of Parasitology*, *56*, 378-383.

Halffter, G., & Arellano, L. (2002). Response of dung beetle diversity to human-induced changes in tropical landscape.*Biotropica*, *34*, 144-154.

Gardner, T. A., Barlow, J., Araujo, I.S., Avila-Pires, T.C., Bonaldo, A.B., Costa, J.E., Esposito, M.C., Ferreira, L.V., Hawes, J., Hernandez, M. I. M., Hoogmoed, M. S., Leite, R. N., Lo-Man-Hung, N. F., Malcolm, J. R., Martins, M. B., Mestre, L. A. M., Miranda-Santos, R., Overal, W.L., Parry, L., Peters, S.L., Ribeiro-Junior, M.A., da Silva, M. N. F. C.. da Silva Motta, C., & Peres, C.A. (2008). The cost-effectiveness of biodiversity surveys in tropical forest. *Ecology Letters*, *11*, 139–150.

Hammer, O., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological Statistics Software Package for Education andData Analysis. *Palaeontologia Electronica*, *4*, 9.

Hanski, I. (1983). Distributional ecology and abundance of dung and carrion-feeding beetles (Scarabaeidae) in tropical rain forests in Sarawak, Borneo. *Acta Zoologica Fennica*, *167*, 1-45.

Hanski, I., & Camberfort, Y. (1991). Dung Beetle Ecology. , Princeton, NJ: Princeton University Press.

Horgan, F. G. (2001). Burial of bovine dung by coprophagus beetles (Coleoptera: Scarabaeidae) from horse and cow grazing sites in El Salvador. *European Journal of Soil Biology, 37*, 103-111.

Horgan, F. G. (2005). Effects of deforestation on diversity, biomass and function of dung beetle on eastern slopes of the Peruvian Andes. *Forest Ecology and Management*, *216*, 117-133.

Horgan, F. G. (2008). Dung beetle assemblages in forest and pastures of El Salvador: a functional comparison. *Biodiversity Conservation, 17*, 2961-2978.

Hosaka, T., Niino, M. Kon, M., Ochi, T. Yamada, T., Fletcher, C., & Okuda, T. (2014). Effects of logging road networks on the ecological functions of dung beetles in Peninsular Malaysia. *Forest Ecology and Management*, *326*, 18-24.

Kanda, N., Yokota, T., Shibata, E., & Sato, H. (2005). Diversity of dung-beetle community in declining Japanese subalpine forest caused by increasing sika deer population. *Ecological Research*, *20*, 135-141.

Klein, A. M., Steffan-Dewenter, I., Buchori, D., & Tscharntke, T. (2002). Effects of land-use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology*, *16*, 1003-1014.

Klein, B. (1989). Effects of forest fragmentation on dung and carrion beetle communities in central Amazonia. *Ecology*, *70*, 1715-1725.

Laidlaw, R. K. (2000). Effects of habitat disturbance and protected areas on mammals of Peninsular Malaysia. *Conservation Biology, 14*, 1639–1648.

Lawton, J. H., Bignell, D. E., Bolton, B. Bloemers, G. F., Eggleton, P., & Hammond, P. M. (1998). Biodiversity inventories, indicator taxa and effects of habitat modification in tropical rain forest. *Nature, 391*, 72-76.

Lee, J. S. H., Lee, I. Q. W., Lim, S. L. H., Huijbregts, J., & Sodhi, N.S. (2009). Changes in dung beetle communities along a gradient of tropical forest disturbance in South-East Asia.*Journal of Tropical Ecology, 25*, 677–680.

Marsh, C. W. & Greer, A.G. (1992). *Forest land-use in Sabah, Malaysia: an introduction to Danum Valley.* Philosophical Transactions of the Royal Society of London B *335*, 331–339.

Mat Salleh, K. (1999). The role and function of Universiti Kebangsaan Malaysia permanent forest reserve in research and education. *Pertanika Journal of Tropical Agricultural Science*, *22*, 185-198.

Md. Zain, B. M., Sha'ari, N. A., Mohd-Zaki, M., Ruslin, F., Idris, N.I., Kadderi, M. D. & Idris, W.M.R. (2010). A Comprehensive Population Survey and Daily Activity Budget on Long-tailed Macaques of Universiti Kebangsaan Malaysia. *Journal of Biological Science, 10*(7), 608-615.

Nichols, E., Larsen, T., Spector, S., Davis, A.L., Escobar, F., Favila, M., & Vulinec, K. (2007). Global dung beetle response to tropical forest modification and fragmentation: A quantitative literature review and meta-analysis. *Biological Conservation, 137*, 1-19.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S., Favila, M.E., & Vulinec, K. (2008). Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation, 141*, 1461–1474.

Nichols, E., Uriarte, M., Bunker, D.E., Favila, M., Slade, E.M., Vulinec, K., Larsen, T., Vaz-de Mello, F.Z., Louzada, J. Naeem, S., & Spector, S.H. (2013). Trait dependent response of dung beetle populations to tropical forest conversion at local to global scales. *Ecology, 94*, 180–189.

Noraini, M. (1990). Profil Hutan Bangi [pp. 125-138]. In A. Latif (Ed.), *Ekologi dan Biologi Hutan Simpan Bangi, Universiti Kebangsaan Malaysia*. UKM, Bangi, Malaysia.

Ochi, T., Kon, M., & Kikuta, T. (1996). Studies on the family Scarabaeidae (Coleoptera) from Borneo. I. Identification keys to subfamilies, tribes and genera. *Giornale Italia Entomologia*, *8*, 37-54.

Ong, X. R., Chua, S.C., & Potts, M.D. (2013). Recent records of the dung beetle *Catharsius renaudpauliani* (Coleoptera: Scarabaeidae) in Singapore. *Nature in Singapore*, *6*, 1-6.

Prize, D. L. (2004). Species diversity and seasonal abundance of scarabaeoid dung beetles (Coleoptera: Scarabaeidae, Geotrupidae and Trogidae) attracted to cow dung in central New Jersey. *Journal of the New York Entomological Society*, *12*, 334-347.

Ridsdill-Smith, T. J., & Hayles, L. (1990). Stages of bush fly, *Musca vetustissima* (Diptera: Muscidae), killed by scarabaeine dung beetles (Coleoptera: Scarabaeidae) in unfavourable cattle dung. *Bulletin of Entomological Research*, *80*, 473-478.

Scheffler, P. Y. (2005). Dung beetle (Coleoptera: Scarabaeidae) diversity and community structure across three disturbance regimes in eastern Amazonia. *Journal of Tropical Ecology*, *21*, 9-19.

Shahabuddin. (2013). Contribution of alpha and beta diversity across land-use type to the regional diversity of dung beetles in Central Sulawesi. *HAYATI Journal of Biosciences, 20*, 72-79.

Shahabuddin, P., Hidayat, S., Manuwoto, W., Noerdjito, T., Tscharntke, T., & C. Schulze. (2010). Diversity and body size of dung beetles attracted to different dung types along a tropical land-use gradient in Sulawesi, Indonesia. *Journal of Tropical Ecology, 26*, 53-65.

Shahabuddin, C. Schulze, H., & Tscharntke, T. (2005). Changes of dung beetle communities from rainforests towards agroforestry systems and annual cultures in Sulawesi (Indonesia). *Biodiversity and Conservation*, *14*, 863-877.

Slade, E. M., Mann, D. J., & Lewis, O. T. (2011). Biodiversity and ecosystem function of tropical forest dung beetles under contrasting logging regimes. *Biological Conservation*, *144*, 166–174.

Spector, S. (2006). Scarabaeine dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae): an invertebrate focal taxon for biodiversity research and conservation. *Coleopterists Bulletin, 5*, 71-83.

Triplehorn, C. A., & Johnson, N. F. (2005). *Borror and DeLong's Introduction to the Study of Insects*. UK: Thomson Learning, Inc..

UKM. (2011). Hutan Simpan Kekal. Retrieved 16 January 2014 from www.ukm.my/hutan/index.php

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science, 277*, 494-499.

Yaakop, S., & Aman, A. Z. (2013). Does the fragmented and logged-over forest show a real hyperdymanism on braconids Species? *Malaysian Applied Biology, 42*(2), 1-5.

Yokoyama, K., & Kai, H. (1993). Distribution and flow of nitrogen in cow dung-soil system colonized by paracoprid dung beetles. *Edaphologia*, *50*, 1-10.

Yokoyama, K., Kai, H., Koga, T., & Aibe, T. (1991). Nitrogen mineralization and microbial populations in cow dung, dung balls and underlying soil affected by paracoprid dung beetles. *Soil Biology and Biochemistry, 23*, 649-653.

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Review of the Compression Moulding of Natural Fiber-Reinforced Thermoset Composites: Material Processing and Characterisations

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ABSTRACT

Compression moulding is generally applied to thermoset-based polymer material composites (PMCs), which consist of a reinforcement phase embedded in a polymer matrix to strengthen the polymer. Thermoset compression-moulded composites have advantageous thermal and mechanical properties. Natural fibres are typically used in composites as a reinforcing material either as continuous (very long) or discontinuous (chopped) fibres. Interest in using natural fibres to make high-performance engineering products is increasing because their mechanical properties are better than those of synthetic fibres. The types of matrix, types of fibre, chemical treatment of fibre, orientation of fibre and processing parameters that reveal converging problems, which can be studied in future research, are still being investigated. This work intends to review current studies on material processing and characterisations in terms of the thermal and mechanical properties of thermoset composites reinforced with natural fibres by compression moulding.

Keywords: Kenaf, thermoset, material processing, mechanical and physical properties

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INTRODUCTION

Research into the use of natural resources has tremendously increased over the past few years (Amel *et al*., 2013). Its applications increase because of its good mechanical and physical properties. Mechanical and physical properties are typically important in engineering because they determine the durability and cost efficiency of the product.

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Synthetic fibres, such as aramid, carbon fibre and glass fibre (Hariharan *et al*., 2005; Ahmed *et al*., 2008) from petroleum also have good mechanical properties but they are not renewable and are tied to the world market. The cost of the composite reinforced with synthetic fibre, therefore, increases (Fulton *et al*., 2011). Natural fibres clearly have potential to replace synthetic fibres.

Plant fibres effectively reinforce thermoplastic and thermoset polymer composites. Polymers (plastic) are generally unsuitable for load-bearing applications because such applications require sufficient stiffness, strength and stable dimension. Natural fibres must thus be embedded in a polymer matrix to strengthen the polymer composite. The polymer matrix typically enables the fibres to adhere in place and reinforce the structural component of the fibre-reinforced polymer composite (Mohanty *et al*., 2005).

Plant fibres have been used in recent years to reinforce polymer composites in various applications, such as automotives, construction, marine and electronic components (Mohanty *et al*., 2005). Its applications increase because its properties, which are light-weight, high specific stiffness and strength, easy production and extensive resistance to fatigue and corrosion, are better than those of synthetic fibres.

The good performance of natural fibre-reinforced composites is typically affected by various factors such as fibre composition, fibre preparation and extraction process. Performance includes thermal, physical and mechanical properties. The physical and mechanical properties of fibres typically depend on the structural and chemical composition, type of fibre, fibre modification etc. during processing (Mohanty *et al*., 2005). This paper, therefore, aims to review current research into material processing and the characterisations of compression-moulded natural fibre-reinforced thermoset composites. This work also aimed to study the material processing and characterisations (mechanical and thermal properties) of polymers reinforced with some of the most popular natural fibres: kenaf, sisal, abaca and pineapple.

NATURAL FIBRE-REINFORCED POLYMER COMPOSITES

Composite materials, also known as engineered materials, are generally fabricated with two or more constituent materials. These constituents have significantly different chemical and physical properties. Constituents are categorised into two groups i.e. matrix and the reinforcement phase, which exist in the form of fibre, particle or flakes.

Types of Natural Fibres(Reinforcement)

Interest in the use of natural fibres as a reinforcement in composite applications has been growing. Composites are basically reinforced with either continuous or discontinuous natural fibres. Natural fibre materials are abundant. Natural fibres are generally classified into three groups based on their sources: plant, animal and mineral (Nguong *et al*., 2013). Plant fibres are widely accepted as reinforcement materials

among these natural fibre groups. A few groups of fibre exist, namely, bast fibre, leaf fibre, seed fibre, fruit fibre, wood fibre, stalk fibre and grass fibre. Common commercially used plant fibres include kenaf, sisal, hemp, flax, abaca, pineapple leaf, and ramie (Aji *et al*., 2009; Akil *et al*., 2011; Nguong *et al*., 2013).

Selecting fibres not only depends on material properties, but also on economic factors and local availability (Fulton *et al*., 2011). Table 1 shows that natural fibres have advantages over the other reinforcement materials in terms of density, tensile strength, Young's modulus etc. A detailed description of the properties of the natural and synthetic fibres is summarised in the table below. The structure and chemical composition of natural fibres depend on their source, processing and growth application. Natural fibres are generally composed of cellulose (51 wt%), hemicelluloses (21 wt%), lignin (10.5 wt%) and pectin (3 wt% to 5 wt%). The major constituents of natural fibres are cellulose, hemicelluloses and lignin. Higher cellulose content generally contributes to higher stiffness.

The utilisation of natural fibres as a reinforcement material in polymer composite is currently gaining interest due to its high strength and stiffness. The properties of the composite are influenced by the fibre itself and by the interfacial adhesion between the fibre and the matrix (Shanmugam *et al*., 2013). The use of the hydrophilic natural fibres in polymers can generally produce bad properties for the composite (Saheb & Jog, 1999) because of the lack of adhesion between the fibre and the matrix in the composite system. Kenaf fibre is usually treated with chemicals to improve interfacial bonding between the fibre and the matrix. Using alkali-treated fibres improves the properties of the fabricated composite and also reduces water absorption of the composite. The orientation of the fibre also affects the mechanical properties of the composite (Aji

TABLE 1
Physical and Mechanical Properties of Natural And Synthetic Fibres (Mohanty *et al.,* 2005)

Fibre	Density (g/cm^3)	Tensile strength (MPa)	Young's Modulus (GPa)	Elongation at Break (%)
Flax	1.5	345-1500	27.6	2.7-3.2
Hemp	1.47	690	70	1.6
Jute	1.3	393-800	13-26.5	1.16-1.5
Kenaf	1.22-1.44	930	53	1.6
Ramie	1.55	400-938	61.4-128	1.2-3.8
Sisal	1.45	468-700	9.4-22	3-7
Coir	1.15-1.46	131-220	4-6	15
E-glass (synthetic)	2.55	3400	73	2.5
Kevlar (synthetic)	1.44	3000	60	2.5-3.7
Carbon (synthetic)	1.78	3400-4800	230-240	1.4-1.8

et al., 2009) because the fibres are difficult to evenly distribute and manually separate during processing. Orientating the fibres in a parallel direction increases Young's modulus and tensile strength.

Kenaf

Kenaf, or *Hibiscus cannabinus* L. Family Malvaceaeis, is planted once a year under a wide range of weather conditions. Kenaf has been cultivated in Asia and Africa a few years ago (Mohanty *et al*., 2005). Kenaf plants were extensively planted in Malaysia by the Tobacco Board of Malaysia (LKTN). The kenaf plant contains two fibre types: long and short fibres. Kenaf plants generally have pale fibres and smaller amounts of noncellullosic materials than jute. These plants have similar breaking strength to low-grade jute and slightly weakens in wet conditions.

Kenaf plant has single and straight stems without branches. Harvested kenaf stems are usually decorticated to separate the stem from the core in producing the kenaf bast fibres (single and bundle bast fibre). The kenaf plantgenerally consists of an inner core and an outer fibrous bark surrounding the core (kenaf bast), as illustrated in Fig.1. Kenaf fibre is typically used for extruded, moulded and nonwoven products because of its higher flexural strength and tensile strength. Kenaf fibre is extensively applied as a reinforcement in door panels, mats, headliners, dashboards, furniture etc. (Ishak *et al*., 2010). Kenaf fibres also have the advantages of biodegradability and renewability, which are essential for making environmentally-friendly products.

Sisal

Sisal, *Agave sisalana*, is widely grown in tropical countries in Africa, Western India and the Far East. Fibres are extracted from the fresh leaves using a decorticator followed by washing and drying under the sun (Mohanty *et al*., 2005). The hard fibre, sisal, is typically extracted from the leaves.

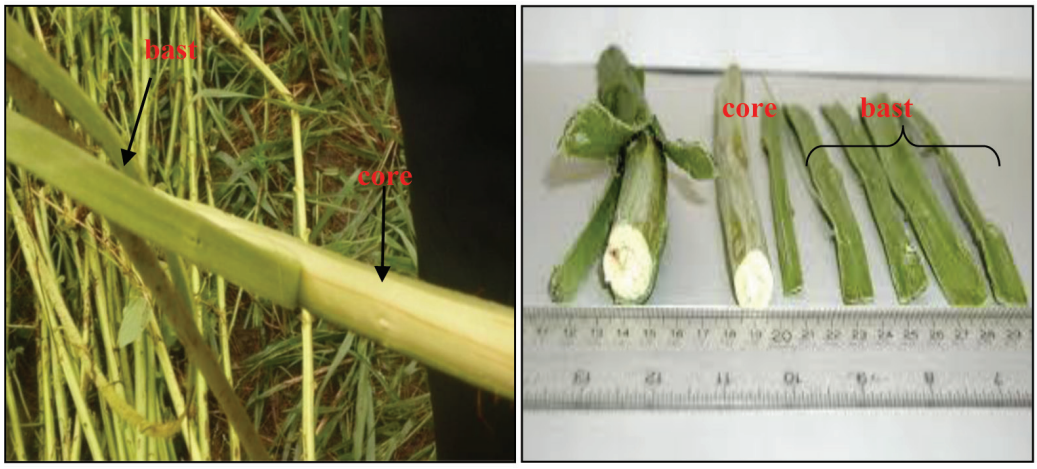

Fig.1: Schematic picture of kenaf plant

The sisal fibre is a bundle of hollow sub-fibres. The cell walls are typically reinforced by spirally orientating a hemicelluloses and lignin matrix. The surface does not form strong bonds with polymer matrices because the external surface of the cell wall is composed of ligninaceous materials and waxy substances that bond the cell to its adjacent neighbours. The strong sisal fibre is traditionally used for making rope, fabrics, rugs, carpet, handicraft etc. The paper industry has become interested in sisal (Saxena *et al*., 2011).

Banana/Abaca

Banana fibre currently is a waste product of cultivation. It is native to the Philippines, and currently is widely planted in Ecuador (Mukhopadhyay *et al*., 2008). The banana plant is durable and resistant to wear. Abaca is used mainly to manufacture rope and handicraft such as bags, doormats and slippers. The banana fibre can be used for various industrial purposes, for instance, in the automotive and construction industries (Samal *et al*., 2009). Various researchers have worked on banana-reinforced polymer composites (Sumaila *et al*., 2013), and most of the research shows that banana fibre can be used as reinforcement, especially in thermoset polymer. Previous studies show that banana is a good reinforcement in polyester resin (Mukhopadhyay *et al*., 2008)

Pineapple leaf

Pineapple fibre (*Ananas comosus*) is typically extracted from the leaves of the tropical pineapple (Shyamraj *et al*., 2013). This fibre is rich in cellulose, and is abundant in Brazil (Faruk *et al*., 2012). The pineapple leaf currently is the most popular waste product from pineapple cultivation, and is relatively cheaper for industrial purposes such as bags, mats and table linen. Pineapple fibre has good potential as reinforcement in thermoset composites. The results of a previous work show that the pineapple leaf strongly influences the tensile properties of the reinforced composite (Vinod & Sudev, 2013). The quality of a pineapple-reinforced composite can generally be improved by surface modification (Vinod & Sudev, 2013). The previous results prove that the modified pineapple leaf fibre composite has the highest tensile and impact strength.

Thermoset Polymer Composite

The matrix phase is important to the performance of polymer composites. The reinforcement materials in the polymer matrix are typically supported and surrounded by the matrix materials to maintain their relative positions. The matrix holds the fibres together. The fibre is embedded in the matrix, such as thermoset and thermoplastic, to make the matrix hold the fibres together and thus strengthen them. Thermosetting plastics are synthetic polymer whose molecules cross-link during processes, and therefore cannot be recycled or reprocessed (Mohanty et al., 2005). Thermoset polymers form three-dimensional molecular chains during cross linking. Thermoset polymer formulations, such as epoxy and polyester, are very complex due to the large number of components involved,

such as the base resin, curing agent, catalyst, flowing agent and hardener.

Chemical curing of the highly cross linked, three-dimensional and network structure of the thermoset polymer increases the toughness and solvent and creep resistance. Thermoset materials, such as epoxy resin, vinylester resin and polyester resin, are generally stronger than thermoplastic polymers due to their 3-D network of bonds. These materials are suitable for high-temperature applications, and then followed to the decomposition temperature of the material. These polymers, therefore, cannot be recycled like thermoplastic polymers, which can be melted and re-molded. Epoxy has unique properties, such as high strength, low creep, low shrinkage and low warping. This material also offers high performance and resistance to environmental degradation. The common properties of thermoset polymers are listed in Table 2.

COMPRESSION MOULDING OF NATURAL FIBRE THERMOSET COMPOSITES

Selecting the suitable processing method for natural composite materials is crucial for the form, performance attributes, cost and ease of manufacturing of the final desired product to obtain the quality, robust and repeatable manufacturing process. Composite processing generally involves equipment with a simple operation, or needs special equipment. There are several types of processing techniques for natural composite materials, such as compression moulding, injection moulding, resin transfer moulding and thermoforming. Compression is widely used among these techniques to manufacture natural fibre composites because of its high reproducibility and low cycle time.

Compression moulding is a conventional processing technique used to manufacture polymer matrix composites under specific temperatures and pressures (Groover, 2007). This technique is commonly used in manufacturing due to its simplicity. The process has advantages in terms of low fibre attrition and speed. Many variations of compression moulding have been developed, including a combination of compression with extrusion and sheet moulding compound (SMC) processes in order to reduce the cost by decreasing the cycle time (Faruk *et al*., 2012). Compression moulding using the thermoset polymer matrix is another major platform used for

TABLE 2
Properties of Typical Thermoset Polymer for Natural fibre Composites

Property	Polyester	Vinylester	Epoxy
Density (g/cm^3)	1.2-1.5	1.2-1.4	1.1-1.4
Tensile strength (MPa)	40-90	69-83	35-100
Compressive strength (MPa)	90-250	100	100-200
Izod Impact Strength (J/m)	0.15-3.2	2.5	0.3

manufacturing in the automotive industry in producing strong, light and thin panels and structures, as shown in Fig.2.

The distribution of the filler for the compression technique is far better than the other techniques because kenaf fibre is difficult to homogeneously distribute in reinforced composites. Compression moulding reduces the changes in the physical properties, and can help retain the isotropic properties of the composites (Aji *et al*., 2009) because it does not change fibre orientation. The mechanical properties of natural fibre composites are basically influenced by the moulding conditions, such as moulding pressure and temperature. The optimum pressure and temperature should therefore be applied to produce good mechanical products because of the problem of assurance of the adhesion of the fibre matrix in manufacturing natural fibre-reinforced composites.

All the previous studies concluded that natural fibre composites may be produced in various ways to gain different thermal and mechanical properties. The compression moulding process produces compression moulded composites with high strengths and impact strengths (Aji *et al*., 2009). The compression moulding process has been tested in some studies on woven jute and jute glass fabric reinforced polyester (Ahmed *et al*., 2008), hybrid kenaf/glass reinforced composite (Ahmed *et al*, 2008) and kenaf fibre with polyurethane (Sapuan *et al*., 2011) to evaluate its tensile, flexural and impact strength.

PROPERTIES OF NATURAL FIBRE-REINFORCED COMPOSITES

Thermal Properties

The thermal properties of natural fibre-reinforced composites were studied. The analyses were essential for distinguishing the behaviour of the fibre-reinforced composite. Three regular characterisation methods, thermogravimetric analysis (TGA), dynamic mechanical analysis (DMA) and differential

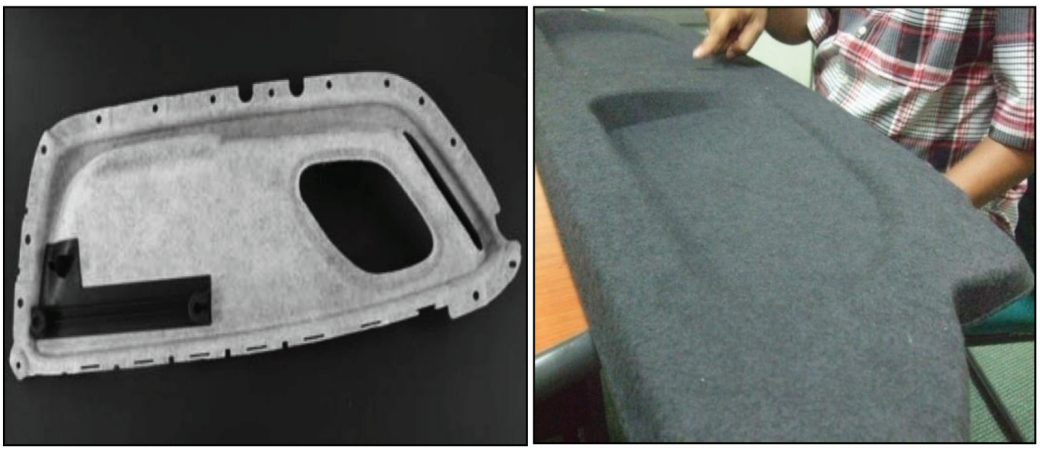

Fig.2: Kenaf natural fibre composite fabricated by compression moulding.

scanning calorimetry (DSC), were used. Several crucial parameters, such as the glass transition temperature (T_g), melting temperature, crystalline level and oxidation, were estimated from the DSC scan. TGA is an experimental technique for determining material stability, in which the weight or the mass of the sample is measured based on temperature changes. This technique is performed in air or at inert temperatures (H_2 and Ag), and the weight is recorded as the temperature increases (Azwa *et al*., 2013). Sublimation, evaporation, decomposition, chemical reaction and the magnetic or electrical transformation of the material usually change the mass, and these are related to thermal stability (Akil *et al*., 2011). The thermomechanical properties of the materials as a function of temperature and deformation can be measured by DMA.

Researchers have studied the thermal properties of the laminate obtained from thermal gravity using differential scanning calorimetry (TG-DSC), showing that the melting point (T_m) of the polypropylene (PP) film laminate decreases and the crystallisation peak increases as the fibre content in the laminates increases (Fulton *et al*., 2011). The DSC analyses of Muhd *et al*. (2010) showed that the endothermic transition temperature of kenaf filled with chitosan biocomposite increases with increasing kenaf content. The TGA result showed that the addition of kenaf dust in the chitosan film did not significantly change the thermal stability of chitosan films. *Samal et al*. (2009) studied the fabrication and performance evaluation of banana/glass fibre-reinforced polypropylene hybrid composites. The results showed that the thermal stability and crystallisation temperature of polypropylene incorporated with maleic anhydride grafted polypropylene (MAPP)-treated banana and glass fibres decreased in contrast to the banana fibre-reinforced polymer composite. The investigation done by Jawaid *et al*. (2012) demonstrated that the incorporation of jute fibres increased the thermal stability of the hybrid composite because the jute fibre had higher thermal stability compared to the empty oil palm fruit bunch. Azwa *et al*. (2013) compared the degradation behaviour of kenaf/epoxy composite and neat epoxy exposed to high temperatures. The investigation revealed that the addition of fibre into the epoxy improved the thermal stability and charring capability of the samples.

Mechanical Properties

Mechanical properties, such as tensile properties, flexural properties and impact properties, are crucial to the performance of materials. Mechanical properties are important in determining the ability of materials, especially under critical conditions. Most studies on natural fibre composites involve mechanical properties. The fibre content, the use of external coupling agents and the effects of various treatments change mechanical properties. Many studies on kenaf fibre-reinforced composites have been conducted during the past few years to study mechanical behaviour. Matrix and reinforcement typically are important

in improving the mechanical properties of the composites (Saheb & Jog, 1999). Some of the mechanical properties of natural fibre-reinforced composites that have been studied include tensile strength, flexural strength and impact strength. The details of the discussion on the mechanical properties are discussed in the following section and are summarised in Table 3.

Tensile Strength

The tensile test predicts the behaviour of materials under different loadings other than uniaxial tension. This test also determines the maximum engineering stress in tension that may be sustained without fracture (Akil *et al*., 2011). Tensile strength is generally more sensitive to the matrix properties, whereas the modulus depends on the fibre properties. The strong interface, low stress concentration, fibre orientation and fibre volume can basically improve tensile strength (Saheb & Jog, 1999).

Davoodi *et al*. (2010) studied the tensile properties of kenaf reinforced with different polymer composites known as polypropylene (thermoplastic) and epoxy (thermoset). The tensile test was performed according to the guidelines of the American Society of Testing Method Standard (ASTM) 3039. Their research shows that fibres reinforced with epoxy are far better because of the unity of the plies and the adhesion between the fibres. The results show that epoxy has higher strength (71.68 MPa) than polypropylene. Saxena *et al*. (2011) proved that the mechanical properties of the polymer composite are more enhanced than the neat polyester composites. The incorporation of the sisal

TABLE 3
Mechanical Properties of Natural Fibre Composites

Reinforcement	Composition (wt %)	Matrix	Tensile (MPa)	Flexural (MPa)	Impact (J/m)	Reference
Kenaf bast fibre	0-50	Poly-lactic acid	223	254	-	(Ochi, 2008)
Kenaf bast fibre	-	Epoxy Polypropylene	71.68 37.2	200-240	20-40	(Davoodi *et al*., 2010)
Coconut particle	5-15	Epoxy	35.48	-	-	(Sapuan *et al*., 2003)
Coconut spathe and coconut fibre	30	Polyester	7.9-11.6 (coconut spathe)	25.6-67.2	-	(Sapuan *et al*., 2005)
Kenaf bast fibre	10-60	Thermoplastic Polyurethane	32-37 (30 wt%)	-	-	(El-Shekeil *et al*., 2011)
Kenaf derived cellulose	40-60	Poly-lactic acid		63.4-98.8	35.3	(Syafinaz *et al*., 2010)
Banana fibre and glass fibre	0-45	Phenol formaldehyde	28 42	50 73	30-40 40	(Joseph *et al*., 2011)

was found to increase the tensile strength and Young's modulus of the epoxy resin.

Sapuan *et al*. (2005) studied the mechanical properties of unsaturated polyester composites reinforced with different percentage weights of kenaf fibre. The results showed that tensile strength increased as the fibre content increased. In another research done by Sapuan *et al*. (2003), the mechanical properties of composites with filler epoxy/coconut particle were studied. The tensile strength of the composites reportedly increased with increasing filler content because coconut filler particle strengthened the interface of the resin matrix and filler materials. The composite with 15% filler showed the highest tensile strength, 35.48 MPa, compared with the other two combinations (5% and 10%).

Sapuan *et al*. (2005) also compared the flexural and tensile strength of epoxy composites reinforced with coconut spathe and coconut fibre. The results showed that the coconut spathe-reinforced composite had higher tensile strength than those reinforced with coconut fibre. The incorporation of spathe fibre within the epoxy thus enhanced the strength of the matrix. Girisha *et al*. (2012) investigated the tensile properties of the epoxy composite reinforced with different types of fibre, coconut spathe, sisal and ridge gourd, with different fractions of fibre loading from 5% to 30%. The results showed that the tensile properties were best at approximately 25% of the weight fraction of the fibres. The values decreased further with increased weight fraction. Research into mechanical properties of polyurethane composites reinforced with different weight percentages of kenaf fibre loading was performed by El-Shekeil *et al*. (2011). Their result showed that the higher strength of 30% contributed to the strong bonding between the fibre and the matrix.

The fibre volume strongly influenced the tensile and Young's modulus of the polymer composite. Saxena *et al*. (2011) found that the tensile and Young's modulus increased with fibre volume increase due to the fibre interaction. The effects of the single and bundle fibre on the tensile modulus were also compared. The results showed that the tensile properties of a single fibre of sisal were better than those of the bundle fibre because the load on the single fibre was not uniform. Saxena *et al.* (2011) also investigated the effect of fibre length on the composite polymer and found that the tensile length increased with the increasing sisal fibre length. This result is consistent with the result obtained by Sumaila *et al.* (2013). The observation on the effect of fibre length with different diameters on the tensile properties of the banana/epoxy composite showed that the percentage elongation increased with increasing fibre length from 5 mm to 15 mm. The tensile strength decreased with increasing fibre length of up to 25 mm afterwards. Vinod and Sudev (2013) also studied the effect of fibre length on the tensile properties of pineapple leaf fibre (PALF). The results revealed that the tensile strength of the fibre increased with increasing fibre length.

Flexural Strength

The type of fibre, orientation (either random or unidirectional), content (fibre or fabric) and type of blending or plasticiser typically influence the flexural and tensile properties of the materials. The three-point bending test method also determines the flexural strength and modulus of the composite by following ASTM D790 standard. The research done by Davoodi *et al*. (2010) showed that the flexural modulus and flexural strength of the kenaf hybrid materials (natural fibre) were higher than those of the glass mat thermoplastics (synthetic fibre). Syafinaz *et al*. (2012) studied the effect of kenaf-derived cellulose content on the physical and mechanical properties of kenaf-derived cellulose (KDC)-filled polyactic acid (PLA) composites. The flexural properties of KDC/PLA composite were found to be improved compared to commercial neat PLA composites.

Joseph *et al*. (2002) also investigated the effect of fibre loading and fibre length on the flexural properties of phenol formaldehyde composites reinforced with banana (natural) fibres and glass (synthetic) fibres. The flexural properties of the composite were found to be dependent on fibre length. Both banana and glass fibre increased in flexural strength with increasing fibre loading. Saxena *et al*. (2011) investigated the effect of sisal fibre length reinforcement on different polymer composite bases, epoxy and polyster. The results showed that the flexural strength of the sisal-reinforced epoxy composite increased with increasing fibre length, whereas the polyster composite did not show any changes.

Impact Strength

Impact strength tests the ability of materials to resist fracture under stress applied at high speeds. The impact properties of polymeric materials are generally strongly related to the overall toughness of the materials. Composite fracture toughness is affected by the interfacial and interlaminar strength parameters. The impact performance of fibre-reinforced composites depend on numerous factors, including the nature of constituent, fibre/matrix interface, the construction and geometry of the composite and test conditions. Joseph *et al*. (2002) studied the influence of fibre length on impact strength. The results revealed that the impact strengths of the composite reinforced with glass fibre and banana fibre increased with the increasing fibre length. Davoodi *et al*. (2010) found that the average impact strength of the kenaf-reinforced epoxy composite was 26 J/m, which is nearly half of that of the common glass mat thermoplastic.

Liu *et al*. (2007) showed that compression moulded biocomposites have higher impact strength than injection moulded samples. The impact strength of the composite reinforced with different fibre lengths and contents were also investigated. The strength of the composite was found to increase with fibre length and content. Hariharan and Khalil (2005) showed that the impact strength (18 kJ/m^2) of oil palm

fibre composites was lower than that of glass fibre (107 kJ/m^2). However, the tensile and impact properties of palm fibre composites have been improved by the hybridisation of oil palm fibres with glass fibres (El-Shekeil *et al*., 2011). Aji *et al*. (2009) reported that the use of short fibres caused lower impact strength than long fibres.

Fibre treatment can generally increase interphase adhesion and cause the penetration of the matrix resin into the fibre. The impact strength of the alkali sisal-treated composite increased because the alkali treatment removed the waxy materials (Saxena *et al*., 2011).

CONCLUSION

Various studies have been conducted on various types of natural fibres, as summarised in Table 4. Natural fibres have better characteristics than synthetic fibres in reinforcement. Different processing methods can produce different products with different properties. For example, compression method improves the mechanical properties of composites. Compression moulding has the advantage of fibre bridging through fibre pullout. Using epoxy thermoset polymer as matrix for the compression moulding offers high performance to the natural fibre-reinforced composite. Epoxy resin potentially eliminates residual stress and also reduces shrinkage and creep of the final product.

There are some problems in using natural fibres, such as interfacial adhesion and water absorption. These problems must be solved before natural fibre composites become fully competitive with synthetic fibres. Further study must, therefore, be conducted to improve the physical and mechanical properties of the kenaf/ thermoset composite to the desired level. Much work must be performed to overcome obstacles, such as moisture absorption, long term performance and toughness, for internal and automotive engine applications.

TABLE 4
Compression Moulded of Thermoset Composite Reinforced with Natural Fibres

Natural Fibre	Matrix	Technique	Tensile Properties (MPa)	Reference
Kenaf fibre	Epoxy Resin	Compression Moulding	71.68	(Davoodi *et al*., 2010)
Kenaf bast fibre	Polyurethane	Compression Moulding	32-37	(El-Shekeil *et al*., 2011)
Banana fibre and glass fibre	Phenol formaldehyde	Compression Moulding	28 & 42	(Joseph *et al*., 2011)
Oil Palm	Epoxy Resin	Compression Moulding	24	(Hariharan & Khalil, 2005)

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REFERENCES

Ahmed, K. S., & Vijayarangan, S., (2008). Tensile, flexural and interlaminar shear properties of woven jute and jute-glass fabric reinforced polyester composites. *Journal of Material Processing*. *207*, 7, 330-335.

Aji, I. S., Sapuan, S. M., Zainuddin, E. S., & Abdan, K. (2009). Kenaf fibres as reinforcement for polymeric composite : A review. *International Journal of Mechanical and Materials Engineering (IJME)*, *4*(3), 239-248.

Akil, H. M., Omar, M. F., Marzuki, A. A. M., Safiee, S., Ishak, Z. A. M., & Bakar, A. A. (2011). Kenaf fibre reinforced composites: A review. *Materials and Design*, *32*(8-9), 4107-4121.

Amel, B. A., Paridah, M. T., Sudin, R., Anwar, U. M. K., & Hussein, A. S. (2013). Effect of fibre extraction methods on some properties of kenaf bast fibre. *Industrial Crops and Products*, *46*, 117-123.

Azwa, Z. N., & Yousif, B. F. (2013). Thermal degradation study of kenaf fibre/epoxy composites using thermo gravimetric analysis. *3rd Malaysian Postgraduate Conference, MPC2013.* (pp. 256-264).

Davoodi, M. M., Sapuan, S. M., Ahmad, D., Ali, A., Khalina, A., & Janoobi, M. (2010). Mechanical Properties of Hybrid Kenaf/Glass Reinforced Epoxy Composite for Passenger Car Bumper Beam. *Materials and Design*, *31*(10), 4927-4932.

El-Shekeil, Y. A., Sapuan, S. M., Zainuddin, E. S., & Abdan, K. (2011). Effect of fibre loading on the mechanical properties of kenaf fibre reinforced thermoplastic polyurethane composite. *Key Engineering Materials*, *471*, 297-302.

Faruk, O., Bledzki, A. K, Fink, H. P., & Sain, M. (2012). Progress in polymer science biocomposites reinforced with natural fibres: 2000-2010. *Progress in Polymer Science*, *37*(11), 1552-1596.

Fulton, I., Qatu, M. S., & Shi, S. (2011). Mechanical properties of kenaf-based natural fibre compsite. *Proceeding of 16th International Conference on Composite Structure (ICCS 16),* p.8.

Girisha, C., Sanjeevamurthy, & Rangassinivas, G. (2012). Tensile properties of natural fibre-reinforced epoxy-hybrid composite. *International Journal of Modern Engineering (IJMER)*, *2*(2),471-474.

Groover, M. P. (2007). *Fundamental of modern manufacturing: materials, procecesses and systems*. John Wiley & Sons, Inc.

Hariharan, A. B. A., & Khalil, H. P. S. A. (2005). Lignocellulose-based hybrid bilayer: Part 1. *Journal of Composite*, *39*, 663-684.

Ishak, M. R., Leman, Z., Sapuan, S. M., Edeerozey, A. M. M., & Othman, I. S. (2010). Mechanical properties of kenaf bast and core fibre reinforced unsaturated polyester composites. *IOP Conference Series: Materials Science and Engineering*, *11*.

Jawaid, M., Khalil, H. P. S. A., Bakar, A. A., Hassan, A., & Dunyani, R. (2012). Effect of jute fibre loading on the mechanical and thermal properties of oil palm-epoxy composites. *Journal of Composite Materials*, *47*(13), 1633–1641.

Joseph, S., Sreekala, M. S., Oomen, Z., Khoshy, P., & Thomas, S. (2002). A comparison of the mechanical properties of phenol formaldehyde composites reinforced with banana fibres and

glass fibres. *Journal of Composites Science and Technology*, *62*, 1857-1868.

Liu, W., Drzal, L. T., Mohanty, A. M., & Misran, M. (2007). Influence of processing methods and fibrelength on physical properties of kenaf fibre reinforced soy based biocomposites. *Journal of Composite Engineering Part B*, *38*, 352-359.

Mohanty, A. K., Misra, M., & Drzal, L. T (2005). *Natural fibre, biopolymer & biocomposite*. Taylor & Francis Group.

Mukhopadhyay, S., Vijay, G., Talwade, R., Dhake, J. D., & Pegoretti, A. (2006). Some studies on banana fibres. *International Conference on Advances in Fibrous Materials, Non Woven and Technical Textiles*. 7-9 August 2006. Coimbatore, India.

Muhd, N., & Akil, H., (2010). Polymer-plastics technology and engineering thermal properties of kenaf-filled chitosan biocomposites thermal properties of kenaf-filled chitosan biocomposites. *Polymer-Plastics Technology and Engineering*, *49*, 37-41.

Nguong, C. W., Lee, S. N. B., & Sujan, D. (2013). A review on natural fibre reinforced polymer composites. *Journal of Engineering and Technology*, *73*, 1123-1130.

Ochi, S. (2008). Mechanical properties of kenaf fibre and kenaf/pla composites. *Mechanics of Materials*, *40*, 446-452.

Saheb, D. N. & Jog, J. P. (1999). Natural fibre polymer composites: A review. *Journal of Advances in Polymer Technology*, *18*(4), 351-363.

Samal, S. K., Mohanty, S., & Nayak, S. K. (2009). Banana/glass fibre-reinforced polypropylene hybrid composites: Fabrication and performance evaluation banana/glass fibre-reinforced polypropylene hybrid composites: Fabrication and performance evaluation. *Polymer-Plastics Technology and Engineering*, *48*, 397-414.

Sapuan, S. M., Zan, M. N. M., & Arora, P. R. (2005). Tensile and flexural strengths of coconut spathe-fibre reinforced epoxy composites. *Journal of Tropical Agriculture, 43*(1-2), 63-65.

Sapuan, S. M., Harimi, M., & Maleque, M. A. (2003). Mechanical properties of epoxy/coconut shell filler particle composite. *Journal of Arabian for Science and Engineering*, *28*(2), 171-181.

Saxena, M., Pappu, A., Haque, R., & Sharma, A. (2011). Sisal fibre based polymer composites and their application. *Cellulose Fibres: Bio- and Nano-Polymer Composites*. Springer-Verlag, Berlin. pp.589-660.

Shanmugam, D., & Thiruchitrambalam, M., (2013). Static and dynamic mechanical properties of alkali treated unidirectional continuous palmyra palm leaf stalk fibre / jute fibre reinforced hybrid polyester composites. *Materials and Design*, *50*, 533-542.

Shyamraj, R., Sivasubramanian, P., & Dhanusha, P. B. (2013). Investigations on mechanical properties of pineapple fibre reinforced polymer composite material. *International Journal of Scientific and Engineering Research*, *4*(8), 4-8.

Sumaila, M., Amber, I., & Bawa, M. (2013). Effect of fibre length on the physical and mechanical properties of random oriented, nonwoven short banana (*Musa balbisiana*) fibre/epoxy composite. *Asian Journal of Natural and Applied Sciences*, *2*(1), 39-49.

Syafinaz, I. M. A. T., Talib, R. A., Abdan, K., & Ling, C. N. (2012). Mechanical and physical properties of kenaf-derived cellulose (kdc)-filled polylactic acid (pla) composites. *Bioresources*, *7*(2), 1643-1655.

Vijay G., Talwade R., Dhake, J. D., & Pegoretti, A. (2006). In Mukhopadhyay S., Some studies on banana fibres *International Conference on Advances in Fibrous Materials, Nonwoven and Technical Textiles*, 7-9 August 2006, Coimbatore, India.

Vinod, B., & Sudev, L. J. (2013). Effect of fibre length on the tensile properties of palf reinforced bisphenol composites. *International Journal of Engineering, Business and Enterprise Applications (IJEBEA), 5*, 158-162.

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Effect of Cutting Speed on Cutting Torque and Cutting Power of Varying Kenaf-Stem Diameters at Different Moisture Contents

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ABSTRACT

This study focused on the development of an efficient cutting system for kenaf harvesters. Laboratory experiments were conducted on cutting kenaf stems of variety V36 using a rotary serrated cutting system. The Torque Trak 10k data acquisition system was used for the experiment. The effect of cutting speed on cutting torque and cutting power of varying kenaf-stem diameters and at different moisture contents was investigated. Four different cutting speeds of 400 rpm, 500 rpm, 600 rpm and 700 rpm were used. The experiments showed that cutting speed had significant effect on cutting torque and cutting power requirements. The cutting speed was directly proportional to the specific cutting power, while the cutting torque was inversely proportional to the moisture content. Increasing the rotational speed from 400 rpm to 700 rpm reduced the cutting torque from 1.91 Nm to 1.49 Nm. The cutting torque was observed to be higher at lower moisture levels of less than 35%. As the moisture content was increased to values greater than 35%, the torque decreased considerably. This invariably indicated that an increase in moisture content reduced cutting torque as shown by the model coefficient of moisture content. Thus, more energy saving and hence, high efficiency, were achieved at high cutting speeds as compared to impact cutting system at similar speeds. Regression equations capable of predicting cutting torque and cutting power at varying stem diameters and cutting speeds, in relation to kenaf stem moisture contents, are presented.

Keywords: Cutting power, cutting speed, cutting torque, harvester, kenaf stem, moisture content

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INTRODUCTION

Kenaf (*Hibiscus cannabinus*), a relatively new crop to Malaysia, is a fibre crop that can grow very well in most parts of the country and matures in 4 to 5 months (Mat Daham, 2009).Kenaf has been known as a fibre crop with high earnings, but the cultivation process, which includes harvesting, transportation, storage and post-harvest, are still labour intensive and takes up a lot of time (Ghahraei *et al*., 2011). The assessment of techniques for kenaf harvesting remains a significant aspect of exploitation (Charles *et al*., 2002; Manuel, 2011; Dauda *et al.*, 2013).

The constituents of harvester power and its theoretical approaches along with results of field experiments on harvesters made the major sources of its power demand known. Cutting power highly relies on chop length and crop moisture content. However, the major constituent of power can be identified as that due to cutting, supplying kinetic energy to the material while overcoming the frictional resistance of the material (O'Dogherty, 1982).

Generally, harvester power consumption has been found to increase linearly with an increasing rate of forage throughput, above a value which represents the no-load power requirement of the machine (O'Dogherty, 1982).

Persson (1987) reviewed various studies on cutting speed and came to the conclusion that cutting speed had slight effect on cutting power; furthermore, power losses resulting from material acceleration often increased when there was an increase in cutting speed. When a critical value of pressure caused by the blade was reached, plant stem cutting occurred and resulted in multiple modes of tissue failure (Persson, 1987; Srivastava *et al*., 2006).

Critical cutting speed of 25-30 m/s for grass was reported by O'Dogherty & Gale, (1991), while speeds lower than that resulted in higher stubble heights and large stem deflections. O'Dogherty and Gale (1991) defined cutting speed as the speed at which there is a rapid increase in stubble length as cutting becomes rapidly less efficient. Many plant parameters influence cutting energy and force these parameters include stem structure, fibre ultimate tensile strength and fibre stiffness (Persson, 1987). Other parameters of importance are the design of the knife blade, cutting material properties and the mode of operation (Prince *et al*., 1958; Suryanto *et al.,* 1993; Womac *et al*., 2005; Ghahraei *et al*., 2011)**.**

However, some researchers studied specific biomass materials; Mesquita and Hanna (1995) studied soybean stalks El Hag *et al*. (1971) studied cotton stalks Prasad and Gupta (1975) studied maize stalks Prince *et al*. (1969) studied alfalfa stems and Chen *et al*., (2004) studied hemp. Igathinathane *et al*., (2008) reported a study on knife grid size reduction of switch grass revealed that cutting energy is related to stem diameter, moisture content, stem shear strength, dry matter density and maximum cutting force.

The thickness of blunt cutting blades over a range 1 to 3 mm had no significant effect on critical cutting speed or on specific cutting energy when cutting at or above the

critical speed. At lower speeds, the 3-mm thick blade required much larger specific cutting energies than the blades of 1 and 2-mm thickness. Blade thickness had no marked effect on specific peak cutting force (O'Dogherty & Gale, 1991).

Low blade velocities are satisfactory for thick-stemmed plants but higher velocities are required for light-stemmed plants such as grass. Thus, disc and drum type rotary mowers typically employ blade velocities of 71-84 m/s. (McRandal & McNulty, 1978).

Cutting energy measurement is considered a significant criterion for comparing any cutting system effectiveness. The cutting element operational principle adopted in any harvesting tool or machine can largely be categorised into two classes: impact cutting and counter edge cutting. Manually operated swinging type tools such as the cradle, scythe, long bladed hoe and power-operated harvesters are such implemets where crops are cut by impact. Generally, scientists are of the resolve that in impact cutting, the energy expended to overcome the stem shearing resistance is similar to the energy needed for quasi static cutting plus the energy expended to overcome friction (Koloor & Kiani, 2007; Reza, 2007).

Data on plant physical and mechanical properties and the power or energy requirement of equipment have been very important to carefully choose design and operational parameters of the equipment (Persson, 1987). Such data are desired for the design of kenaf harvesters in order to achieve appropriate machine functions and efficient energy utilisation. The objective of this study was to determine the cutting torque and power requirements to cut kenaf stems using a rotary serrated cutting system.

MATERIALS AND METHODS

Kenaf variety V36 from INTROP/TPU research field located at 2°58.844'N, 101°42.722'E, Universiti Putra Malaysia was used for the experiment. Kenaf stems were manually harvested at 12 weeks after planting (WAP). The stems were cut close to the ground, leaving stubble about 10 cm in height. Moisture content (Mc), weight (W), height (H) and diameter (D) of the stems were recorded. The diameter was measured using Mitutoyo absolute digimatic vernier callipers (precision 0.010). The diameter was measured at the point where the cutting blade was expected to cut the stem each time in the experiment. The moisture content (Mc) was determined using the oven-dry method at 104°C for 24 hours (ASABE, 2008, 2012). The stems were divided into three major parts: bottom, middle and top (Ghahraei *et al*., 2011). As the stem size differed, the cutting torque was determined per unit area of the cut stem measured over the cutting plane and expressed as the specific cutting torque.

The torque required to cut the stems was determined using a rotary serrated cutting system with 25° knife edge angle (Ghahraei *et al*., 2011) incorporated in the stem cutting setup (Suryanto *et al*., 2009). The setup was driven by a low voltage speed drive Toshiba model VFNC1S-2015P-W-1HP-200V,

single phase input and three phase output. The speeds were varied at 400, 500, 600 and 700 rpm.

The kenaf stems were manually fed into the rotating blade as shown in Fig.1. A torque data acquisition system TorqueTrak 10k telemetry system was used to acquire the data at Gain and Transmitter settings of 4000. During the cutting tests, kenaf stem samples were brought to the biomaterial processing laboratory of the Department of Biological and Agricultural Engineering, Universiti Putra Malaysia. Some of the stems were stored in the cold room at an average temperature of 4°C to monitor the stems' moisture contents. At each test, voltage signal on the TorqueTrak digital receiver and rotational speeds were recorded and AutoZero turned on to zero out the offset voltage at zero torque in relation to the installed strain gauge.

A full-scale torque calculator available at http:www.binsfeld.com/calculators/tt10k_range (Binsfeld, 2013) was used to determine the full-scale torque on the shaft and the sensitivity per unit volt output (Fig.2) and the cutting power was calculated using equation 1.

$$P_{rot} = M \chi \omega \qquad \text{Eqn. 1}$$

Where:

P_{rot} = rotational mechanical power (watts)

M = torque (Nm)

ω = angular velocity (rads/sec)

In calculating rotational power, it is necessary to convert the velocity from rpm to rads/sec. Therefore,

$$\omega_{rad/\sec} = \omega_{rpm} \chi \frac{2\pi}{60} \qquad \text{Eqn. 2}$$

The experiments were replicated three times.

Fig.1: Kenaf stem cutting setup.

Statistical Analysis for Cutting Speeds, Cutting Torque and Cutting Power Requirements for Cutting Blade

All data generated were subjected to analysis of variance (ANOVA) to evaluate the level of significance (p = 0.05) and the trend of linear relationship between various parameters of kenaf stems and cutting speeds estimated using SPSS statistics software version 20.

A single-factor-ANOVA was run to evaluate the effect of different levels of cutting speed on the cutting torque, cutting power and moisture content. For this purpose, the cutting speeds used for the experiment were set at 400, 500, 600 and 700 rpm. Furthermore, a simple trend

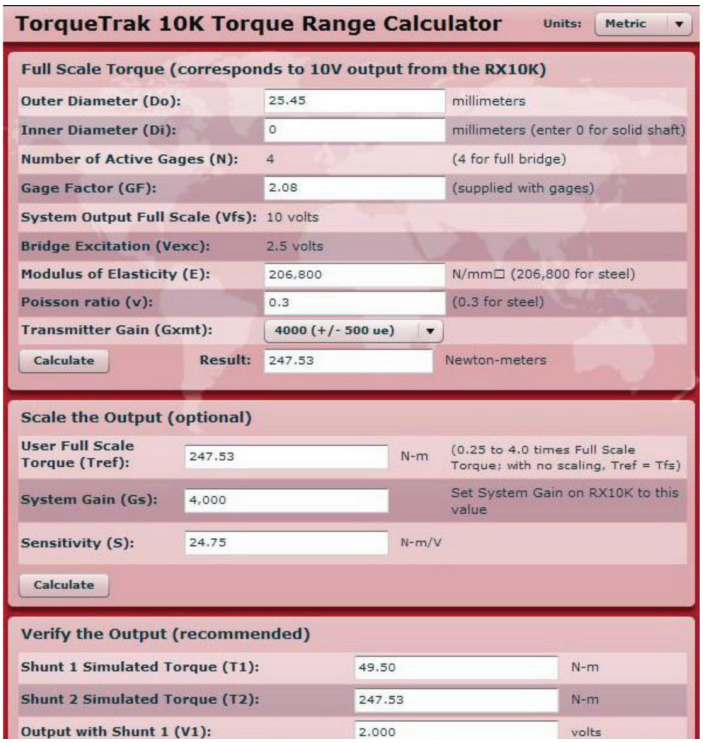

Fig.2: Torque range calculator. Source: (Binsfeld, 2013).

analysis was performed to fit the data among variables such as cutting speed (rpm), torque (N.m), cutting power (W), moisture content (%) and stem diameter (mm).

The data generated were configured in a Randomized Complete Block Design (RCBD) in which Analysis of Variance (ANOVA) was performed to determine the statistical significance of the independent factors of study, namely, cutting speed (cs) and stem diameter (sd) on cutting torque (ct) and cutting power (cp), respectively. Duncan's Multiple Range Test (DMRT) was performed in each case for factors that were significantly different. All tests were conducted at 5% levels of significance. The need for a predictive model that would also offer an explanation for the relationship between the variables of the study gave rise to the use of multiple and simple regression analyses. These models were:

$$m_1: ct = f(cs, sd) + \varepsilon_1 \quad \text{Eqn. 3}$$

$$m_2: cp = f(cs, sd) + \varepsilon_2 \quad \text{Eqn. 4}$$

$$m_3: ct = f(mc) + \varepsilon_3 \quad \text{Eqn. 5}$$

$$m_4: ct = f(cs) + \varepsilon_4 \quad \text{Eqn. 6}$$

$$m_5: cp = f(cs) + \varepsilon_5 \quad \text{Eqn. 7}$$

The direction and magnitude of the coefficient enabled the interpretation of existing relationships along with the correlation matrix. Basically, two forms of relationship were of interest, namely, positive (direct relationship) or negative (inverse relationship).

Model Development on Cutting Torque (ct) and Cutting Power (cp)

Randomized Complete Block Design (RCBD) with replication was used in designing the experiment. The experimental factors considered were cutting speed (cs) at 4 levels and kenaf stem diameter (sd) at 3 levels, namely, bottom (1), middle (2) and top (3) with the 3 replicates at each condition of cutting speed and stem diameter, respectively for cutting torque (ct) and cutting power (cp), respectively. The model corresponding to the RCBD with replication is given as follows (Equations 8 and 9):

$$y_{ij} = m + cs_i + sd_j + \varepsilon_{ij} \quad \text{Eqn. 8}$$

$$y_{ij} = m + t_i + b_j + \varepsilon_{ij} \quad \text{Eqn. 9}$$

Where:

m = overall mean effect

t_i = treatment effect due to cutting speed

b_j = treatment effect due to stem diameter

ε_{ij} = random error which is being minimised

Testing

$$H_{01}: T_1 = T_2 = T_3 + T_4 \quad \text{Eqn. 10}$$

(non significant)

$$H_{11}: T_1 \neq T_2 \neq T_3 \neq T_4 \quad \text{Eqn. 11}$$

(significant)

$\alpha = 5\%$

Consequently, H_0 is rejected if $F_c > F_\alpha$ (v1,v2) ($P < 0.05$).

RESULTS AND DISCUSSION

Analysis of Variance on Cutting Torque (ct)

From the result, the model was significant with $F = 261.407$ ($P < 0.05$) as shown in Table 1. Similarly, both content term and levels of cutting speed were statistically significant with $P < 0.05$. However, stem diameter was not significant with $F = 1.114$ and $P=0.341 > 0.05$.

It was therefore worthwhile at this point to investigate the level of cutting speed that made the difference, so Duncan's Multiple Range Test (DMRT) was performed in investigation.

TABLE 1
Analysis of Variance Dependent Variable: Cutting Torque (ct)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.883[a]	5	.177	261.407	.000
Intercept	104.721	1	104.721	154972.321	.000
cs	.882	3	.294	434.936	.000
sd	.002	2	.001	1.114	.341
Error	.020	30	.001		
Total	105.625	36			
Corrected Total	.903	35			

a. R Squared = .978 (Adjusted R Squared = .974), cs = cutting speed, sd = stem diameter

$H_0: T_1 = T'_1$ Eqn. 12

The results in Table 2 suggest that subset 1 level 4 (700 rpm) provided the most significant difference since the interest was on minimum torque, thus cutting torque was most significant. It also follows that levels 3 (600 rpm), 2 (500 rpm) and 1 (400 rpm) were significant in that order.

TABLE 2
Duncan's Multiple Range Test on Cutting Torque (ct)

Duncan a, b

cs	N	Subset			
		1	2	3	4
700	9	1.4878			
600	9		1.6344		
500	9			1.8067	
400	9				1.8933
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = .001.

Linear Regression Model on Cutting Torque

Model 1:

$$ct = f(cs, sd, \varepsilon) \quad \text{Eqn. 13}$$

The coefficient of multiple determinations predicted a measure of goodness of fit of the model Equation 13. This gave the predictive power of the model specified. The closer R^2 is to 1, the more adequate is the model.

H_0: the model is not significant
H_1: the model is significant

From Table 3, $F_c = 422.323 > F_{0.05\,(2,33)} = 19.46$ or $P < 0.05$. H_0 was rejected and it can be concluded that the model was statistically significant at 5% level as shown in Table 3. The level of relativity of this was 0.960, which was an indication that the model provided a good fit.

The model is thus given as:

$$ct = 2.412 + 0.003sd - 0.001cs \quad \text{Eqn. 14}$$

TABLE 3
Cutting Torque Model

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.981[a]	.962	.960	.03208	.962	422.323	2	33	.000

a. Predictors: (Constant), cs1, sd1

TABLE 4
Cutting Power Model

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.972a	.945	.941	2.74229	.945	281.536	2	33	.000

a. Predictors: (Constant), cs1, sd1

Testing $H_{0(1)}$: β(sd) = 0 (not significant)
$H_{0(2)}$: β(cs) = 0 (not significant)

Against

$H_{1(1)}$: β(sd) ≠ 0 (significant)
$H_{1(2)}$: β(cs) ≠ 0 (significant)

Therefore, the statistic test was:

$$t = \frac{\beta}{s\beta} \quad \text{Eqn. 15}$$

It also means that H_0 was rejected if $t_c > t_{\alpha, (2,33)}$ or (P < 0.05). Here, it was clear that the constant was significant. Similarly, β(cs) was significant but negative. The implication was that cutting speed constituted the significant factor but was inversely related with cutting torque. This was also evidenced by the correlation matrix, indicating that the correlation coefficient between cutting torque and cutting speed was 0.98, which was a very strong negative correlation.

Model 2:

$$\text{cutting power} = f(cs, sd, \varepsilon) \quad \text{Eqn. 16}$$

The model is given as:

$$cp = 39.070 + 0.220sd + 0.097cs \quad \text{Eqn. 17}$$

The coefficient of determination was 0.941, indicating a good fit (Table 4). Thus from the ANOVA and model summary statistics, $F_c = 281.536 > F = 19.46$ (P < 0.05) also indicated that the model was good.

On the model parameters, it was clear that the coefficient of cutting speed was significant and positive while that of the stem diameter was not significant. Obviously, cutting speed had a strong positive correlation with cutting power. Consequently, as cutting speed increased, cutting power also increased.

Analysis of Variance on Cutting Power (cp)

The model is:

$$y_{ij} = m + cs_i + sd_j + \varepsilon_{ij} \quad \text{Eqn. 18}$$

$$y_{ij} = m + t_i + b_j + \varepsilon_{ij} \quad \text{Eqn. 19}$$

The results in Table 5 show that the overall model was significant with F = 410.107 (P < 0.05). The intercept, which was the mean effect, was also significant with F = 155375.287 (P < 0.05). Similarly, cutting speed was significant with F = 682.808 (P

TABLE 5
Analysis of Variance Table Dependent Variable: Cutting Power (cp)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4417.916[a]	5	883.583	410.107	.000
Intercept	334758.674	1	334758.674	155375.287	.000
Cs	4413.366	3	1471.122	682.808	.000
Sd	4.551	2	2.275	1.056	.360
Error	64.636	30	2.155		
Total	339241.226	36			
Corrected Total	4482.552	35			

a. R Squared = .986 (Adjusted R Squared = .983)

TABLE 6
Duncan's Multiple Range Test on Cutting Power (cp)

		Duncan[a,b]			
cs	N	Subset			
		1	2	3	4
400	9	79.4311			
500	9		94.5767		
600	9			102.6767	
700	9				109.0378
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = 2.155.
a. Uses Harmonic Mean Sample Size = 9.000.
b. Alpha = 0.05

< 0.05). However, stem diameter was not significant with F = 1.056 (P > 0.05), and this suggested that the independent variable cutting speed was statistically significant.

On the level of cutting speed that was significantly different, Duncan's Multiple Range Test was performed in investigation as shown in Table 6. Since the interest here was in the minimum power, subset 1 on level 1(400 rpm) provided the minimum power required while subset 4 on level 4 (700 rpm) provided the maximum power required to cut the kenaf stems.

Effects of Cutting Speed on Cutting Power

From the results obtained from the experiment to determine the effect of cutting speed on cutting power, it could be deduced from Fig.3 that the best rotational speed ranged between 600 and 700 rpm. From the regression analysis conducted, it could be seen that cutting speed had strong positive correlation with cutting power. Consequently, as cutting speed increased, cutting power also increased. At speeds less than 600 rpm, the cutter tended to break the smaller stems rather than cut them. This possibly occurred due to the fact that at lower speeds, the cutting impact was less, sufficiently causing tissue failure in the stem. Power consumption of the cutting knife increased as the speed also increased from 79.99 W at 400 rpm to 109.2 W at 700 rpm. The increase in power may be attributed to increase in speed. This was in conformity with studies conducted by Gupta and Oduori (1992), Persson (1987) and Veikle (2011).

Effects of Cutting Speed on Cutting Torque

Regression analysis conducted on the data indicated a correlation between the blade rotational speed and the specific cutting torque. The cutting torque linearly decreased from about 1.9 Nm/cm^2 to 1.5 Nm/cm^2 as the speed increased from 400 rpm to 700 rpm (Fig.4). This trend of result was in agreement with a similar study conducted

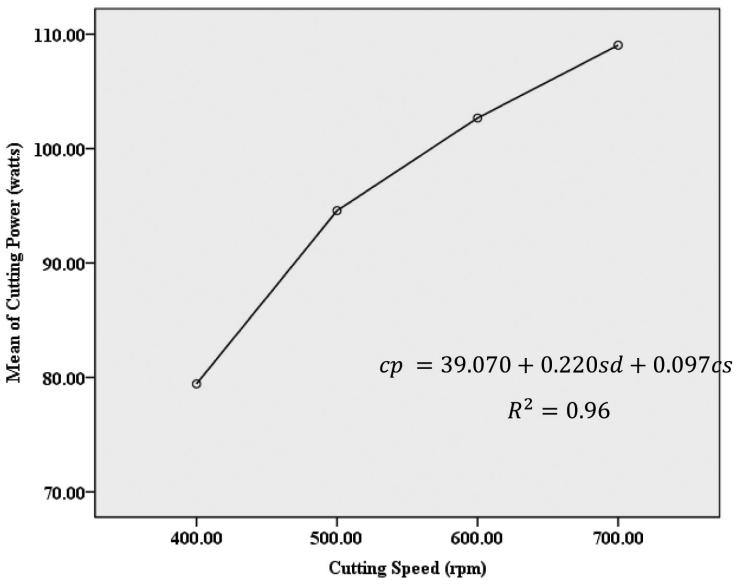

Fig.3: Effect of cutting speed on cutting power.

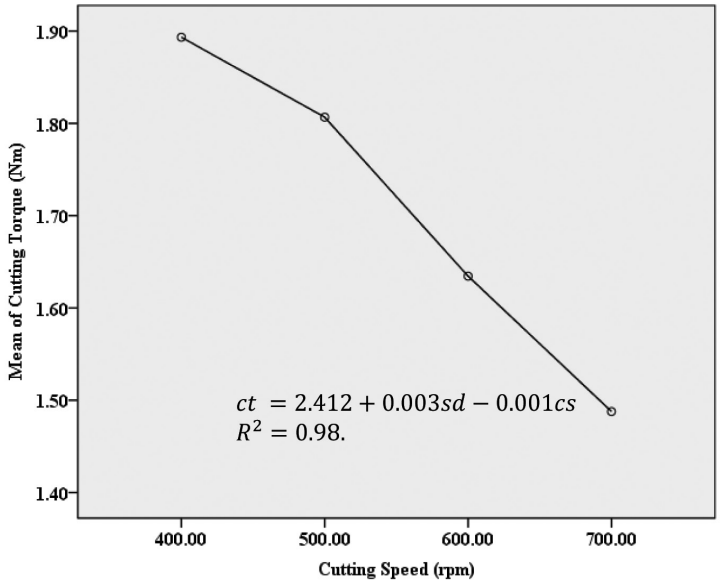

Fig.4: Effect of cutting speed on cutting torque.

on impact cutting of kenaf stems to predict the cutting torque with regard to the blade rotational speed by Ghahraei *et al*. (2011). In comparison with the results obtained by Ghahraei *et al*. (2011), it was discovered that using a rotary serrated cutting blade reduced the cutting torque and the cutting power requirement by about 60%.

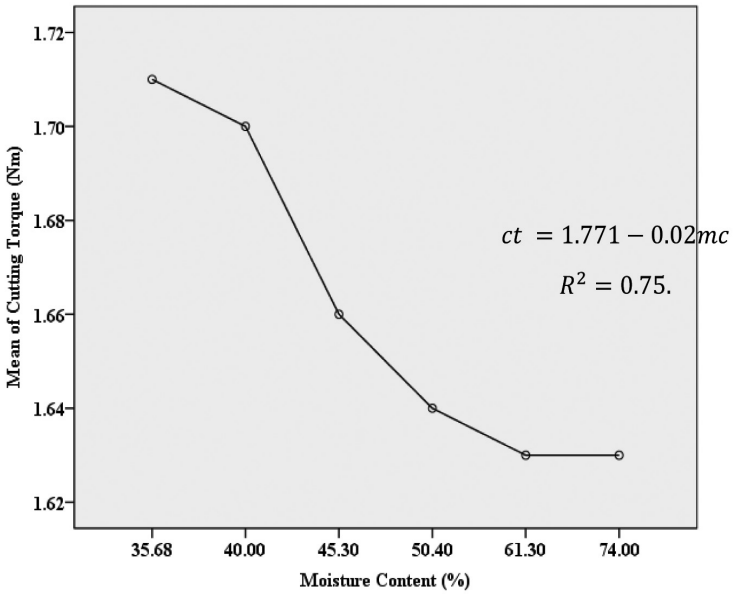

Fig.5: Effect of stem moisture content on cutting torque.

In determining the relationship between kenaf stem moisture content and cutting torque, the regression model is in the form of Equation 20:

$$ct = 1.771 - 0.02mc \qquad \text{Eqn. 20}$$

The result showed that an increase in moisture content reduced cutting torque as shown in the model coefficient of moisture content (Fig.5).

In the analysis of variance at 0.05 level, the cutting torque was observed to be higher at lower moisture levels of less than 35%. As the moisture content increased to values greater than 35%, the torque decreased to a constant value at about 50% (Fig.5), indicating that the power requirement reduced with increase in moisture content. This result conforms to similar studies conducted by Nowakowski (2012a, 2012b) and Veikle (2011). It was also observed that the highest value of cutting power required was recorded at the lowest moisture contents.

CONCLUSION

Kenaf stem cutting speed studied in this research greatly influenced cutting torque and cutting power with varying stem diameters and moisture contents. Higher cutting speed resulted in a decrease in cutting torque from 1.91-1.49 Nm. It was also observed that cutting torque was higher at lower moisture levels of less than 35%. As the moisture content increased to values greater than 35%, the cutting torque decreased to a constant value of about 50%. The effect of cutting speed on cutting torque and cutting power was statistically significant. The data generated will help engineers in developing effective harvesting machinery for kenaf stems.

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REFERENCES

ASABE. (2008). S358. 2–moisture measurement–forages, ASABE Standards, 608. *American Society of Agricultural and Biological Engineers,* St. Joseph, MI.

ASABE. (2012). *Adjusting forage harvester test data for varying crop moisture.* Retrieved from https://elibrary.asabe.org/abstract.asp?aid=41267&t=2&redir=&redirType=

Binsfeld. (2013). *Full scale torque calculator.* Available at *http:www.binsfeld.com/calculators/tt10k_range accessed 12th March, 2012*.

Charles, L. W. I., Venita, K. B., & Robert, E. B. (2002). Kenaf harvesting and processing. *Trends in new crops and new uses. Janick, J., & Whipkey, A. (Eds.). ASHS Press, Alexandria, VA.*

Chen, Y., Gratton, J. L., & Liu, J. (2004). Power requirements of hemp cutting and conditioning. *Biosystems Engineering*, *87*(4), 417-424.

Dauda, S. M., Ahmad, D., Khalina, A., & Jamarei, O. (2013). Performance evaluation of a tractor mounted kenaf harvesting machine. *Academic Research International*, *4*(2), 70-81.

El Hag, H. E., Kunze, O. R., & Wilkes, L. H. (1971). Influence of moisture, dry-matter density and rate of loading on ultimate strength of cotton stalks. *Transactions of the ASABE*, *13*, 713-716.

Ghahraei, O., Ahmad, D., Khalina, A., Suryanto, H., & Othman, J. (2011). Cutting tests of kenaf stems. *Transactions of the ASABE, 54*(1), 51-56.

Gupta, C., & Oduori, M. (1992). Design of the revolving knife-type sugarcane basecutter, *Transactions of the ASAE, 35,* 1753-1759.

Igathinathane, C., Womac, A. R., Sokhansanj, S., & Narayan, S. (2008). Knife grid size reduction to pre-process packed beds of high- and low-moisture switchgrass. *Bioresource Technology*. *99*(7), 2254-2264.

Koloor, R. T., & Kiani, G. (2007). Soybean stems cutting energy and the effects of blade parameters on it. *Pakistan Journal of Biological Sciences*, *10*(9), 1532.

Manuel, S. (2011). Insights on microbial and biochemical aspects of retting for bast fiber plant processing in a bioreactor. *Titolo Della Tesi Di Dottorato*. Universita Di Fermia.

Mat Daham, M. (2009). Prospects of commercializing mechanized kenaf production and processing. *Proceedings of the National Conferenceon Agricultural and Food Mechanization, Malaysian Agricultural Research and Mechanization Institute (MARDI). 23-25 June*, 64-66.

McRandal, D. M., & McNulty, P. B. (1978). Impact cutting behaviour of forage crops I. Mathematical models and laboratory tests. *Journal of Agricultural Engineering Research*, *23*(3), 313-328.

Mesquita, C. M., & Hanna, M. A. (1995). Physical and mechanical properties of soybean crops. *Transactions of the ASABE*, *38*, 1655-1658.

Nowakowski, T. (2012a). Energetic loads of header cutting unit for harvesting the energetic plants. *Anals of Warsaw University of Life Sciences – SGGW*, *60*, 35-49.

Nowakowski, T. (2012b). Specific energy for cutting stems of the basket willow (*Salix vimilalis* L.). *Anals of Warsaw University of Life Sciences – SGGW*, *60*, 25-33.

O'Dogherty, M. J. (1982). A review of research on forage chopping. *Journal of Agricultural Engineering Research*, *27*(4), 267-289.

O'Dogherty, M. J., & Gale, G. E. (1991). Laboratory studies of the effect of blade parameters and stem configuration on the dynamics of cutting grass. *Journal of Agricultural Engineering Research*, *49*, 99-111.

Persson, S. (1987). *Mechanics of cutting plant material*. American society of agricultural and biological engineers, ASABE. St. Joseph, Michigan.

Prasad, J., & Gupta, C. P. (1975). Mechanical properties of maize stalk as related to harvesting. *Journal of Agricultural Engineering Research*, *20*(1), 79-87.

Prince, R., Wheeler, W., & Fisher, D. (1958). Discussion on energy requirements for cutting forage. *Agricultural Engineering*, *39*(10), 638.

Prince, R. P., Bartok, J. W., & Bradway, D. W. (1969). Shear stress and modulus of elasticity of selected forages. *Transactions of the ASABE*, *12*, 426-429.

Reza, T. K. (2007). Paddy stems cutting energy and suggested blade optimum parameters. *Pakistan Journal of Biological Sciences*, *10*(24), 4523-4526.

Srivastava, A. K., Carroll, E. G., Roger, P. R., & Dennis, R. B. (2006). *Engineering principles of agricultural machines,* (2nd Ed.). ASABE, St. Joseph, Michigan.

Suryanto, H., Ahmad, D., Yahya, A., Akande, F., & Syahrita, K. (2009). Cutting tests of oil palm empty fruit bunches. *Transactions of the ASABE*, *52*(3), 723-726.

Suryanto, H., Bardaie, M. Z., Ahmad, D., & Yahya, A. (1993). Force and energy requirement for cutting oil palm fresh fruit bunch. *Planter*, *69*, 399-407.

Veikle, E. (2011). *Modeling the power requirements of a rotary feeding and cutting system.* Doctoral dissertation, University of Saskatchewan.

Womac, A., Yu, M., Igathinathane, C., Ye, P., Hayes, D., Narayan, S., Sokhansanj, S., & Wright, L. (2005). Shearing characteristics of biomass for size reduction. *ASAE Paper,* 56058.

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Reinforcing Mechanical, Water Absorption and Barrier Properties of Poly(Lactic Acid) Composites with Kenaf-Derived Cellulose of Thermally-Grafted Aminosilane

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ABSTRACT

The effects of filling poly(lactic acid) (PLA) composites with cellulose thermally-grafted with hydrolysed 3-aminopropyltriethoxysilane (APS) were investigated. Composites containing 30 wt% of kenaf-derived cellulose (C) and silane-grafted cellulose (SGC) were melt-blended into a PLA matrix before being hot-pressed into 0.3 mm sheets. The tensile strength of neat PLA was 47 MPa. With addition of C and SGC, the tensile strength was improved by 13% and 23%, respectively. The tensile modulus was approximately doubled for both of the composites. PLA/C and PLA/SGC composites remained brittle with marginally lowered elongation at break. The addition of C and SGC significantly increased the oxygen barrier of PLA with the reduction of oxygen transmission rate (OTR) of PLA at 76.6 cc/m^2/day to 42.2 cc/m^2/day and 40.3 cc/m^2/day, respectively. This was due to the tortuous path created and crystallites induced by the fillers. The water vapour transmission rate (WVTR) for PLA, PLA/C and PLA/SGC was in the range of 21-23 g/m^2/day. From the water absorption test, PLA/SGC reported slightly better water resistance as compared to PLA/C. The reinforcing results from these bio-based materials may suggest contribution towards packaging oxygen and moisture sensitive food.

Keywords: Cellulose, kenaf, mechanical barrier, poly(lactic acid), silane coupling agent, water absorption

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INTRODUCTION

When environmental conservation had not yet become an issue, many manufacturers used manmade fibres as fillers in composites due to their superior composite reinforcing ability (Mohanty *et al*., 2000). It was not until the late 1980s that research into natural fibres as composite fillers became important (Westman *et al.,* 2010). Specifically in the plastic packaging field, research works on natural fibre reinforced petroleum-derived plastics, the so called "big four" polyethylene (PE), poly(propylene) (PP), polystyrene (PS) and poly(vinyl chloride) (PVC), became prevalent (Mohanty *et al*., 2000). In recent years, with the intensifying environmental concern and stringent government regulations especially in the Asian and European countries, development of packaging materials that are attuned to the environment such as bio-derived polymers, recyclable materials and reusable packaging have become a main focus (Holbery & Houston, 2006; Satyanarayana *et al*., 2009; Johansson *et al*., 2012).

Noticing the increasing demand of "greener" products, it became the impetus of the present research to produce an entirely bio-sourced composite specifically for food packaging applications. Kenaf-derived cellulose and poly(lactic acid) (PLA) were chosen as the filler and matrix for the present research. However, as was agreed by various authors, the major drawback of using natural fibres as composite filler is their marked hydrophilicity and polar nature (Khan & Hassan, 2006; Masirek *et al*., 2007; Xie *et al*., 2010; Pang & Ismail, 2013) as this leads to other problems, such as poor interfacial adhesion with the non-polar polymeric matrixes (Abdelmouleh *et al*., 2007; Lu *et al*. 2008; Kalia *et al*., 2009), agglomeration and uneven dispersion of fibres within the matrix (Kazayawoko *et al*., 1999; Masirek *et al*., 2007) and poor resistance towards moisture (Bisanda & Ansell, 1991; Castellano *et al*., 2004; Kalia *et al*., 2009).

To improve the compatibility of natural fibres and polymer matrix, fibre and/or matrix modification with physical or chemical treatments can be done (George *et al*., 2001). Among other modifications, treating fillers with a silane coupling agent was frequently practised by various authors (Khan & Hassan, 2005; Huda *et al*., 2008; Lee *et al*., 2009). However, thermal grafting of the hydrolysed silane or silanol onto the fillers before addition into matrix was comparatively little. Thermal grafting was highlighted in this research as a procedure to enable chemical condensation of the silanol and siloxane (-Si-O-Si-) polymer networks onto the fibres, which was initially only hydrogen bonded (Si-OH). Unlike hydrogen bonding, the siloxane bridges (Si-O-C) from the condensation cannot be hydrolysed off and thus desorption of the coupling agent is diminished (Brochier Salon *et al*., 2005). An in-depth review with regards to this study was done by Xie and co-workers (2010).

Recently, the authors reported elsewhere the thermal properties of PLA composites that were reinforced with aminosilane-grafted cellulose derived from kenaf fibres (Tee *et al*., 2013). As the composites

were characterised towards packaging applications, the barrier properties of the material became customary in maintaining the quality and shelf life of the food product (Siracusa, 2012). At least to our knowledge, little work on the barrier properties of a thermoplastic composite was published. Thus, the objective of the present work was to investigate the mechanical, water absorption and barrier properties of the PLA composites reinforced with silane grafted cellulose.

MATERIALS AND METHOD

Materials

Poly(lactic acid) (PLA) resin (Ingeo 2003D, with MFI of 6 g/10 min at 210°C and bulk density of 0.85 g/cm^3) was purchased from NatureWorks LLC, USA. Kenaf bast fibre (KBF) was provided by the Institute of Tropical Forestry and Forest Products (INTROP), Malaysia. Reagent-grade acetic acid (CH_3COOH) and sodium hydroxide (NaOH), technical-grade sodium chlorite ($NaClO_2$) of 80% purity and 3-aminopropyltriethoxysilane (APS) of 99% purity were purchased from Fisher Chemicals Sdn. Bhd., Malaysia.

Preparation of Fillers and PLA Composites

A detailed methodology of preparations was reported elsewhere (Tee *et al*., 2013). Derivation of cellulose (C) from kenaf bast fibres was done via chlorination followed by 2 h of soaking in 5% w/v of NaOH solution. Preparation of silane-grafted cellulose (SGC) was done via treatment with 5 wt% of APS coupling agent followed by thermal treatment at 120°C under vacuum of 2 mm Hg for 2 h. Preparation of PLA/C and PLA/SGC composites at 70:30 w/w loading with 0.3-mm thickness was done via melt blending and hot pressing.

Characterisation

Tensile testing

A rectangular strip with 100 mm x 15 mm x 0.3 mm dimensions was cut from each film specimen and was subjected to tensile measurement using a Universal Testing Machine (Instron Model 4301, USA) with a load cell of 1 kN. The test was performed at a cross-head speed of 5 mm/min. The average values of seven repetitions of each specimen were reported with standard error.

Scanning electron microscope

The fracture surface of the PLA and composite films after the tensile test was observed using a variable pressure scanning electron microscope (LEO, 1455 VP SEM, England) at an accelerated voltage of 20 kV. Each sample was sputter-coated with gold before scanning.

Water absorption

Five rectangular strips with 30 mm x 10 mm x 0.3 mm dimension were cut from each film specimen and oven dried at 50°C for 24 hours to a constant weight and measured as the initial weight, W_1. Then, they were immersed in distilled water at room temperature. At regular time intervals (every 24 h), each strip was taken out,

pressed dry with a cloth and subsequently weighted for up to 70 days. The amount of water absorbed by the specimen was calculated using Equation [1],

$$W_t(\%) = \frac{W_2 - W_1}{W_1} \times 100 \qquad [1]$$

where W_t the total water absorbed by the specimen, and W_1 and W_2 are the weights of the specimen before and after immersion in the water respectively. The average values of five repetitions of each specimen were reported.

Oxygen transmission rate. Oxygen transmission rate (OTR) of the film specimens was acquired using Oxygen Permeability Analyser (Mocon®, Oxtran 2/21, USA) in accordance with ASTM D 3985-05. The test area of the films was 5cm^2. The test was run at 23.0^{o}C, 0% RH, barometric pressure of 755 to 759 mmHg with nitrogen flow rate of 10 sscm.

Water vapour transmission rate. Water vapour transmission rate (WVTR) of the film specimens was acquired using Water Vapour Permeability Analyser (Mocon®, Permatran W3/33B, USA) in accordance with ASTM F 1249-06. The test area of the films was 5cm^2. The test was run at 37.8^{o}C with 90± 2% RH at the permeant side.

RESULTS AND DISCUSSION

Tensile Properties

Table 1 shows the tensile properties of PLA, PLA/C and PLA/SGC films. The tensile strength of neat PLA was 47 MPa. The addition of 30 wt% of C increased 13% of the tensile strength while the addition of SGC further improved the tensile strength to 58 MPa, with 23% of total increment. The improvement of tensile strength indicated effective stress transfer from the good interfacial bonding between the fillers and PLA matrix (El-Shekeil *et al*., 2012). For PLA/C, this could be due to the rough and grooved surface topography of the cellulose derived through alkali treatment as was previously defined elsewhere (Tee *et al*., 2013); which enabled the formation of mechanical interlocking to the PLA matrix (Avella *et al*., 2009; Tawakkal *et al*., 2012). As for PLA/SGC, the tensile strength was further improved from the enhanced interfacial adhesion between the silane-grafted cellulose and PLA matrix (Khan & Hassan, 2006; Lee *et al*., 2008). The amine groups of the hydrolysed APS could have formed hydrogen bonds with the carboxylic sites on the hydrolysed PLA backbone (Ghosh *et al*., 2010) while siloxane bridges (S-O) were formed between the hydrolysed APS and the hydroxyl groups of cellulose (Brochier Salon *et al*., 2005; Zhao *et al*., 2012). Authors have reported an increase in tensile strength of composites in the range of 0.5 MPa to 8 MPa from the silane treatment (Abdelmouleh *et al*., 2007; Khan & Hassan, 2006; Pang & Ismail, 2013; Wang *et al*., 2011; Zhao *et al*., 2012). Compared with the current research, the increase in composites' tensile strength from the silane grafting was rather high at 5 MPa and this could be further changed with different amounts of silane. Zhao *et al.* (2012) treated poly(butylene succinate)/rice straw fibre composites with different amount of APS

coupling agent and reported an increase in tensile strength in the range of 4 MPa to 6 MPa.

The tensile modulus of neat PLA was 7 GPa. With C added, the tensile modulus of PLA almost doubled at 13 GPa. With SGC, the tensile modulus was further increased to 15 GPa. The increase in stiffness of the composites was contributed by the comparatively higher stiffness of the cellulose (Luz *et al*., 2008). El-Shekeil and co-workers (2012) termed it as a 'logical trend' that a composite becomes stiffer with the addition of natural fibres. The further increment in stiffness with silane treatment was also reported by several authors (Abdelmouleh *et al*., 2007; Huda *et al*., 2008; Lee *et al*., 2009). This could be due to the better adhesion from the treatment which restricts the polymer chain mobility and further hinders the ability of the polymer to deform.

TABLE 1
Tensile Properties of PLA, PLA/C, and PLA/SGC Films

Samples	Tensile strength (MPa)	Tensile modulus (GPa)	Elongation at break (%)
PLA	47(±1.0)	7(±0.1)	1.6(±0.09)
PLA/C (70/30)	53(±2.3)	13(±0.3)	0.6(±0.02)
PLA/SGC (70/30)	58(±1.8)	15(±0.1)	0.6(±0.02)

Standard error in parenthesis ( )

As seen in Table 1, the elongation at break of neat PLA was 1.6%, which was, as expected, low due to its brittle nature. Both PLA/C and PLA/SGC composites further lowered the elongation at break to 0.6%. As the rigid fillers were added to the PLA, they suppressed the PLA matrix to elongate further (Tawakkal *et al*., 2012). The voids between the fillers and matrix interface may cause an early rupture or premature composite failure, thus decreasing the elongation at break (Luz *et al*., 2008).

The micrographs of tensile fracture surface of the film samples are shown in Fig.1 to Fig.3. The fracture surface for PLA was clear cut and smooth, representing its brittle nature. As for the composites, the cellulose was rod shaped with different diameters and randomly dispersed in the matrix. From the PLA/SGC micrograph in Fig.3b, it can be seen that the cellulose was surface-coated, which could be the grafted silane (Koga *et al*., 2011; Tee *et al*., 2013). From the PLA/C micrograph in Fig.3, aside from some good physical interlocking between the cellulose and the matrix, there were quite a number of filler-related failures such as cellulose pullout, gaps between cellulose and PLA matrix interface and cellulose-matrix debonding, which resulted in voids. Although the filler-related mechanisms similar to PLA/C were also seen in the PLA/SGC micrographs, it was significantly less in comparison. Moreover, good interfacial adhesion and compatibility between the interfaces was seen as there was better wetting out of the PLA matrix onto the SGC and this improved the tensile properties.

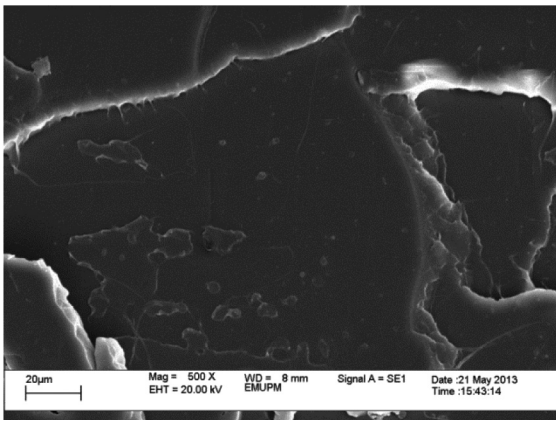

Fig.1: Tensile fracture surface of PLA film at 500× magnification.

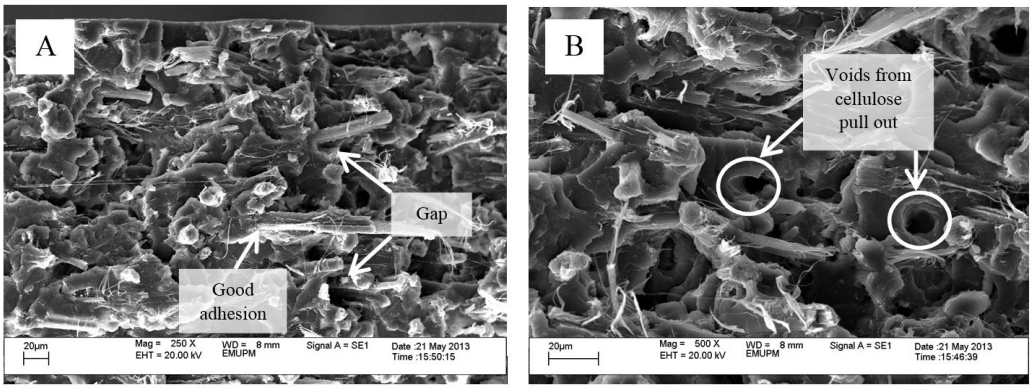

Fig.2: Tensile fracture surface of PLA/C composite films at A) 250× magnification, and B) 500× magnification.

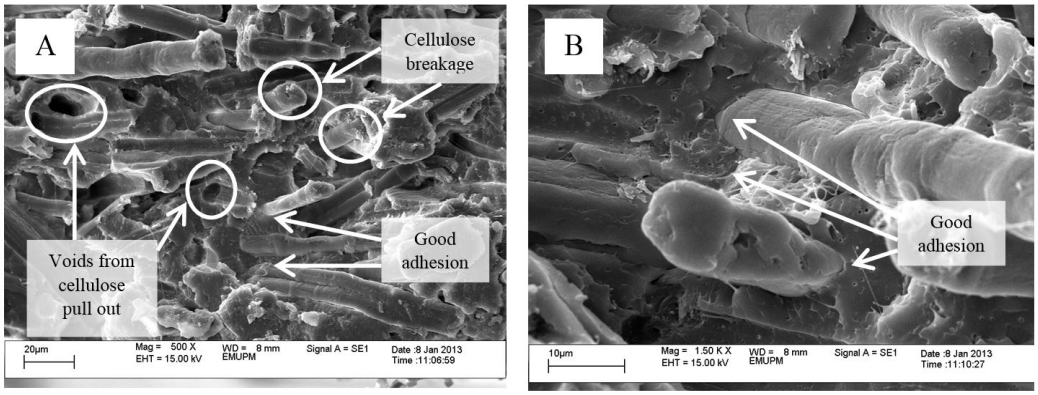

Fig.3: Tensile fracture surface of PLA/SGC composite films at A) 500× magnification, and B) 1500× magnification.

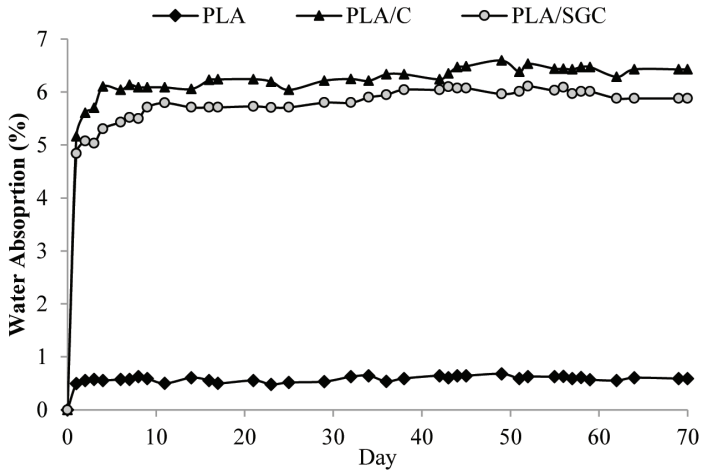

Fig.4: Water absorption of PLA, PLA/C and PLA/SGC composite films.

Water Absorption

Fig.4 shows the water absorption of the PLA and composite films measured throughout 70 days of water immersion. For the PLA film, equilibrium was reached within 2 days with low water absorption of 0.6%. For the composite films, there was a rapid increase in water absorption in the first 2 days. The following days showed a gradual increase until equilibrium was reached. PLA/C absorbed 6.5% of moisture. Comparatively, PLA/SGC slightly improved the water resistance with 5.9% of water absorption. Few authors have reported the effect of silane treatment on the water absorption of natural fibre-related composites. The range of water reduction was reportedly low at the range of 0.2 to 1.3% by the authors (Abdelmouleh *et al*., 2007; Zhao *et al*., 2012; Pang *et al*., 2013).

Water can penetrate into composite materials via three mechanisms whereby water molecules can either diffuse through micro gaps between polymer chains, capillary transported into gaps between the fillers-matrix interfaces from incomplete wetting or transported through the matrix micro cracks or pores formed during compounding (Zhao *et al*., 2012). The free hydroxyl groups in cellulose readily form hydrogen bonds with the water molecules and this expands the cells until they are saturated with water (Rampinelli *et al*., 2010; Zhao *et al*., 2012). The cell expansion may generate even more micro cracks and fibre-matrix debonding, which causes further water penetration (Mat Taib *et al*., 2008). At similar filler loadings, composites with SGC had lower water absorption, which indicated the presence of the silane coupling agent in enhancing the filler-matrix interfacial adhesion. Lee *et al.* (2009) called it better wetting out of the polymer matrix onto the fibres. From the previous finding via Fourier transform infrared spectroscopy (FTIR), silane-grafted cellulose had comparatively fewer free –OH

groups as they were chemically grafted with the silanol of the ethoxysilane (Tee *et al*., 2013). This also contributed to the decreased amount of equilibrium water absorption by PLA/SGC.

Barrier Properties

Table 2 shows the barrier properties of PLA, PLA/C and PLA/SGC films. The average oxygen and water vapour transmission rate of the PLA films was 76.6 cc/m^2/day and 23.05 g/m^2/day. The incorporation of 30 wt% of cellulose resulted in OTR of 42.2 cc/m^2/day, with 45% reduction. The incorporation of similar loading of SGC further reduced the OTR to 40.3 cc/m^2/day. As for WVTR, it was reported that PLA/C only slightly reduced 2% of PLA films' WVTR. PLA/SGC further reduced 8% of the WVTR, resulting 20.85 g/m^2/day. While the incorporation of cellulose had more influence as compared to the effect of silane grafted onto the cellulose in retarding the oxygen permeation of the film, it was vice versa in terms of water vapour. At least to our knowledge, not many authors reported the barrier properties of polymer-cellulose composites. Laxmeshwar and co-workers (2012) reported the trend of OTR and WVTR of PLA reinforced with treated microcrystalline cellulose at different loading. They had the same finding whereby the fillers had more significant influence in increasing the oxygen barrier properties of the composites as compared to the water vapour barrier properties. The slight reduction of WVTR could be due to the initial PLA's WVTR that was already low. The reduction of OTR and WVTR from the addition of fillers was due to the decrease in the polymer chain flexibility (Laxmeshwar *et al*., 2012). Similar to stiffness, the polymer chain flexibility was further reduced with SGC due to the improved interfacial adhesion, and thus further improved the barrier properties. The addition of fillers also created a blocking effect as they forced the permeant to follow the tortuous pathway during diffusion (Majeed *et al*., 2013). According to Shogren *et al.* (1997), crystallites impose barriers to permeant diffusion, which also explains the improved barrier properties of PLA/C and PLA/SGC as cellulose is known to be highly crystalline (Mwaikambo & Ansell, 2002). Other studies report that cellulose and SGC were shown to be crystal nucleating agents, which further supports the role of these fillers in increasing the barrier of the composite films (Tee *et al*., 2013).

TABLE 2
Barrier properties of PLA, PLA/C, and PLA/SGC Films

Samples	Oxygen transmission rate (cc/m^2/day)	Water vapour transmission rate (g/m^2/day)
PLA	76.6(±6.5)	23.1(±0.5)
PLA/C (70/30)	42.2(±4.6)	22.7(±0.2)
PLA/SGC (70/30)	40.3(±1.8)	20.9(±1.8)

Standard error in parenthesis ( )

CONCLUSION

In the current work, improvement in tensile properties was attained with the addition of cellulose into a PLA matrix and they

were further enhanced with silane-grafted cellulose. The rigidity of the fillers slightly reduced the elasticity of PLA, which was brittle in nature. Increment in water absorption was reported in PLA/C. However, composites with silane-grafting reported slightly better water resistance from the improved interfacial compatibility and reduced free –OH groups in the cellulose. While the incorporation of C and SGC into the PLA matrix increased the oxygen and water vapour barrier properties, in particular, SGC was more effective in improving the water vapour barrier properties of the composites. This may suggest the potential of these bio-based materials for packaging oxygen and moisture-sensitive food.

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REFERENCES

Abdelmouleh, M., Boufi, S., Belgacem, M. N., & Dufresne, A. (2007). Short natural-fibre reinforced polyethylene and natural rubber composites: Effect of silane coupling agents and fibres loading. *Composites Science and Technology, 67*(7-8), 1627-1639.

Avella, M., Buzarovska, A., Errico, M. E., Gentile, G. & Grozdanov, A. (2009). Eco-challenges of bio-based polymer composites. *Materials, 2*(3), 911-925.

Bisanda, E. T. N., & Ansell, M. P. (1991). The effect of silane treatment on the mechanical and physical properties of sisal-epoxy composites. *Composites Science and Technology, 41*(2), 165-178.

Brochier Salon, M. C., Abdelmouleh, M., Boufi, S., Belgacem, M. N., & Gandini, A. (2005). Silane adsorption onto cellulose fibres: Hydrolysis and condensation reactions. *Journal of Colloid and Interface Science, 289*(1), 249-261.

Castellano, M., Gandini, A., Fabbri, P., & Belgacem, M. N. (2004). Modification of cellulose fibres with organosilanes: Under what conditions does coupling occur? *Journal of Colloid and Interface Science, 273*(2), 505-511.

El-Shekeil, Y. A., Sapuan, S. M., Abdan, K., & Zainuddin, E. S. (2012). Influence of fibre content on the mechanical and thermal properties of kenaf fibre reinforced thermoplastic polyurethane composites. *Materials and Design, 40*(11), 299-303.

George, J., Sreekala, M. S., & Thomas, S. (2001). A review on interface modification and characterization of natural fibre reinforced plastic composites. *Polymer Engineering and Science, 41*(9), 1471-1485.

Ghosh, S. B., Bandyopadhyay-Ghosh, S., & Sai, M. (2010). Composites. In R. Auras, R., Lim, L. T., Selke, S. E. M., & Tsuji, H. (Eds.), *Poly(lactic acid): Synthesis, structures, properties, processing, and applications* (2nd ed.). (pp. 239-310). United States: John Wiley & Sons.

Holbery, J., & Houston, D. (2006). Natural-fibre-reinforced polymer composites in automotive applications. *JOM, 58*(11), 80-86.

Huda, M. S., Drzal, L. T., Mohanty, A. K., & Misra, M. (2008). Effect of fibre surface treatments on the properties of laminated biocomposites from poly(lactic acid) (pla) and kenaf fibres. *Composites Science and Technology, 68*(2), 424-432.

Johansson, C., Bras, J., Mondragon, I., Nechita, P., Plackett, D., Simon, P., Svetec, D. G., Virtanen, S., Baschetti, M. G., Breen, C., Clegg, F., & Aucejo, S. (2012). Renewable fibres and bio-based materials for packaging applications - A review of recent developments. *BioResources, 7*(2), 2506-2552.

Kalia, S., Kaith, B. S., & Kaur, I. (2009). Pretreatments of natural fibres and their application as reinforcing material in polymer composites - A review. *Polymer Engineering and Science, 49*,(7) 1253-1272.

Kazayawoko, M., Balatinecz, J. J., & Matuana, L. M. (1999). Surface modification and adhesion mechanisms in woodfibre-polypropylene composites. *Journal of Materials Science, 34*(24), 6189-6199.

Khan, M. A., & Hassan, M. M. (2006). Effect of γ-aminopropyl trimethoxy silane on the performance of jute–polycarbonate composites. *Journal of Applied Polymer Science, 100*(5), 4142-4154.

Koga, H., Kitaoka, T., & Isogai, A. (2011). In situ modification of cellulose paper with amino groups for catalytic applications. *Journal of Materials Chemistry, 21*(25), 9356-9361.

Laxmeshwar, S. S., Viveka. S., Kumar, D. J. M., & Nagaraja, G. K. (2012). Preparation and properties of composite films from modified cellulose fibre-reinforced with PLA. *Der Pharma Chemica, 4*(1), 159-168.

Lee, B.-H., Kim, H.-S., Lee, S., Kim, H.-J., & Dorgan, J. R. (2009). Bio-composites of kenaf fibres in polylactide: Role of improved interfacial adhesion in the carding process. *Composites Science and Technology, 69*(15-16), 2573-2579.

Lee, S. Y., Kang, I. A., Doh, G. H., Yoon, H. G., Park, B. D., & Wu, Q. (2008). Thermal and mechanical properties of wood flour/talc-filled polylactic acid composites: Effect of filler content and coupling treatment. *Journal of Thermoplastic Composite Materials, 21*(3), 209-223.

Lu, J., Askeland, P., & Drzal, L. T. (2008). Surface modification of microfibrillated cellulose for epoxy composite applications. *Polymer, 49*(5), 1285-1296.

Luz, S. M., Del Tio, J., Rocha, G. J. M., Gonçalves, A. R., & Del'Arco Jr., A. P. (2008). Cellulose and cellulignin from sugarcane bagasse reinforced polypropylene composites: Effect of acetylation on mechanical and thermal properties. *Composites: Part A, 39*(9), 1362-1369.

Majeed, K., Jawaid, M., Hassan, A., Abu Bakar, A., Abdul Khalil, H. P. S., Salema, A. A., & Inuwa, I. (2013). Potential materials for food packaging from nanoclay/natural fibres filled hybrid composites. *Materials and Design, 46*(1), 391-410.

Masirek, R., Kulinski, Z., Chionna, D., Piorkowska, E., & Pracella, M. (2007). Composites of poly(L-lactide) with hemp fibres: Morphology and thermal and mechanical properties. *Journal of Applied Polymer Science, 105*(1), 255-268.

Mat Taib, R., Ramarad, S., Ishak, M., & Todok, M. (2008, 15-19 June 2008). *Water absorption and tensile properties of kenaf bast fibre-plasticized poly(lactic acid) biocomposites*. Paper presented at the Polymer Society 24th Annual Meeting (PPS-24), Salerno, Italy.

Mohanty, A. K., Misra, M., & Hinrichsen, G. (2000). Biofibres, biodegradable polymers and biocomposites: An overview. *Macromolecular Materials and Engineering, 276-277*(1), 1-24.

Mwaikambo, L. Y, & Ansell, M. P. (2002). Chemical modification of hemp, sisal, jute, and kapok fibres by alkalization. *Journal of Applied Polymer Science*, *84*(12), 2222-2234.

Pang, A. L., & Ismail, H. (2013). Tensile properties, water uptake, and thermal properties of polypropylene/waste pulverized tire/kenaf (PP/

WPT/KNF) composites. *BioResources, 8*(1), 806-817.

Rampinelli, G., Landro, L. D., & Fujii, T. (2010). Characterization of biomaterials based on microfibrillated cellulose with different modifications. *Journal of Reinforced Plastics and Composites, 29*(12), 1793-1803.

Satyanarayana, K. G, Arizaga, G. G. C. & Wypych, F. (2009). Biodegradable composites based on lignocellulosic fibres - An overview. *Progress in Polymer Science, 34*(9), 982-1021.

Shogren, R. (1997). Water vapor permeability of biodegradable polymers. *Journal of Environmental Polymer Degradation, 5*(2), 91-95.

Tawakkal, I. S. M. A., Talib, R. A., Abdan, K., & Chin, N. L. (2012). Mechanical and physical properties of kenaf-derived cellulose (KDC)-filled polylactic acid (PLA) composites. *BioResources, 7*(2), 1643-1655.

Tee, Y. B., Talib, R. A., Khalina, A., Chin, N. L., Kadir Basha, R., & MdYunos, K. F. (2013). Thermally grafting aminosilane onto kenaf-derived cellulose and its influence on the thermal properties of poly(lactic acid) composites. *BioResources, 8*(3), 4468-4483.

Wang, Y., Qi, R., Xiong, C., & Huang, M. (2011). Effects of coupling agent and interfacial modifiers on mechanical properties of poly(lactic acid) and woodflour biocomposites. *Iranian Polymer Journal, 20*(4), 281-294.

Westman, M. P., Fifield, L. S., Simmons, K. L., Laddha, S. G., & Kafentzis, T. A. (2010). *Natural fibre composites: A review*. (No. 19220). Springfield, U.S.A.: Pacific Northwest National Laboratory.

Xie, Y., Hill, C. A. S., Xiao, Z., Militz, H., & Mai, C. (2010). Silane coupling agents used for natural fibre/polymer composites: A review. *Composites: Part A, 41*(7), 806-819.

Zhao, Y., Qiu, J., Feng, H., & Zhang, M. (2012). The interfacial modification of rice straw fibre reinforced poly(butylene succinate) composites: Effect of aminosilane with different alkoxy groups. *Journal of Applied Polymer Science, 125*(4), 3211-3220.

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Tensile Strength of Some Natural-Fibre Composites

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ABSTRACT

The conversion of natural fibre into biocomposites is rapidly being exploited globally. Locally, there are some viable natural fibres that can be utilised for this. In this research project, several types of composites were produced, all of which were made from natural fibres. Four different natural cellulosic fibres were chosen, namely, kenaf, pineapple (pina), banana and coir. Hybrids of kenaf blended with each of these fibres were also woven. The samples were woven manually as weft yarn while the warp yarn used plied polyester thread. The samples were fabricated into composites using four types of matrices which were epoxy, polyester, polypropylene and polyethylene. The composites were fabricated using the manual compression method. These composites were then tested for their tensile strength in weft direction. All results were also analysed using Analysis of Variance (ANOVA) and ranked accordingly. It was found that woven samples made of 100% kenaf fibre exhibited the best tensile strength for all types of resin while coir was found to be the poorest. All kenaf hybrid composites mostly exhibited better results than the non-hybrid composites.

Keywords: Banana fibres, biocomposites, coir, kenaf, pina, pineapple fibres, tensile strength

Agriculture is an important sector contributing to economic growth in many countries around the world. Traditionally, the waste produced by this sector was just burnt away. One of the possible prospects is to convert agricultural waste into a composite material for technical applications because fibre can be a source of reinforcement filler for composites (Salleh *et al*., 2004). For example, after harvesting pineapples, the leaves of the plant were normally burnt. Producing fabric from pineapple leaves as done in the Philippines to create yarn and fabric involved laborious work. Producing fibres for composites, however, is a much simpler process.

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There are many types of fibre waste such as coir, banana fibre, paddy straw, pineapple leaf fibre, kenaf, sugarcane and many more. In recent years there have been studies to investigate the use of this waste as biocomposite materials (Rowell, 1992; Bledzki & Gassan, 1999; Summerscales *et al*., 2010). The advantages offered here are the low cost of materials, renewable resources and low specific weight, which resulted in fairly good tensile properties (Mohanty, 2000; Badros, 2007). Nevertheless, composites made from these are only suitable for use in the low performance applications. (Shibata *et al*., 2003; Burgueno *et al*., 2004; O'Donnel *et al*., 2004; Morye & Wool, 2005; Badros, 2007).

A bio-based composite can be defined as a material that includes some types of natural materials in its structure (Sanadi *et al*., 2004). The biocomposites from cellulosic materials include those materials obtained from kenaf, pineapple, hemp, flax, bamboo, bast and core leaf plants (for instance, sisal and abaca), and agricultural fibres such as rice straw, corncobs, sugarcane baggase and coconut hair (coir). Many studies were also made on hybrids of natural fibre composites. It was said that hybridisation can be a cost-effective approach (Venkateshwaran, 2012).

Wan Ahmad *et al*. (2004) found that bio-based fibres could be used for composite production and kenaf was found to possess good properties for composites. A blend of kenaf and cotton was also found to possess good mechanical properties. Wambua *et al*. (2003) said that composites made from natural cellulosic fibres namely kenaf, coir, sisal, hemp and jute (except for coir) yielded similar tensile strength with glass fibre. They concluded that natural-fibre composites had the potential of replacing glass fibre in several applications. Shibata *et al*. (2003) also found that kenaf fibre could be a good reinforcement candidate for high performance biodegradable polymer composites. Nevertheless, Akil *et al*. (2011) cautioned the setback of kenaf, which was the lack of good interfacial adhesion between the fibre and the polymer matrix. In solving this, Asumani *et al*. (2012) and Yousif *et al*. (2012) found that tensile and flexural properties of kenaf can be improved by treating the fibres with sodium hydroxide and silane.

In this research, natural fibre waste generated from agricultural practice was woven into fabric and then fabricated into biocomposite. The fibres used were kenaf, pineapple (pina), banana stem and coir. The fibres were then fabricated with two thermoset and two thermoplastic resins to produce biocomposites. The tensile properties of each were evaluated and compared.

MATERIALS AND METHODS

Materials

Kenaf, pineapple leaf, coir, banana stem and hybrids of 50/50% kenaf/pineapple and 50/50% kenaf/banana and 50/50% kenaf/coir fibres were used in this research project. Each material was first cut and the fibres were extracted through retting, crushing, combing and then dried. The fibres were then woven as weft yarns to form a fabric as

shown in Fig.1. This was to take advantage of the parallel weft, which was supposed to perform better than the randomly laid nonwoven. The warp used was polyester spun threads of 40/2's.

For the hybrids, the weft picks were arranged alternately during weft insertion. Each of the weft was measured in terms of its linear density (0.3 gm each) before alternately inserted as weft. The average thread densities for warps and wefts of the fabric were 6 ends and 12 picks per 2.5 cm.

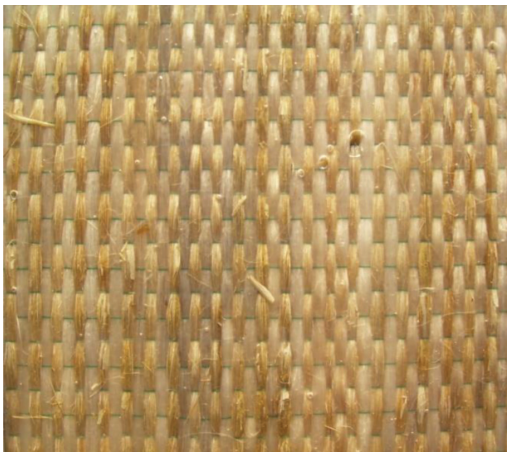


Fig.1: Woven materials.

Fabrication Methods

Two thermoset and two thermoplastic resins were used in the experiments, namely polyester and epoxy for the thermoset, and high density polyethylene (HDPE) and polypropylene for the thermoplastic. The thermoplastic resins were in pallet form, and these were melted to form sheets before the lay-up process.

For composite fabrication, two layers of fabrics were laid on an aluminium plate and the resin with the hardener (where needed) was applied evenly on the fabric using a roller. Once completed, another aluminium plate was placed on top of the resinated fabric. Force was applied using a G-clamp to the aluminium plates as shown in Fig.2 so that the resin would be distributed evenly among the fibres. Spacers of 2-mm thickness were placed in between the aluminium plates to ensure even thickness of the composites.

The polyester resin samples were left to cure at room temperature for 24 hours and the epoxy samples were put under a hot press for 8 hours at 120°C. The thermoplastic samples were cured under hot press at 190°C for 5 minutes. The samples were left to cool down for another 24 hours before the assembly was dismantled. Four composites for each resin treatment with dimensions of 26 cm x 26 cm were made.

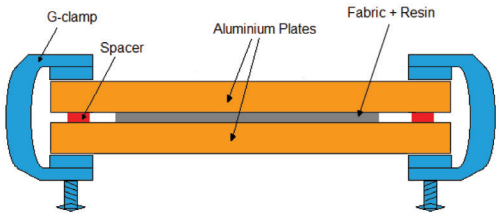


Fig.2: Fabrication arrangement.

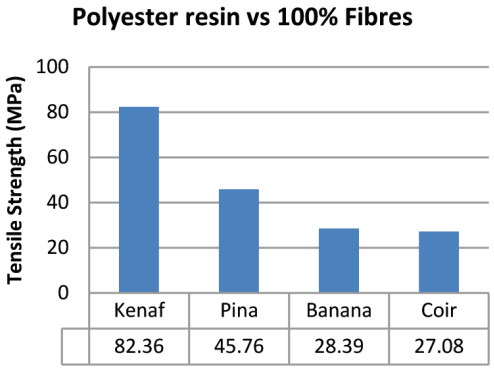
Testing

Tensile testing was done in accordance to Composites Research Advisory Group 302 (CRAG 302). Since the fibres used were all in the weft direction, testing was conducted with the weft direction only. Five samples for each resin treatment were cut using a rotary cutter with size 20 mm x 150 mm. Nevertheless, the sample where tensile breakage occurred at the tab was discarded and replaced with another sample. The

gauge length was 100 mm and the crosshead speed was 5 mm/min. Analysis of variance (ANOVA) was conducted to rank the result of tensile strength of those composites.

RESULTS AND DISCUSSIONS

The results for the composites made from 100% natural fibres are presented separately from those obtained using the blended or hybrid fabrics. Fig.3a and 3b show the results of these composites using polyester resin. From Fig.3a and 3b it can be seen that composites made from 100% kenaf exhibited the highest tensile strength at 83 MPa compared to other 100% fibre composites. For the hybrids, kenaf/pina showed the highest tensile strength at 58 MPa. It is interesting to note that the kenaf/pina hybrid recorded tensile properties higher than the 100% pina fibres. All the hybrid composites showed that kenaf fibres had a strong influence on the strength of these composites. Composites from coir displayed the weakest tensile results, and this included its hybrid with kenaf.



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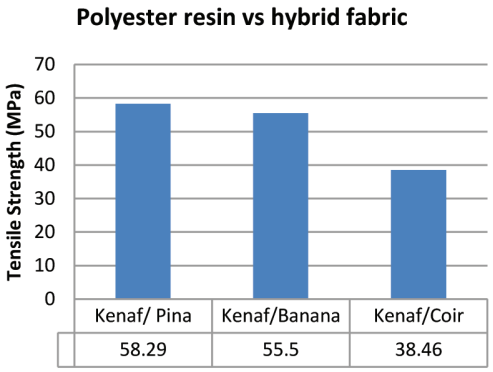
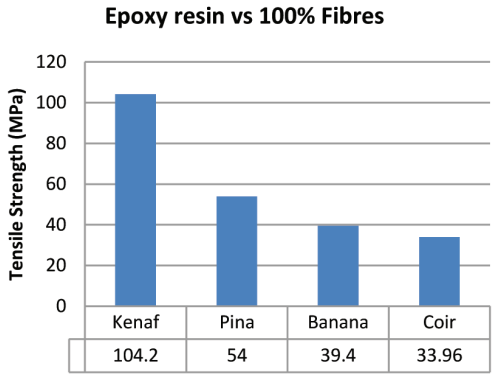


(b)

Fig.3: Tensile strength using polyester resin

Fig.4a and 4b show the results of tensile test using epoxy resin. Again, it can be seen that 100% kenaf exhibited the highest tensile strength at 104 MPa followed by pina, banana and coir again the lowest. With the hybrids, kenaf/pina showed the highest properties and, similar to composites with polyester resin, the strength of kenaf/pina was higher than that of 100% pina, which were 73 MPa and 54 Mpa, respectively. In fact, kenaf/coir hybrid strength was almost reaching the strength of 100% pina composites. Therefore, with epoxy resin, kenaf also played an important role in strengthening the hybrid composites.



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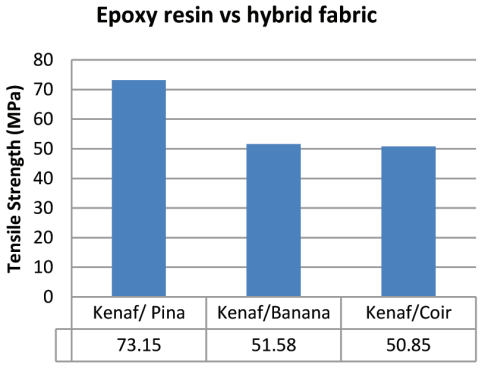
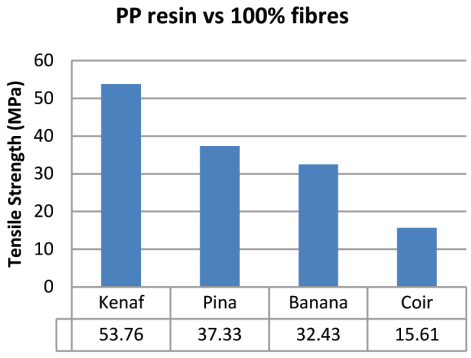


(b)

Fig.4: Tensile strength using epoxy resin.



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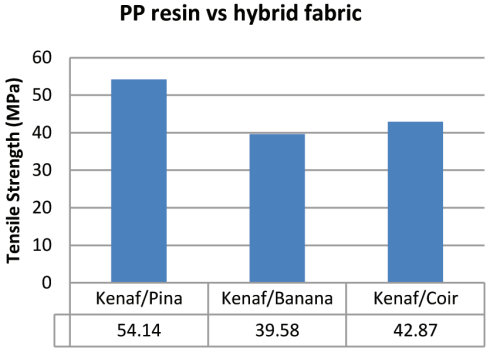
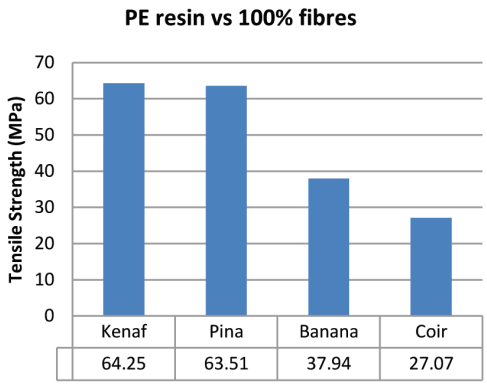


(b)

Fig.5: Tensile strength using polypropylene resin.

The results for tensile test using polypropylene resin for the composites made from 100% natural fibres and the hybrids fabrics are shown in Fig.5a and 5b, respectively. Using the polypropylene also, composites made from 100% kenaf showed the highest tensile strength followed by pina, banana and coir. Similar to the thermosets, the hybrids also behaved in the same way. However, it was noticed that the kenaf/coir hybrid exhibited higher tensile properties than the 100% pina composites.

Fig.6a and 6b exhibit tensile test results from using polyethylene resin. With this resin also, 100% kenaf composites demonstrated the highest in tensile strength. Nevertheless, 100% pina fibres also showed quite a remarkable tensile strength compared to kenaf. Analysis of this result showed that there was no significant difference between the two. As for the hybrids, the results were similar to the other resins.



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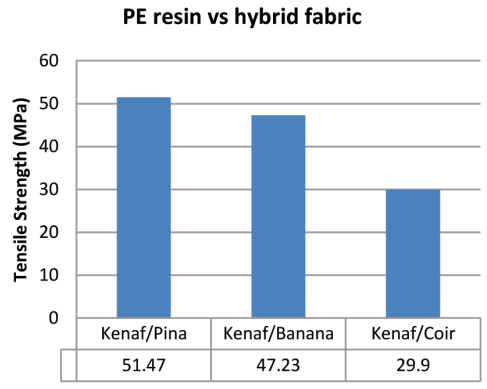
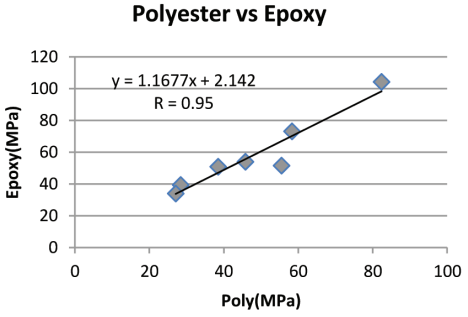


(b)

Fig.6: Tensile strength using polyethylene resin.

Analysis of variance conducted to rank the tensile strength confirmed that the highest tensile strength was exhibited by 100% kenaf followed by pina, banana and coir. With the exception of polyethylene resin, 100% kenaf was significantly higher in tensile properties when it was compared to other natural-fibre composites in this project. This was in agreement with the result of Wan Ahmad *et al*. (2004). With the hybrids, kenaf/pina hybrid was significantly higher in tensile strength to other hybrids, but the ranking between kenaf/ banana and kenaf/coir was a little inconsistent with the epoxy and polypropylene resins.

Correlation analyses were also conducted between the tensile results of the thermoset and the thermoplastic resins for all the composites fabricated and shown in Fig.7a and 7b, respectively. This was done to examine the effect of composite fabrication for each fabric and resin. It can be seen that the tensile test results using the two thermoset resins correlated very well with R = 0.95. This showed that the fabrication and the fibres had been consistent for all the composites. However, the correlation for the composites using the thermoplastic resin was moderate with R = 0.63. The result for 100% pina with polyethylene resin and the result for kenaf/coir for polypropylene resin could have caused this irregularity. It was probably because the thermoplastic resins were applied using sheets and not liquid. The thickness of the polypropylene sheets was not that consistent. It also could be due to lack of interfacial adhesion as mentioned by Akil *et al*. (2011).



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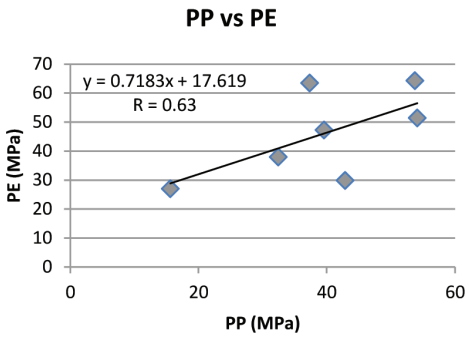

(b)

Fig.7: Correlation analysis between the resins.

CONCLUSION

The results of this research project demonstrated that functional composites can be developed using local cellulosic fibres as reinforcing materials for biocomposites. Kenaf was found to be the best reinforcing fibre compared to pineapple fibres, banana and coir in terms of tensile strength. Its tensile properties were consistent with all types of resin used in this project. Next to kenaf was pina which has good potential as reinforcing material for composites.

It is possible to produce a good hybrid composite by weaving these fibres to form fabric. Blending of kenaf with other fibres shows that kenaf fibres has very strong influence on the strength of these composites. Kenaf/pina is consistenly higher in tensile properties compared to other hybrids. Almost all kenaf hybrid composites exhibit tensile results higher than the non-hybrid composites.

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REFERENCES

Akil, H. M., Omar, M. F., Mazuki, A. A. M., Safiee, S., Ishak, Z. A. M., & Abu Bakar. A. (2011). Kenaf fibre reinforced composites: A review. *Materials and Design*, *32*, 4107-4121.

Asumani, O. M. L., Reid, R. G., & Paskaramoorthy, R. (2012). The effects of alkali-silane treatment on the tensile and flexural properties of short fibre non-woven kenaf reinforced polypropylene composites, *Composites: Part A*, *43*, 1431-1440.

Bledzki, A. K., & Gassan, J. (1999). Composites reinforced with cellulose based fibres. *Progress in Polymer Science*, *24*, 221-74.

Bodros, E., Pillin, I., Montrelay, N. & Baley, C. (2007). Could biopolymers reinforced by randomly scattered flax fibers be used in structural applications. *Composites Science Technology*, *67*, 462-470.

Burgueno, R., Quagliata, M. J., Mohanty, A. K., Mehta, G. M., Drzal, L. T., & Misra, M. (2004). Load bearing natural fiber composite cellular beams and panels. *Composites Part A*, *35*, 645-656.

Mohanty, A. K., Misra, M., & Hinrichsen, G. (2000). Biofibers, biodegradable polymers and biocomposites: An overview. *Macromolecular Materials and Engineering, 276/277*, 1-24.

Morye, S. S., & Wool, R. P. (2005). Mechanical properties of glass/flax hybrid composites based on a novel modified soyabean oil matrix material. *Polymer Composite*, *26*, 146-407.

O'Donnel, A., Dweib, M. A., & Wool, R. P. (2004). Natural fiber composites with plant oil based resin. *Composite Science Technology*, *64*, 1135-1145.

Rowell, R. M. (1992). *Opportunities for value added bio-based composites*. The pacific rim bio-based composites, symposium; 1992, November 9-13; Rotorua, New Zealand, FRI Bulletin 177. Rotorua, New Zealand: New Zealand Forest, Research Institute, 244-252.

Salleh, J., Ahmad, W. Y. W., Ahmad, M. R., Yahya, M. F., Ghani, S. A., & Misnon, M. I. (2004). *Tensile and flexural properties of composites made from spinning waste.* Exotextile '04. Manchester.

Sanadi, A. R., Caufield, D. F., & Rowell, R. M. (1994). *Plastic Engineering*, *4*, 27.

Shibata, M., Ozawa, K., Teramoto, N., Yosomiya, R., & Takeishi, H. (2003). Biocomposites made from short abaca fiber and biodegradable polyesters. *Macromolecular Materials and Engineering*, *288*, 35-43.

Summerscales, J., Dissanayake, N., Virk, A., & Hall, W. (2010). A review of bast fibres and their composites. Part 2-Composites. *Composites: Part A*., *41*, 1336-1344.

Venkateshwaran N., Elayaperumal, A., & Sathiya, G. K. (2012). Prediction of tensile properties of hybrid-natural fiber composites. *Composites: Part B, 43*, 793-796.

Wambua, P., Ivens, J., & Verpoest, I. (2003). Natural fibres: Can they replace fibre reinforced plastics? *Composites Science and Technology*, *63*, 1259-1264.

Wan Ahmad, W. Y., Salleh, J., Yahya, M. F., Ab Kadir, M. I., & Misnon, M. I. (2004). *Kenaf and kenaf combined waste composites.* Ecotextiles, Manchester, United Kingdom, July 7-8.

Yousif B. F., Shalwan, A., Chin, C. W., Ming, K. C. (2012). Flexural properties of treated and untreated kenaf/epoxy composites. *Materials and Design*, *40*, 378-385.

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Effect of Filler Loading and NaOH Addition on Mechanical Properties of Moulded Kenaf/Polypropylene Composite

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ABSTRACT

Natural fibre composites can have varying combinations of physical and mechanical properties, such as low density, low cost, high stiffness and strength. Kenaf *(Hibiscus cannabinus)* was used in this study as a natural fibre to reinforce polypropylene (PP) in the fabrication of polymer composite materials. The injection moulding method, with an injection temperature of 170°C, was used in this study. This study aimed to investigate the effect of sodium hydroxide (NaOH) on the mechanical properties of kenaf/PP using the injection moulding method. PP was mixed with different compositions (5, 10, and 15 wt%) of kenaf particles with increasing concentrations of NaOH as a treatment agent to enhance the adhesion between kenaf and PP. Morphological and structural changes of the sample fracture were observed under a scanning electron microscope (SEM). The results showed that the mechanical properties of the composite were increased when the percentage of kenaf composition was increased, and decreased when NaOH concentration was increased. The highest tensile value of the sample was 21.93 MPa at 15 wt% composition of kenaf particles, while the lowest value of 16.42 MPa was observed when NaOH was present. The improvement of flexural strength was highlighted, in 5 wt% composition of kenaf-reinforced PP with NaOH that was 32.07 MPa, but when the NaOH concentration was increased to over 10%, the flexural value decreased to 26.97 Mpa. Based on the results, the researchers concluded that NaOH treatment may increase the bond strength of kenaf composite; however, increasing the NaOH concentration can lead to a decrease in mechanical properties.

Keywords: Injection molding, natural fibre polymer composite, kenaf, mechanical properties, physical properties

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INTRODUCTION

The demand for the development of high-performance engineering products from sustainable materials made from natural resources has recently increased worldwide. The merging of reinforcements, such as fibres and matrix, in composites can provide a way to expand and improve composite properties that can fulfill the requirements of most engineering applications (Akil *et al.*, 2011). The research and development of natural fibres used as filler or reinforcement has gained momentum in the last decade. Natural fibre polymer composites offer a sustainable and renewable alternative to commercial composite materials (Pothan *et al*., 2003).

Kenaf plant is one of the many types of natural resources that have been extensively investigated over the past few years. *H. cannabinus* L. is a biodegradable and environment-friendly plant. The use of kenaf, a natural fibre-based composite, has increased because the material is readily available, lightweight, biodegradable, non-abrasive, non-toxic, low cost and has low density with high specific strength (Nishino *et al*., 2003)

Poor merging is the major problem encountered when the natural fibres in polymer composite are not compatible with the matrix (El-Shekeil *et al*., 2011). The inherent high moisture absorption of the product generated by the dimensional changes of the fibres can lead to microcracking of the composites and degradation of the mechanical properties (Edeerozey *et al*., 2007). Kenaf fibres are hydrophilic in nature, whereas the polymer matrix is hydrophobic. Polar hydroxyl groups on the surface of kenaf fibre make it difficult to form an interphase surface with the non-polar polymer matrix relative. The hydrogen bonds in kenaf fibres prevent the surface from getting wet, resulting in lack of interfacial adhesion between both fibres and polymer matrix. The lack of adhesion between them results in weak of mechanical and physical properties in the final product (Tserki 2006; Vilay *et al*., 2008). Various chemical treatments have been used by researchers to enhance the mechanical performance of natural fibres. In this study, the researchers used one of the most common and effective modifications applied to kenaf fibres, which is the alkaline treatment with various concentrations of NaOH (Aziz *et al*., 2004). The treatment results were assessed by tensile and flexural testing, which showed the mechanical properties of the kenaf composites with different compositions.

Our study aimed to fabricate kenaf reinforced polypropylene (PP) composites using the injection moulding method and determine the effect of NaOH on the strength bonding of fibre-matrix composites. In addition, the suitable composition of kenaf exhibiting better mechanical properties was determined by examining the composition of the mechanical properties of each kenaf particle.

MATERIALS AND METHODS

Kenaf particles in size 40 mesh were used in this study. Kenaf particles were supplied by the National Kenaf and Tobacco Board (Lembaga Kenaf dan Tembakau Negara) (Fig.1). PP and NaOH pellets were supplied by Sigma-Aldrich. The prepared feedstock was composed of PP, kenaf particles and different NaOH percentages (Table 1). The materials were mixed using the Brabender® internal mixer at optimum processing conditions. Reaction temperature, time and rotating speed were 180°C, 30 min and 30 rpm, respectively. Mixing temperature was based on the melting temperature of polypropylene, which is 171°C. The mixing temperature should be higher than the melting point of the polymer matrix to ensure that the polymer is homogenously mixed with the fibre particles. PP was initially placed in the mixer, and the fibre particles were then added when torque stabilised.

TABLE 1
Composition of Materials Used in the Experiment

Kenaf particles (wt%)	Polypropylene (wt%)	Existence of NaOH (%)
0	100	No / 0
5	95	No / 0
10	90	No / 0
15	85	No / 0
5	90	Yes / 5
10	80	Yes / 10
15	70	Yes / 15

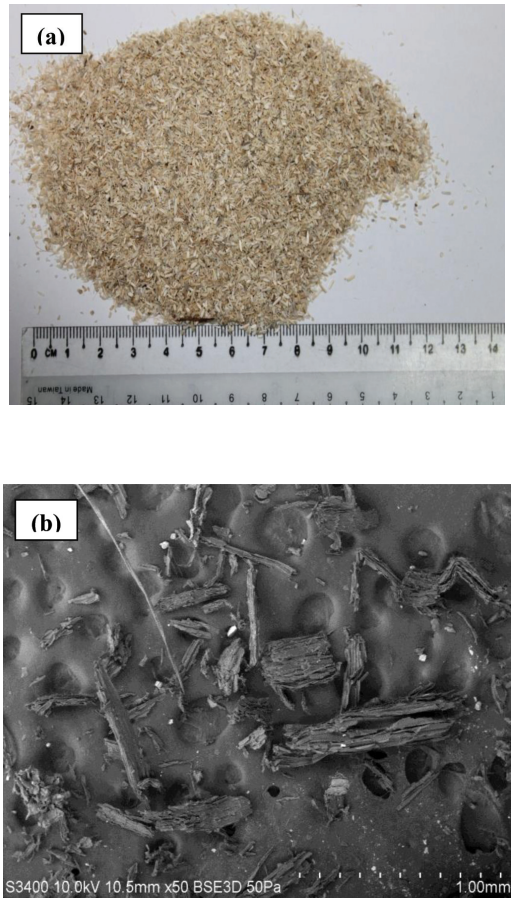

Fig.1: 40 mesh kenaf particles: (a) Digital image; (b) SEM image.

Two types of dumbbell-shaped samples were prepared by injecting kenaf/PP composites and kenaf/PP composites treated with NaOH from different weight compositions into the mould.

All samples were tested by the universal tensile strength and three-point bending flexural strength test according to ASTM D 638 (ASTM, 2000a) and ASTM D 790 (ASTM, 2000b), respectively.

The surface morphology of the fracture specimens were then investigated under a scanning electron microscope to visualise the effect of NaOH on kenaf/PP composites through interfacial adhesion of the samples.

RESULTS AND DISCUSSION

Mechanical Properties of Composites

The universal tensile strength test determines the in-plane tensile properties of the polymer matrix composite materials that are reinforced by high-modulus fibres. The samples were fractured and failed through three possible ways, which may break off at the top, middle or bottom of the beam. Fig.2 shows the middle of the break off of the samples.

Fig.3 shows the effect of the kenaf particle content on the tensile strength of the kenaf/PP composite with NaOH. The mechanical property results were compared with the PP strength properties. Comparison of the histogram patterns showed that the tensile value increased from 5 wt% to 15 wt% composition of kenaf. The highest tensile value of kenaf/PP composite was 21.92 MPa. Loh *et al*. (2001) reported that the addition of micro-powder can increase the hardness and density of the composite. However, the tensile value began to decrease and reached the lowest value of 16.42 MPa at 15 wt% composition of kenaf when 15% NaOH was added. In summary, the tensile strength test results showed that the strength of composite was increased by higher percentage of kenaf composition, but decreased by higher NaOH concentration.

Fig.2: Specimen after tensile test

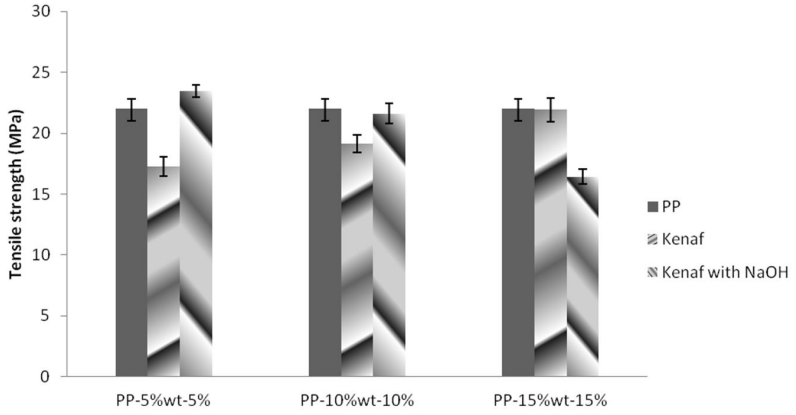

Fig.3: The variation of tensile strength against kenaf particles content with concentration of NaOH.

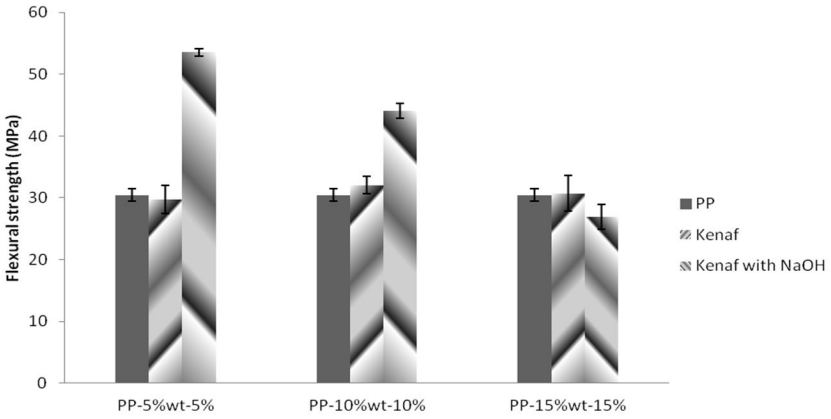

Fig.4: The variation of flexural strength against kenaf particles content with concentration of NaOH.

The flexural strength results shown in Fig.4 indicate that the strength of the composites slightly improved (32.07 MPa) with the addition of 10 wt% kenaf particles. The 15 wt% kenaf sample exhibited slightly decreased flexural strength because of insufficient PP content. The lack of PP in the matrix leads to poor adhesion between the fibres and the matrix. Furthermore, the matrix was not able to hold the fibres during loading, which in turn decreased the utility of the fibres in the load transfer process (Ibraheem *et al*., 2011). The improvement of flexural strength was highlighted in 5 wt% composition of kenaf-reinforced PP with NaOH, because of the removal of impurities in the treated particles of kenaf. However, when the NaOH concentration was increased to over 10%, the value of the flexural strength decreased to 26.97 MPa because 15% NaOH concentration may have been too strong and could have damaged the particles, thereby resulting in decreased mechanical properties and damaged fibres (Mwaikambo & Ansell, 2002).

Fig.5 shows the tensile modulus results reflecting the effect of changes in the content percentage of the treated and untreated kenaf particles. The Young's modulus value of the kenaf/PP composite increased from 5 wt% to 15 wt% composition of kenaf, where the highest value was 1079.61 MPa at 5 wt% of composition. The treated kenaf particles showed stronger strength in the Young's modulus at 5% concentration of NaOH (1079.68 MPa), but the value decreased when the concentration of NaOH was increased (807.12 MPa). According to Nishino *et al*. (2003), the increase in percentage of natural fibres enhanced the properties of the Young's modulus. The increasing concentration of NaOH led to decreased tensile modulus.

Morphological of Fracture Surface of Flexural Specimens

Morphological and structural changes of the fracture surface were observed under SEM. The fracture surface of 5 wt% kenaf/PP composite is illustrated in Fig.6(a). The

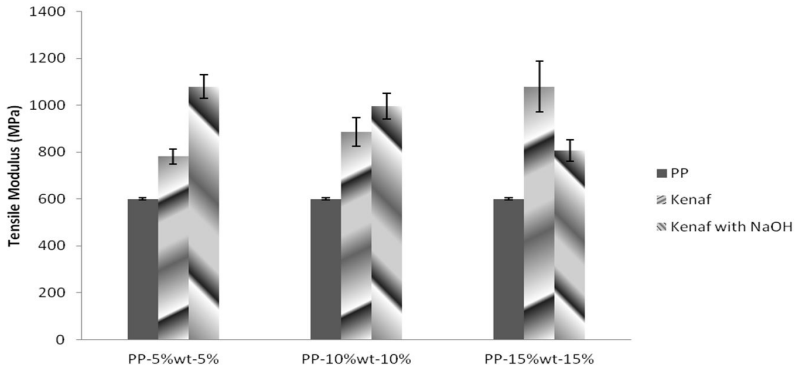

Fig.5: The variation of tensile modulus against kenaf particles content with concentration of NaOH.

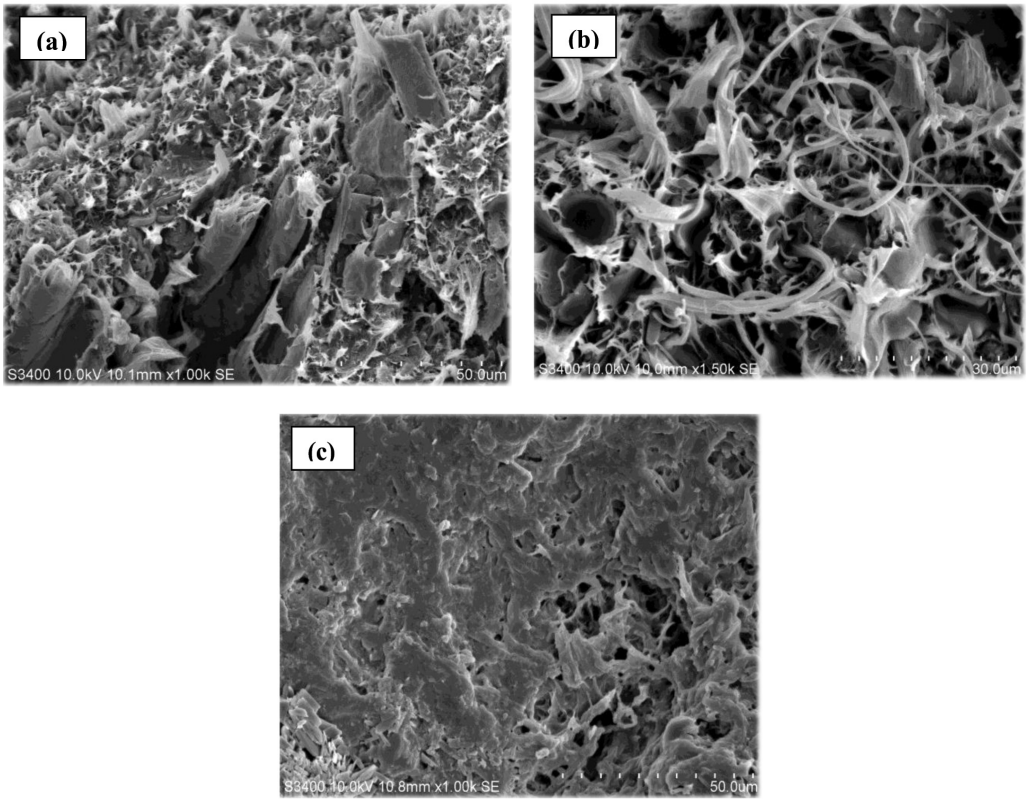

Fig.6: SEM micrograph for flexural fracture structure of kenaf/PP composites: (a) 5%wt kenaf/PP; (b) 10%wt kenaf/PP; (c) 10%wt kenaf/PP with NaOH.

combination of kenaf particle breakage and the particle pull-out indicates that the adhesion of kenaf/PP was adequate. Fig.6(b) shows the fracture surface of 10 wt% kenaf/PP composite and reveals a strong bond between PP and kenaf particles. The content of particle loading is higher and well coated with a polymer. The melted PP was distributed effectively and penetrated the kenaf particles. Treatment on kenaf/PP composites with NaOH showed poor fibre-matrix adhesion, as shown in Fig.6(c). The fracture surface of the specimens was fully covered with the NaOH residues, and the strength continuously decreased with increasing NaOH concentrations.

CONCLUSION

Our study examined the application of Kenaf as a natural fibre reinforced with PP in the fabrication of polymer composite materials. Kenaf/PP was treated with different NaOH concentrations. After treatment, the samples were subjected to mechanical testing and their morphology was examined under SEM. The strength of the kenaf/PP composite decreased with increasing NaOH concentrations. At 15% of NaOH, the concentration proved to be too strong and caused damage to the particles, resulting in decreased mechanical properties of the sample. Nevertheless, the incorporated kenaf particles acted as good reinforcement in the polymer composites and improved the mechanical property strength of the composites with kenaf particle increment loading.

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REFERENCES

Akil, H. M., Omar, M. F., Mazuki, A. A. M., Safiee, S., Ishak, Z. A. M., & Abu Bakar, A. (2011). Kenaf fibre reinforced composites: A review. *Materials & Design, 32*(8-9).

ASTM. (2000a). *Standard test method for tensile properties of plastics* (pp. 13). United States. An American National Standards.

ASTM. (2000b). *Standard test methods for flexural properties of unreinforced and reinforced plastics and electrical insulating materials* (p. 9). United States An American National Standards.

Aziz, S. H., & Ansell, M. P. (2004). The effect of alkalization and fibre alignment on the mechanical and thermal properties of kenaf and hemp bast fibre composites: Part 1 - polyester resin matrix. *Composites Science and Technology, 64*(9), 1219-1230.

Edeerozey, A. M. M., Akil, H. M., Azhar, A. B., & Ariffin, M. Z. (2007). Chemical modification of kenaf fibres. *Materials Letters, 61*(10), 2023-2025.

El-Shekeil, Y. A., Sapuan, S. M., Zainudin, E. S., & Abdan, K. (2011). Effect of fibre loading on the mechanical properties of kenaf fibre reinforced thermoplastic polyurethane composite. *Composite Science and Technology, 471-472*, 1058-1063.

Ibraheem, S. A., Ali, A., & Khalina, A. (2011). Development of green insulation boards from kenaf fibres part 1: Development and characterization of mechanical properties.

Fracture and Strength of Solids, 462-463, 1343-1348.

Loh, N. H., Tor, S. B., & Khor, K. A. (2001). Production of metal matrix composite part by powder injection molding. *Journal of Materials Processing Technology, 108*(3), 398-407.

Nishino, T., Hirao, K., Kotera, M., Nakamae, K., & Inagaki, H. (2003). Kenaf reinforced biodegradable composite. *Composites Science and Technology, 63*(9), 1281-1286.

Nishino, T., Hirao, K., Kotera, M., Nakamae, K., & Inagaki, H. (2003). Kenaf reinforced biodegradable composite. *Composites Science and Technology, 63*(9), 1281-1286.

Pothan, L. A., Oommen, Z., & Thomas, S. (2003). Dynamic mechanical analysis of banana fibre reinforced polyester composites. *Composites Science and Technology, 63*(2), 283-293.

Tserki, V., Matzinos, P., & Panayiotou, C. (2006). Novel biodegradable composites based on treated lignocellulosic waste flour as filler Part II. Development of biodegradable composites using treated and compatibilized waste flour. *Composites Part A-Applied Science and Manufacturing, 37*(9), 1231-1238.

Vilay, V., Mariatti, M., Taib, R. M., & Todo, M. (2008). Effect of fibre surface treatment and fibre loading on the properties of bagasse fibre-reinforced unsaturated polyester composites. *Composites Science and Technology, 68*(3-4), 631-638.

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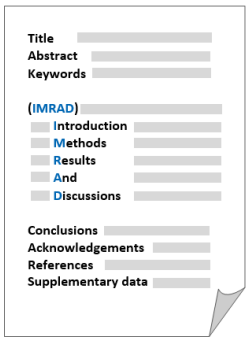

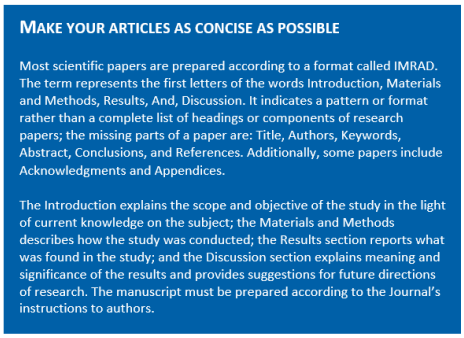

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