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Editorial Statement

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EDITORIAL OFFICE

JOURNAL DIVISION
Office of the Deputy Vice Chancellor (R&I)
1st Floor, IDEA Tower II
UPM-MTDC Technology Centre
Universiti Putra Malaysia
43400 Serdang, Selangor Malaysia.
Gen Enq.: +603 8947 1622 | 1619 | 1616
E-mail: executive_editor.pertanika@upm.my
URL: www.journals-jd.upm.edu.my

PUBLISHER

Kamariah Mohd Saidin
UPM Press
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor, Malaysia.
Tel: +603 8946 8855, 8946 8854
Fax: +603 8941 6172
E-mail: penerbit@putra.upm.edu.my
URL: http://penerbit.upm.edu.my

ABSTRACTING/INDEXING

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Foreword

Welcome to the **Fourth Issue 2014** 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 **nine articles,** out of which **one** is a review article and **eight** are regular research papers. The authors of these articles are from **Malaysia, Germany, Norway, Bangladesh, India** and **Indonesia**.

The review article discusses the current scenario and risk factors of streptococcosis in tilapia and suggests the control and prevention measures for this disease (*Zamri-Saad, M., Amal, M. N. A., Siti-Zahrah, A.* and *Zulkafli, A. R.*). The preventive measures focus on combined aspects of selecting farm location, applying good aquaculture farm practices, utilization of antibiotics and proper vaccination programme. A combination of all these measures will perhaps be the key to improve the health of cultured tilapia and prevent the infection by *S. agalactiae*, which in turn will increase the economic profit of tilapia farm operators

The eight research papers cover a wide range of topics. The first research paper, a collaborative work between Universiti Sains Malaysia, National Prawn Fry Production and Research Centre and World Fish Centre, Malaysia reports on the performance of the Genetically Improved Farmed Tilapia (GIFT) strain over ten generations of selection in Malaysia (*Azhar Hamzah, Raul W. Ponzoni, Nguyen Hong Nguyen, Hooi Ling Khaw, Hoong Yip Yee* and *Siti Azizah Mohd Nor*). The second research paper presents an empirical approach in attaining sufficient yield of sugarcane in Bangladesh (*Rana, M. S., Hossain, F.* and *Roy, S. S.*). The next research paper from the School of Biological Sciences, Universiti Sains Malaysia reports on the occurrence of *Fusarium* spp. on vegetable crops and provides an assessment of its pathogenicity (*Nurul Huda Mohamad Saseetharan* and *Latiffah Zakaria*).

In the next research paper, researchers from Universiti Putra Malaysia submit their findings after comparing preference for *Molineria latifolia* var. megacarpa and *Rhodomyrtus tomentosa* as native urban landscape plants (*Sarah, B., Thohirah, L. A., Mustafa Kamal, M. S.* and *Rosenani A. B.*) while another research paper, also from Universiti Putra Malaysia highlights timber use practices in Malaysia's construction industry (*Mohamed, S.* and *Abdullah, R.*).

Another research paper examines the molecular characterisation of fowl Adenoviruses isolated from inclusion in body hepatitis outbreaks in commercial broiler chickens in Malaysia (*Juliana, M. A., Nurulfiza, I., Hair-Bejo, M., Omar, A. R.* and *Aini, I.*). The seventh research paper discusses a comparative study on callus induction, proliferation and plantlets regeneration in two cultivars of *Stevia rebaudiana* Bertoni, which is known as the only non-caloric natural sweetener (*Sharuti Rathore, Kuldeep Yadav, Narender Singh* and *S. K. Singh*). The last research paper reports on a new latent lovastatin producer viz. *Fusarium pseudocircinatum* IBRL B3-4, produced through laboratory tray system (*Syarifah, A. R., Darah, I.* and *I Nyoman, P. A.*).

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.

Chief Executive Editor
Nayan Deep S. KANWAL, FRSA, ABIM, AMIS, Ph.D.
nayan@upm.my

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Review Article

Control and Prevention of Streptococcosis in Cultured Tilapia in Malaysia: A Review

Zamri-Saad, M.[1], Amal, M. N. A.[2]*, Siti-Zahrah, A.[3] and Zulkafli, A. R.[4]

[1]Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Malaysia
[3]National Fish Health Research Centre, Fisheries Research Institute, 11960 Batu Maung, Penang, Malaysia
[4]Freshwater Fisheries Research Center, Fisheries Research Institute, 71600 Jelebu, Negeri Sembilan, Malaysia

ABSTRACT

Streptococcosis in cultured fishes has been reported to cause severe economic losses to the aquaculture industry worldwide. Lancefield group B *Streptococcus agalactiae* has been recognised as the main pathogen in cultured tilapia. This review discusses the current scenario and risk factors of streptococcosis in tilapia and suggests the control and prevention measures for this disease. The preventive measures focus on combined aspects of selecting farm location, applying good aquaculture farm practices, utilization of antibiotics and proper vaccination programme. A combination of all these measures will perhaps be the key to improve the health of cultured tilapia and prevent the infection by *S. agalactiae*, which in turn will increase the economic profit of tilapia farm operators.

Keywords: Control, prevention, streptococcosis, tilapia, aquaculture, Malaysia

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E-mail addresses:
mzamri@upm.edu.my (Zamri-Saad, M.),
mnamal@upm.edu.my (Amal, M. N. A.),
siti.zahrah.abd@gmail.com (Siti-Zahrah, A.),
zulkaflirashid@gmail.com (Zulkafli, A. R.)
* Corresponding author

INTRODUCTION

Streptococcosis is an infection by Gram-positive bacteria of the genus *Streptococcus*. In cultured fish industry, infections by *Streptococcus* sp. have been reported to cause outbreaks leading to considerable morbidity and mortality worldwide. Klesius *et al*. (2008) estimated that worldwide annual losses due to streptococcosis alone

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were USD150 million in 2000 and exceeded USD250 million in 2008. Currently, *Streptococcus agalactiae*, *S. iniae* and *S. dysgalactiae* have been identified as the main pathogens that cause diseases, leading to severe economic losses in the aquaculture and fisheries industry throughout the world (Evans *et al.,* 2006; Amal & Zamri-Saad, 2011; Costa *et al.,* 2013).

Disease outbreaks following infections by *S. agalactiae* have been reported in various species of marine and freshwater fishes such as silver pomfret (*Pampus argenteus*), golden pompano (*Trachinotus blochii*), seabream (*Sparus auratus*), wild mullet (*Liza klunzingeri*), Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), ya-fish (*Schizothorax prenanti*), wild giant Queensland grouper (*Epinephelus lanceolatus*), estuary ray (*Dasyatis fluviorum*), mangrove whipray (*Himantura granulata*) and eastern shovelnose ray (*Aptychotrema rostrata*) (Evans *et al.,* 2002; Duremdez *et al.,* 2004; Suanyuk *et al.,* 2005, 2008; Hernandez *et al.,* 2009; Mian *et al.,* 2009; Geng *et al.,* 2011; Amal *et al.,* 2012; Azad *et al.,* 2012; Bowater *et al.,* 2012). Recently, streptococcal disease in cultured tilapia has become an emergence problem and is among the leading disease that causes severe economical impact worldwide. Therefore, *S. agalactiae* has been identified as one of the important tilapia pathogens among the streptococcal species that affects various species of fishes in the world.

In Malaysia, *Streptococcus* outbreak was first recorded in the late 1990s. The first outbreak of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.) was observed in 1997 in Pahang river, Pahang. The disease affected tilapia weighing between 300 and 400g causing 60% mortality. Subsequently in 2000, outbreaks of *S. agalactiae* infection were reported in Kenyir Lake, Terengganu and Pergau Lake, Kelantan, killing approximately 50% of the cultured tilapia population. The outbreaks were observed between March and June of the year (Siti Zahrah *et al.,* 2004, 2005). Lately, reported cases of *S. agalactiae* infection, which included the wild and cultured tilapia, are widespread, covering almost all over Peninsular Malaysia (see Fig.1) (Amal, 2007, 2011; Najiah *et al.,* 2009; Nur-Nazifah *et al.,* 2009; Siti-Zahrah *et al.,* 2009; Zulkafli *et al.,* 2009; Amal *et al.,* 2010ab, 2013abc; Zamri-Saad *et al.,* 2010).

Affected tilapias showed either red discolouration of the skin, erratic swimming, whirling, corneal opacity, eye haemorrhage, cataract, exopthalmia, occasional sunken body or acute inflammation along the base of the pectoral and ventral regions, skin haemorrhages around the anus or at the base of anus, congested visceral organs (particularly liver, spleen and kidney), while the brain appeared soft and occasionally oedematous (Najiah *et al.,* 2009; Ali *et al.,* 2010; Zamri-Saad *et al.,* 2010).

Introduction of contaminated water and/or fry into the farm, high stocking density, poor husbandry management, deterioration of water qualities such as slow flowing water, high water temperature, high ammonia, low dissolved oxygen and unsuitable pH

and salinity in the culture system were reported to be the risk factors and stressors that increased the susceptibility of fish to streptococcal infection (Bunch & Bejerano, 1997; Bowser *et al.,* 1998; Shoemaker *et al.,* 2000; Nguyen *et al.,* 2002; Yanong & Floyd, 2002; Mian *et al.,* 2009; Amal, 2011; Amal *et al.,* 2013a; Milud *et al.,* 2013).

This report analyses and discusses the potential control and prevention measures for *S. agalactiae* infection in cultured tilapia (*Oreochromis* sp.), which can be used as a guide for the farmers and researchers based on previous reports and experiences of streptococcosis outbreaks in Malaysia.

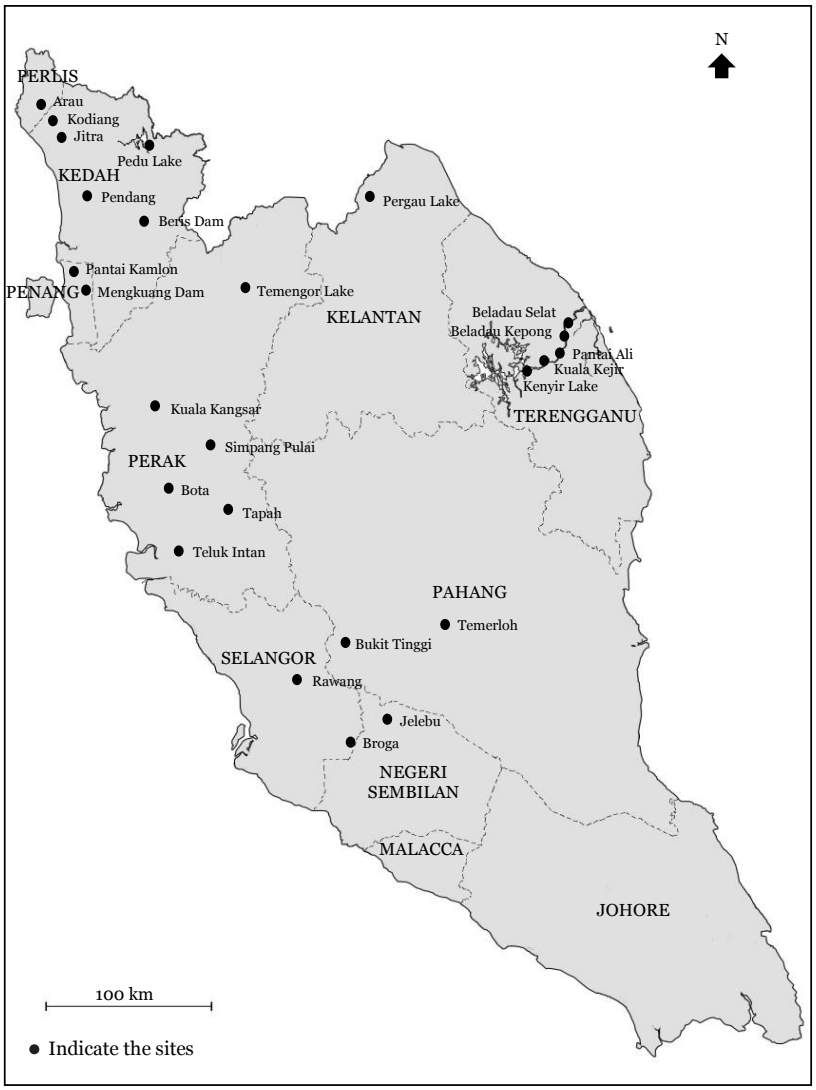

Fig.1: Sites with reported cases of *S. agalactiae* infection in wild and cultured tilapia in Peninsular Malaysia

POTENTIAL CONTROL AND PREVENTION MEASURES

Selection of Hatchery and Farm Sites

Siti-Zahrah *et al.* (2009) stated that cases of streptococcosis in tilapia were significantly higher in floating net cages kept in rivers, ponds and dams. *Streptococcus agalactiae* was also successfully isolated from the red tilapia cultured in commercial earthen pond farms (Najiah *et al.,* 2009; Nur-Nazifah *et al.,* 2009; Ali *et al.,* 2010). More recently, Amal (2011) reported that the mean prevalence of *S. agalactiae* infections in tilapia in Malaysia was significantly higher in floating net cage culture in huge-sized reservoirs (9.68±10.42%) and moderate-sized river (2.57±3.59%) compared to small-sized irrigation canal (0.28±0.9%), pond (0.69±2.77%) and ex-mining pool (0.17±0.82%). Moreover, infections by *S. agalactiae* have also been reported from various types of water bodies and culture methods such as raceway, hatchery, marine water floating net cage, freshwater floating net cage, shaded outdoor tank and open sea (Glibert *et al.,* 2002; Duremdez *et al.,* 2004; Mian *et al.,* 2009; Azad *et al.,* 2012). It seemed that certain types of water bodies and fish culture methods enhanced susceptibility of tilapia to *S. agalactiae* infections. However, no prohibition or preferable place was suggested as suitable culture site from previous studies.

Therefore, a good tilapia hatchery or farm should have electricity supply, proper roads, continuous water supply and water reservoirs, feed store, treatment or quarantine tanks, proper water aeration system, good water filters, etc. (Klesius *et al.,* 2008). Nevertheless, based on the observation of streptococcosis outbreaks in this country, farmers are advised not to choose huge size water body with extremely slow water flow rate such as big reservoirs or downstream huge rivers as site for tilapia culture due to retention of the heat at the upper water column during hot and dry seasons (Amal, 2011). Preferable culture sites for irrigation canal, raceway, river and sea should have flowing water, while for hatchery, indoor tank, shaded outdoor tank and earthen pond, the site should have continuous sources of water or water reservoir for partial or total water exchange in order to maintain a good water quality of their culture site (El-Sayed, 2006). Moreover, floating net cage culture site in rivers and reservoirs also must be equipped with excellent facilities mentioned above as an early step to prevent and control this disease.

Treatment of Water Supply and Fish Fry

In hatchery, farmers who culture their fry or adult tilapia in small ponds, concrete cemented ponds or tanks should filter and treat the water supply before channeling it to the culture system. Moreover, the farmers should ensure that the water is not or less contaminated by pathogens by using commercially available filters such as ozone and ultraviolet. In addition, the treatment of water can also be conducted using commercial biological filters and several types of solid removal filtration. It is important for fry production in hatchery

and small-scale farm where the input and output of water at the culture system can be manually controlled (pond, fiber tank, etc). By separating and treating the water supply, farmers can minimize the transmission of pathogenic bacteria from the water or wild fishes into the hatchery or culture system (Evans *et al*., 2002).

Previous study revealed that the wild fishes collected nearby the fish culture farms were infected by the same *S. iniae* strain, indicating the transmission of the pathogen from the wild to the cultured fish (Colorni *et al.,* 2002). Similarly, Bromage and Owen (2002) also reported that fish cohabiting barramundi pens had the same *S. iniae* strain infection as those of the barramundi. Moreover, Glibert *et al.* (2002) suggested that the transmission of *S. agalactiae* to the cultured fish was believed to have occurred via several routes and *Streptococcus* might have already present for a long period of time within the ecosystem (water, wild or cultured fish). Xu *et al.* (2007) also demonstrated that concurrent infection of tilapia with *Gyrodactyus niloticus* and *S. iniae* resulted in a significant increase in susceptibility to *S. iniae* disease. The authors also showed that *G. niloticus* could harbor *S. iniae* and might be a vector of infection for tilapia. Concurrent infection with *Trichodina* spp. and *S. iniae* or *S. agalactiae* also increased the susceptibility of channel catfish to both these streptococci (Evans *et al.,* 2007). Their results demonstrated that external parasites might play a role in the susceptibility of fish to *S. iniae* and *S. agalactiae* infections.

In order to treat the newly arrival fish fry before introducing them into the farm from most common fish parasites, Siti-Zahrah and Rokiah (1996) suggested to quarantine the fish using formalin and salt in concentrations of 25ppm and 1500ppm, respectively. The treatment should be conducted for three consecutive days in a week and repeated (if necessary) for the following week.

Currently, Arechavala-Lopez *et al.* (2013) reported the possibilities of reared fish, farmed escapees and wild fish stocks, which could contribute to a triangle of pathogen transmission. Thus, farmers should prevent the diseased fry or fish cultured in their hatchery or farm to not escape and transmit the diseases to the wild population, whether for same or different wild species of fishes.

Recently in Malaysia, Amal *et al*. (2013c) reported a case where *S. agalactiae* was transmitted to a newly established red hybrid tilapia farm in a reservoir via carrier fish fry and water from hatchery. Moreover, preventing the introduction of the wild fish into the culture system by additional nets surrounding the farm or cages with regular monitoring and by using improved netting materials for sea-cages (Jensen *et al.,* 2010), quarantine and treatment of the new stocks of fish fry for bacterial and parasitic diseases, and purchasing fish fry from a disease-free hatchery are important practices in order to control and prevent the transmission of streptococcosis.

Frequent Monitoring of Water Quality

Water quality is an important part of any aquaculture system. Successful aquaculture depends on the water quality (Boyd & Tucker, 1998). Water quality plays a major role in fish health because deterioration in water quality causes stress to the fish and leads to disease outbreaks. As *Streptococcus* spp. are opportunistic pathogens that are widely spread in aquaculture environment, they depend on stress to assert pathogenicity (Bunch & Bejerano, 1997). Therefore, farmers should always monitor and maintain water quality during the culture period. It is therefore necessary to understand the major water quality parameters and their interrelationships (relationship within the water quality parameters) which affect fish growth and health, and to determine the failure or success of the overall culture practices (El-Sayed, 2006).

For tilapia culture, the recommended water quality parameters suggested by Department of Fisheries, Malaysia are 6.5 – 8.5 for pH, >5mg/l for dissolved oxygen, 25 – 32^{0}C for water temperature, <0.02mg/l for ammonia, <3mg/l for nitrate, <2mg/l for iron and <120mg/l for alkalinity (Zulkafli, 2013). However, the water quality parameters may vary depending on the species, strains, sizes, duration of exposure, other environmental factors, culture system, geographical system, etc. (El-Sayed, 2006).

Environmental conditions surrounding the culture sites may affect water quality, and this will in turn stress the cultured fish due to disturbance on their physiology (Amal *et al.,* 2013a). This eventually lowers immune response and triggers bacterial infection, which are already in the water or the carrier fish leading to disease outbreaks. Even with low concentration of *S. agalactiae* in the water of culture system, bacteria are able to infect fish and express their virulency. This happens when water quality parameters are not at the optimum condition (Le Morvan *et al.,* 1998; Langston *et al.,* 2002).

There are a number of studies that evaluate the effects of water quality parameters on streptococcosis in tilapia. Some stressors that have been associated with *Streptococcus* outbreaks include high water temperature (>26°C) (Mian *et al.,* 2009; Amal *et al.,* 2013a; Milud *et al.,* 2013), high salinity (>15ppt) (Milud *et al.,* 2013) and pH (6-8) (Zhou *et al.,* 2008; Amal *et al.,* 2013a), low dissolved oxygen concentration (<3.4mg/l) (Bunch & Bejerano, 1997), high ammonia (>0.18mg/l) (Hurvitz *et al.,* 1997) or nitrite concentration (>10mg/l) (Bunch & Bejerano, 1997), slow flowing water (<0.25m/s) (Amal, 2011) and very high clarity of water (>334cm) (Amal *et al.,* 2013a).

An experimental study involving red hybrid tilapia (*Oreochromis* sp.) revealed that environmental temperature of 33°C, water salinity of 15ppt and water pH of 6 increased the susceptibility of the fish to *S. agalactiae* (Milud *et al.,* 2013). A field study conducted in Malaysia revealed that tilapia cultured in deep reservoirs were exposed to high water temperature (>29^{0}C) for up to 8m deep for a long period of time (during hot and dry season) due to extremely high water clarity (>425cm) and very slow water flow

(<0.01cm/s) increased their susceptibility to *S. agalactiae* infection (Amal *et al.,* 2010b). Moreover, Amal *et al.* (2013a) found that high water temperature (>27^{0}C), clarity (>334cm) and pH (7.37±0.53) of lake water, as well as high ammonia (>0.2mg/l), temperature (>29^{0}C) and low dissolved oxygen (<5mg/l) in down-stream river showed significant correlation with the presence of *S. agalactiae* in the cultured tilapia farms. Since it is not always possible to properly manage water temperature and dissolved oxygen levels, the conditions that favour disease outbreaks should always be kept minimal.

Controlled water bodies and culture systems such as earthern pond, tank, pool and hatchery, frequent partial or total water exchange are very important. According to Sipauba-Tavares *et al.* (2000), continuous water exchange increases the growth performance of Nile tilapia compared to no water exchange treatment when cultured in earthern pond. If this practice is not effective and practical in certain area due to water limitation and water cost, partial exchange and continuous water aeration are recommended. This practices are very important to remove and also lower the level of faeces and other total solids and dissolved metabolites such as ammonia and nitrite in the culture system, which negatively affect the culture performance.

For uncontrolled water bodies such as river and reservoir, lowering the fish culture density may help to reduce the stress of fish, which could lead to disease outbreaks. Sipauba-Tavares *et al.* (2000) revealed that pH, dissolved oxygen, carbon dioxide, nitrite, ammonia, phosphorus and chlorophyll a did not differ across the treatment of tilapia that received no water exchange, no water exchange but provided with aeration at night, or continuous aeration due to low fish stocking density (1.3 fish/m^3). The recommended stocking density is discussed later in this review.

Good Management Practice in Tilapia Hatchery and Farm

Farmers should minimize unnecessary handling or transportation of fish to reduce the stress of fish, which may contribute to disease outbreaks. It is also recommended to keep the hatcheries and farms clean all the time in order to reduce the risks of disease transmission and outbreaks. Furthermore, periodic cleaning and disinfection of all production units and equipment are also suggested as these will decrease the transmission of pathogens. In farm, for example, cleaning of the culture net cages, tanks and ponds should be conducted before the introduction of new fry by physical cleaning and drying, while drying and liming (calcite or dolomite) for the ponds. Equipment such as scoop nets, buckets, small tanks, containers, etc., should be regularly cleaned using the recommended commercial bleach (sodium hypochlorite at 200 - 500ppm) (Dvorak, 2009). Purchasing specific pathogen-free stocks and proper quarantine of newly arrived fish stocks are highly recommended while screening the new arrival fish stocks against *Streptococcus* is a compulsory (Dvorak, 2009). This is

because once the disease is introduced into the culture systems, it is difficult to eradicate them.

Maintenance and monitoring of fish production and health records can also help to detect disease problems and highlight their severity that often provides clues for disease diagnoses. In fact, maintaining accurate records of fish illnesses or deaths, while keeping records on fish production parameters such as water quality, growth and feed conversion ration also aids in detecting subclinical disease problems. Moreover, it is suggested that farmers record all new introductions or returning fish, their sources and movements on or off the hatcheries and farms. This practice can actually help identify potential disease entry points in the event of a disease outbreak (Dvorak, 2009).

High water temperature is a stress for fish but a preferential condition for the bacteria. Therefore, lowering the water temperature is extremely important and this can be achieved by recirculation systems. Small-size ponds and net cage farms often utilize sunscreens and water sprinklers to reduce water temperature. Activating paddle wheels during hot day is an additional method that can help reduce water temperature and increase the concentration of dissolved oxygen. Floating net-cage culture involving huge water bodies like reservoirs and dams, high-pressure multijet air under the cages is quite practical in reducing the water temperature and improving supply of the dissolved oxygen. However, these practices are costly and can cause upwelling in shallow water bodies and they can be deleterious. Thus, utilization of sunscreens is widely practiced in this country.

Reasonable Fish Stocking Density in the Culture System

Farmers should reduce fish stocking density to be reasonable according to the size of cages, size of fish or type of culture systems. High productivity in tilapia farming is achieved by balancing stocking density with a good survival rate. When mortality increases, lowering the stocking density helps to lower both stress level and pathogen load within the population.

Extensive research has been carried out on the effects of stocking density on tilapia production in different intensive culture systems (El-Sayed, 2002). However, the results revealed controversial outcomes and this is probably due to the differences in species, sexes, life stages, sizes, social hierarchies, nutrition, feeds, feeding regimes, culture systems and water qualities (Huang & Chiu, 1997; Muir *et al*., 2000). Nevertheless, El-Sayed (2006) concluded that tilapia are known to tolerate a high stocking density and can withstand extreme crowding condition. In contrast, Shoemaker *et al.* (2000) reported that high stocking density (≥11.2g/l) significantly affects the rate of mortality in tilapia exposed to *S. iniae*. In addition, Xu *et al.* (2007) revealed that the infection of this pathogen could involve direct infection through wounds and abrasions of the skin. This mechanism was reported to occur when the fish were

cultured in high densities. By reducing the stocking density, outbreaks of this disease could probably be controlled or at least minimized.

The relationship between stocking density and individual fish growth is generally negative (Sin & Chiu, 1983), while there is a positive correlation between stocking density and tilapia yield (i.e., high fish density leads to high yield) (Siddiqui *et al.,* 1997). According to El-Sayed (2006), farmers should therefore carefully define their target consumers and determine the appropriate stocking density that will produce the size of the fish preferred by the consumers. In addition, farmers should also adopt the stocking density that satisfies maximum production efficiency and the production of fish of a uniform size. Farmers should also determine whether they want to produce a high yield but small individual fish to be sold in rural areas at low prices, or produce larger individual fish but with a lower yield for restaurants or export purposes at higher prices.

In Malaysia, farmers usually stocked their fish based on the size of cage and fish. In theory, the bigger the size of the fish, the stocking density should be reduced accordingly. It is recommended to stock the cultured tilapia at 13 fish/m^3 of water (approximately 1500 fish in a cage of 6m length x 5m width x 4m depth) until the fish reached 100g and at the stocking rate should be 8 fish/m^3 of water (approximately 1000 fish in a cage of 6m length x 5m width x 4m depth).

Feed and Feeding

Partial reduction or completely stop feeding helps to control or reduce the mortality during streptococcosis outbreaks. This is because feeding facilitates proliferation of bacteria in the water. Furthermore, uneaten or excessive feed leads to deterioration of water quality. Besides, infected fish are low in appetite until they are recovered from the infection or in other words, the sick fish lose their appetite and will not eat. Therefore, feed reduction is one of the factors that can reduce and control the mortality rate of streptococcosis.

The use of contaminated thrash fish as feeds has also been implicated in the outbreaks of *Streptococcus* in Korea (Kim *et al.,* 2007), especially in the marine water fish aquaculture. Therefore in Malaysia, farmers are advised to feed tilapia with boiled visceral organ of chickens or trash fishes. This is to ensure that the feeds are free of pathogens which may contribute to disease outbreaks.

Susceptible Fish Size and Critical Period of Tilapia Farming

Study on streptococcosis in Malaysia revealed that tilapia weighing between 100 and 300g (10 - 30cm of total body length) were more susceptible to *S. agalactiae* infection (Zulkafli *et al.,* 2009; Amal, 2011; Amal *et al.,* 2013a). In addition, when tilapia of this size are cultured in huge water bodies (e.g., reservoirs and dams) with extremely slow water movement and high water temperature (≥30^0C) during the hot and dry months (April to September), the tilapia are

more susceptible to streptococcosis. Thus, avoiding fish of susceptible size during the critical months might be beneficial in reducing streptococcosis. For example, when a farmer starts culturing tilapia in early January by introducing fingerling of approximately ±20g, the harvesting size of ±500g can be reached in 5 to 6 months at the growth rate of approximately 3g/day. Therefore, fish should be weighing between 100 and 300g (susceptible size) in the months of February to April (non-critical period). This simple management practice avoids rearing susceptible tilapias size during the critical period of April to September and thus reduces chances of disease outbreaks. However, the success of this control measure depends on the growth rates, strains of tilapia, feeding regimes, nutrition, stocking densities, water qualities and other husbandry practices. Experienced farmers can manage their own strategies but the concept remains that they should avoid rearing susceptible size during critical period.

Practising "break cycle" Method

Another potential husbandry management that could be considered is breaking the disease cycle by not culturing tilapia during the critical period of hot months. By using this method, farmers can avoid the risks of economic losses due to streptococcosis.

Water temperature influences the presence of *S. agalactiae* in cultured red hybrid tilapia (Amal *et al.,* 2013a). Thus, by not culturing tilapia during hot seasons, the presence or population of *Streptococcus* in cultured fish, culture systems and farm water bodies could be reduced as well. Moreover, if abnormal mortality is detected, early precaution should be taken by harvesting the fish in order to reduce the economic losses and to break the cycle of fish culture. Indeed, they should avoid culturing the susceptible size of fish during the critical period. However, farmers are recommended to culture other fish species during this critical period.

Practising the "all-in-all-out" Method

Farmers may also control streptococcosis by practicing the all-in-all-out method. It is suggested that new tilapia fry (non-critical size) be introduced in November (non-critical period) before all fish are harvested in April (critical period). The fish reach susceptible size (critical size) in January or February (non-critical period). Following harvest, cages must be cleaned before another batch of fry (non-critical size) is introduced in May (critical period), reach susceptible size in July (critical period) and harvested in October (non-critical period). This method of culture can effectively reduce disease incidence. The newly introduced fish is the most important factor in introduction of *Streptococcus* into a fish farm while some fish survived an outbreak can harbour the bacteria and transmit them to susceptible tilapia (Nguyen *et al.,* 2002). However, this all-in-all-out method may have some difficulties in implementation particularly the consistent supply of the fry.

Farmers are also recommended to practice monosex culture for tilapia. According to El-Sayed (2006), monosex tilapia showed high growth rates and feed utilization efficiency, greater uniformity of size at harvest, high tolerance to severe environmental conditions or water qualities, high resistance to stress and diseases, etc., and all-male populations are more preferable. Moreover, in this country, farmers prefer practicing monospecies culture, namely the red hybrid tilapia (*Oreochromis* sp.) due to their higher price, demands and easily available fry compared to the black or Nile tilapia and GIFT (Genetically Improved Farmed Tilapia). Combination of monosex and monospecies culture practices could also be concurrently practiced with the all-in-all-out culture method to minimize disease outbreaks and increase farmers' economic profits.

Early Marketing

The suggested break cycle and the all-in-all-out methods of rearing will eventually result in rearing susceptible fish during the critical period. Therefore, early marketing may be considered where fish are sold when they reach <350g during critical period (Siti-Zahrah *et al.,* 2009). When farmers sell their fish of susceptible size upon entering the critical period, they could minimize the risk of disease outbreaks. Moreover, tilapia consumers in Malaysia prefer fish of the sizes between 250 and 350g (Siti-Zahrah *et al.,* 2009). This practice may reduce the cost of feeding through shortened rearing period.

Immediate Disposal of Dead Tilapia

Many researchers speculate on the mode of transmissions of *Streptococcus*. Transmissions of *Streptococcus* may be due to inhabitation of cage culture environment, carried over by contaminated fish fry or disseminated by reservoir adult fish (Nguyen *et al.,* 2002; Najiah *et al.,* 2009). Experimental cohabitation of dead infected fish with healthy fish resulted in infection of many healthy fish. Horizontal transmission of the pathogens between fish is another likely mechanism of dissemination (Xu *et al.,* 2007). Moreover, cannibalism of infected dead fish is an important issue in lateral disease transmission. The dead fish tissue in water is soft and sometimes attacked by the other fish will possibly disseminate the *Streptococcus*. Thus, in order to prevent outbreaks, it is important to remove dying and dead fish as promptly and frequently as possible. Farmers should bury or burn the dead infected tilapia to avoid transmission of the bacteria. The increasing pathogen load and deterioration of water quality could be easily observed in tilapia culture sites when farmers failed to remove the dead fish.

Antibiotics

Antibiotics are only effective in treating outbreaks of streptococcosis if the treatment is applied early. In most cases, oral antibiotic treatments are ineffective as the infected fish have a reduced appetite. Therefore, antibiotics are only able to partially control mortality rates during the period of

application. Once the course of antibiotic is over, mortality usually increases again. This phenomenon leads to non-sustainable behaviour; as mortality increases again after a normal antibiotic course, farmers are tempted to extend the duration of antibiotic application to longer periods or use higher doses. This in turn increases selection pressure toward resistant bacteria. The negative consequences of using antibiotics, such as emergence of antibiotic resistant bacteria and antibiotic residues in meat, must be carefully evaluated.

In Malaysia, farmers tend to use erythromycin and oxytetracycline to treat streptococcosis in tilapia as well as a prophylactic agent in healthy fish. These antibiotics are usually sprayed onto fish pellets and given orally to fish. Therefore, *S. agalactiae* isolated from tilapia in Malaysia were found to be resistant to spiramycin, oleandomycin, sulphamethoxazole, oxolinic acid, kanamycin and nalidixic acid, probably due to the previous overuse or misuse of these drugs to combat bacterial diseases in farms (Najiah *et al.,* 2009). Therefore, antibiotic treatment is generally ineffective and the need for proper vaccine has become necessary (Klesius *et al.,* 2000b).

Darwish and Griffin (2002) found that oxytetracycline is effective in controlling *S. iniae* in blue tilapia (*O. aureus*). Oxytetracycline was incorporated into the feed at 0, 25, 50, 75 and 100mg/kg fish body weight for 14 consecutive days. The 75 and 100mg doses significantly increased the survival rate of the fish from 7% in the infected non-medicated to 85% and 98%, respectively. Other reports concluded that erythromycin-incorporated feed is effective against streptococcal infections in cultured yellowtails (Shiomitsu *et al.,* 1980) and rainbow trout (Kitao *et al*., 1979) at the doses of 25-50mg/kg fish body weight for 4 to 7 consecutive days. Darwish and Hobbs also (2005) revealed that oral administration of amoxicillin-medicated feed for 12 consecutive days at a daily rate of 10, 30 and 80mg/kg fish body weight significantly increased the survival of *S. iniae* infected tilapia from 3.8% in the challenged, non-medicated positive control to 45, 75 and 93.8%, respectively. The survival rate was significantly higher in the 80mg treatment (93.75%) than the 10mg treatment (45%) but did not differ significantly between the 10mg (45%) and 30mg (75%) treatments. In conclusion, they stated that no carriers were detected in any challenged group receiving amoxicillin-medicated feed, whereas the bacterium was recovered from the non-medicated, challenged survivors of the infection.

Several studies revealed the efficacy of florfenicol in controlling *S. iniae* infection in Nile tilapia, blue tilapia and sunshine bass (*Morone chrysops* x *M. saxatilis*) (Darwish, 2007; Bowser *et al.,* 2009; Bowker *et al.,* 2010; Darwish, 2010; Gaunt *et al.,* 2010; Gaikowski *et al.,* 2013). Gaunt *et al.* (2010), in a study to determine the dosage of florfenicol in feed to control *S. iniae*-associated mortality in Nile tilapia, found that cumulative mortality was 20.5±2.0% in the challenged, unmedicated group; 11.0±2.1% in the 10mg florfenicol/kg group;

and 5.5±2.4% in the 15mg florfenicol/kg group following intracoelomic injection with 0.1ml of 10^5 colony-forming units (CFU)/ml of *S. iniae* to the fish. The fish were given their treatment feed once per day at 2.5% body weight for 10 consecutive days after the infection. Moreover, the mortality was significantly less in the medicated groups than in the challenged, unmedicated control group (10mg/kg: p=0.0270; 15mg/kg: p=0.0007). Similarly, Darwish (2007) suggested the optimum therapeutic daily dose of florfenicol was between 10 and 15 mg/kg body weight for 10 consecutive days. Their studies also concluded that florfenicol was palatable, safe, and efficacious for control of Nile tilapia mortality due to *S. iniae* infection.

Recently, Lee and Park (2014) evaluated the combination of intramuscular amoxicillin-florfenicol in controlling *S. iniae* in olive flounder (*Paralichthys olivaceus*). They found that a single IM delivery at 10mg/kg body weight of the amoxicillin-florfenicol combination in olive flounder infected with 4.56 x 10^5 CFU/ml of *S. iniae* resulted in an increased rate of survival (77.5% after 5 days and 62.5% after 14 days) compared to the challenged but untreated control group.

The main issue in antibiotic utilization is the tissue withdrawal period of antibiotic. Bowser *et al*. (2009) showed a trend toward shorter half-lives of elimination of florfenicol in the smaller fish compared to those bigger sizes (100, 250 and 500g of experimental fish weight). The elimination times in muscle-skin and half-lives were 9.2 and 1.2 days (100g), 8.6 and 1.7 days (250g), and 12.7 and 2.2 days (500g), respectively. SEAFDEC (2000) and Serrano (2005) listed several antibiotics that are used in aquaculture and their withdrawal periods, ranging from five days (amoxicillin, ampicillin and florfenicol) to 30 days (erythromycin, spiramycin, oxytetracycline hydrochloride, sulfadimethoxine and oxolinic acid). However, in order to prevent development of antibiotic resistant bacteria and antibiotic residues in meat, farmers must practice prudent use of antibiotics, while referring to the local Fisheries Department Officer, veterinarians, fish health specialist and experienced aquaculturist is also recommended.

Vaccinations

Vaccination has been recognised as an important method of prevention of infectious diseases in farmed fish (Lombard *et al.,* 2007). The objective of vaccination is to provide a strong immune response against an administered antigen that is able to produce long-term protection against a pathogen (Klesius *et al.,* 2008). Killed and modified live vaccines have been developed for use in aquaculture. The type of immunity needed, antibody or cell mediated against a particular pathogens is among the deciding factor in the development of a vaccine.

The understanding on response of immune system of fish in relation to vaccination is very important. In general, vaccines are designed to protect fish from the consequences of infectious diseases. This is accomplished by exposing the

fish to inactivated, attenuated or other forms of the pathogen, giving rise to antibodies (including B cells, T cells, IgM, gut associated lymphoid tissue, and etc. in the serum, mucus and gut lavage fluid of the vaccinated fish) that protect the fish from the debilitating and often life-threatening consequences of infectious diseases. Vaccines are unique among modern medications in that they offer effective protection against the onset and progression of specific infectious diseases. Most other medications are therapeutic, i.e., they are used to treat the disease and/or its symptoms; few are preventative. Vaccination is also unique in harnessing the cells, tissues and molecules of fish immune system to mediate this protection through a variety of natural mechanisms and processes that are fundamental to fish biology (Anon, 2014).

Killed vaccines are usually administered by IM or intraperitoneal (IP) of individual fish. Injection is the least cost effective in terms of labour and time. Killed vaccines are considered safer than modified live vaccines, which may revert to virulence. Future trends in vaccination include oral and immersion delivery of killed vaccine, development of modified live vaccines and multivalent vaccines that require improved vaccine adjuvants and immunostimulant. Vaccine prevents disease and mortality but may not completely eliminate streptococci in surviving fish (Klesius *et al.,* 2008). To date, several vaccines have been developed against streptococcosis in fish (Eldar *et al.,* 1997; Klesius *et al.,* 2000a; Evans *et al.,* 2004; Shoemaker *et al.,* 2006).

There are several available commercial vaccines for *Streptococcus* disease in fish, such as:

- Vaccine from formalin killed *S. agalactiae* and administrated to fish by injection (United States Department of Agriculture).
- AQUAVAC® Strep Sa vaccine from attenuated *S. agalactiae* and addition of oil adjuvant against *S. agalactiae* Biotype 2. Administrated to fish by injection (Merck Animal Health Company, USA).
- Aquavac™ Garvetil™, vaccine against *Lactococcus* and *S. iniae* via immersion dip administration (Intervet/Schering-Plough Animal Health).
- Aquavac™ Garvetil™, an oral vaccine against *S. iniae* which was applied by mixing the vaccine with the feed (Intervet/Schering-Plough Animal Health).
- NORVAX® STREP Si vaccine, a monovalent vaccine containing an inactivated strain of *S. iniae* (Merck Animal Health Company, USA).

The administration of vaccine is another issue to be considered especially where a large number of fish is involved. Many possible routes of administration have been studied to facilitate vaccination, and these include immersion, IP injection, IM injection and oral. Immersion is one route that is suitable to be applied on fry in the sizes between 1 and 5g (Le Breton, 2009). Intraperitoneal and IM injections are

normally being used on large sized, valuable fish such as brood stocks and ornamental fishes. Oral administration fits to be used on all sizes of fish and fish that are cultured in large water bodies such as lakes and open sea cages (Le Breton, 2009). Oral feed vaccination is preferable route since it consumes less time, less labour work, low cost and easy to perform by farmers.

In Malaysia, several oral and spray vaccines against *S. agalactiae* infection in tilapia have been developed, even though at laboratory scale. The newly developed feed-based vaccines showed promising early results (Firdaus-Nawi *et al.,* 2011, 2012). According to Firdaus-Nawi *et al.* (2012), in their study to determine the systemic, mucosal immunity and protective capacity of the feed-based adjuvant vaccine (FAV) of *S. agalactiae* following oral vaccination against streptococcosis in tilapia, the FAV group had a significantly higher protection level compared to only feed-based vaccine and control group, which were 100%, 50% and 12.5% survival, respectively, after being challenged with 3.4 x 10^9 CFU/ml of live virulent *S. agalactiae*.

Moreover, in other study, Noraini *et al.* (2013) revealed that the formalin-killed cells of *S. agalactiae* vaccine with single spray exposure was able to induce IgM, giving moderate to high protection following the immersion (80% survival) and IP (70% survival) challenge with virulent strain of *S. agalactiae* compared to unvaccinated group (0% survival). They concluded that the spray administration of vaccines had a moderate to high protection level in tilapia by producing higher antibody responses in mucus and serum. Moreover, they also suggested that in order to further increase the length of protection against streptococcosis to a period longer than 2 months in cage-cultured tilapia (during the critical months), spray vaccination should be combined with oral vaccine (Firdaus-Nawi *et al.,* 2012).

Recently, Nur-Nazifah *et al.* (2014) successfully developed and evaluated the efficacy of feed-based recombinant vaccine encoding the cell wall surface anchoring the family protein of *S. agalactiae* against streptococcosis in *Oreochromis* sp. Their results showed that tilapia immunized with the feed-based recombinant vaccine developed a strong and significantly higher IgM antibody response in serum, mucus and gut lavage fluid samples compared to formalin killed of *S. agalactiae* feed-based vaccine and unvaccinated group. Following heat intervenes and IP challenge, the rate of survivors was 70% for the recombinant vaccinated group and 0% for the rest of the groups. Moreover, their study also showed that the newly developed vaccine significantly provided high protection against high dose challenge in heat stress environment and enhanced the production of the mucosal and humoral immunity.

Based on the study on the epidemiological pattern of *Streptococcus* infection among tilapia in Malaysia, vaccination is suggested to be conducted prior to the critical period on tilapia that have reached the susceptible size (100 - 300g) (Amal, 2011; Amal *et al*., 2013a). Immersion vaccination is recommended for fry and fingerling tilapia

of hatchery stage (Le Breton, 2009; Merck, 2013). After introducing fingerlings into cultured farms (floating net cage, pond, tank, etc.), the vaccine should be given when they enter the susceptible size (100±50g) before the hot seasons. Booster dose must be given on day 14 after the first vaccination (Firdaus-Nawi *et al.,* 2012). Interestingly, Noraini *et al.* (2013) and Nur-Nazifah *et al.* (2014) revealed that their newly developed vaccines could provide protection for about 8 weeks and thus protecting the fish during the critical hot season.

CONCLUSION

A combination of good aquaculture farm practices, selective utilization of antibiotics and proper vaccination programme are keys to improved fish health, reducing disease outbreaks and decreasing the devastating economic impact on tilapia farming in Malaysia.

Considering the prevalence and risk factors discussed earlier, the following combined control measures are suggested:

- Tilapia farm should be established at a site with moderate rate of water flow. The most suitable sites are the upstreams of river, irrigation canal and other small water bodies with moderate water flow and humanly controlled.
- The source of tilapia fry must be from disease-free hatcheries.
- Tilapia should be kept at reasonable stocking density at all time. As the size of fish increases, stocking density must also be modified accordingly.
- Always aware of the farm environment. This includes regular monitoring of water quality, proper disposal of dead fish and removal of debris.
- If possible, practice all-in-all-out management system. Otherwise, be aware of the critical fish size during critical months.
- Use proper antibiotics and vaccination regime.

Adopting biosecurity measures by all hatcheries and farms is recommended. Biosecurity involves practices, procedures and policies used to prevent the introduction of infectious diseases (Dvorak, 2009). Also, the spread of disease causing organisms (e.g., bacteria, viruses, fungi and parasites), as well as many aquatic invasive species, should be considered. Routine use of biosecurity measures can reduce the risks of introduction and reduce economic impacts of the diseases. Fish movement, water sources, fish health, equipment/vehicles and vectors (human and animal) should always be monitored and examined as they are the main risk factors for disease introduction and spread in aquaculture facilities. The use of biosecurity measures on the hatcheries and farms can help farmers to prevent the disease introduction and spread, and thus protect their fish and investment.

REFERENCES

Ali, A., Hassan, D., Saleha, A. A., Siti-Khairani, B., & Milud, A. (2010*). Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances*, *9*, 2640-2646.

Amal, M. N. A., & Zamri-Saad, M. (2011). Streptococcosis in tilapia (*Oreochromis niloticus*): a review. *Pertanika Journal of Tropical Agricultural Science, 34*, 195-206.

Amal, M. N. A. (2007). *Isolation and identification of Streptococcus* spp*. isolated from red tilapia (Oreochromis* sp.*)*. Undergraduate thesis, Bachelor of Science in Agrotechnology (Aquaculture), Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu.

Amal, M. N. A. (2011). *Prevalence, risk factors and transmission of Streptococcus agalactiae in the red hybrid tilapia (Oreochromis* sp.*)*. PhD thesis. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor.

Amal, M. N. A., Zamri-Saad, M., Iftikhar, A. R., Siti-Zahrah, A., Aziel, S., & Fahmi, S. (2012). An outbreak of *Streptococcus agalactiae* infection in cage-cultured golden pompano, *Trachinotus blochii* (Lacépède), in Malaysia. *Journal of Fish Diseases*, *35,* 849-852.

Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., & Zulkafli, A. R. (2013a). Water quality influences the presence of *Streptococcus agalactiae* in cage cultured red hybrid tilapia, *Oreochromis niloticus* x *Oreochromis mossambicus*. *Aquaculture Research*. doi: 10.1111/are.12180.

Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., & Zulkafli, A. R. (2013c). Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.) x *Oreochromis mossambicus* (Peters), farm. *Journal of Fish Diseases, 36*, 735-739.

Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, A. R., & Nur-Nazifah, M. (2013b). Molecular characterization of *Streptococcus agalactiae* strains isolated from fishes in Malaysia. *Journal of Applied Microbiology*, *115*, 20-29.

Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M., & Shahidan, H. (2010a). Prevalence of *Streptococcus agalactiae* in tilapia from flowing water, ponds, rivers and reservoirs. *Online Journal of Veterinary Research*, *14,* 153-162.

Amal, M. N. A., Zamri-Saad, M., Zulkafli, A. R., Siti-Zahrah, A., Misri, S., Ramley, B., Shahidan, H., & Sabri, M. Y. (2010b). Water thermocline confirms susceptibility of tilapia cultured in lakes to *Streptococcus agalactiae. Journal of Animal and Veterinary Advances, 9,* 2811-2817.

Anon. (2014). *How vaccines work. NPI reference guide on vaccine and vaccine safety*. Retrieved from http://www.path.org/vaccineresources/files/How_Vaccine_Work.pd.

Arechavala-Lopez, P., Sanchez-Jerez, P., Bayle-Sempere, J. T., Uglem, I., & Mladineo, I. (2013). Reared fish, farmed escapees and wild fish stocks - a triangle of pathogen transmission of concern to Mediterranean aquaculture management. *Aquaculture Environment Interactions*, *3*, 153-161.

Azad, I. S., Al-Marzouk, A., James, C. M., Almatar, S., Al-Gharabally, H. & Qasem, J. A. (2012). Outbreak of natural Streptococcosis in hatchery produced silver pomfret (*Pampus argenteus* Euphrasen) larvae in Kuwait. *Aquaculture*, 330-333, 15-20.

Bowater, R. O., Forbes-Faulkner, J., Anderson, I. G., Condon, K., Robinson *et al*. (2012). Natural outbreak of *Streptococcus agalactiae* (GBS) infection in wild giant Queensland grouper, *Epinephelus lanceolatus* (Bloch), and other wild fish in northern Queensland, Australia. *Journal of Fish Diseases*, *35*, 173-186.

Bowker, J. D., Ostland, V. E., Carty, D., & Bowman, M. P. (2010). Effectiveness of Aquaflor (50% florfenicol) to control mortality associated with *Streptococcus iniae* in freshwater-reared subadult sunshine bass. *Journal of Aquatic Animal Health*, *22*, 254-265.

Bowser, P. R., Kosoff, R. E., Chen, C. Y., Wooster, G. A., Getchell, R. G., Craig, J. L., Lim, P., Wetzlich, S. E., Craigmill, A. L., & Tell, L. A. (2009). Florfenicol residues in Nile tilapia after 10-d oral dosing in feed: effect of fish size. *Journal of Aquatic Animal Health, 21*, 14-17.

Bowser, P. R., Wooster, G. A., Getchell R. G., & Timmons, M. B. (1998). *Streptococcus iniae* infection of tilapia *Orechromis niloticus* in a recirculation production facility. *Journal of World Aquaculture Society*, *29*, 335-339.

Boyd, E. C., & Tucker, C. S. (1998). *Pond aquaculture water quality management*. Kluwer Academic Publisher, Massachusetts, USA.

Bromage, E. S. & Owens, L. (2002). Infection of barramundi *Lates calcarifer* with *Streptococcus iniae*: effect of different routes of exposure. *Diseases of Aquatic Organisms, 52*, 199-205.

Bunch, E. C. & Bejerano, Y. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* to Streptococcosis. *Israeli Journal of Aquaculture, 49*, 56-61.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H., & Zlotkin, A. (2002). *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Diseases of Aquatic Organisms, 49,* 165-170.

Costa, F. A. A., Leal, C. A. G., Leite, R. C., & Figueiredo, H. C. P. (2013). Genotyping of *Streptococcus dysgalactiae* strains isolated from Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases*, doi: 10.1111/jfd.12125.

Darwish, A. M. (2007). Laboratory efficacy of florfenicol against *Streptococcus iniae* infection in sunshine bass. *Journal of Aquatic Animal Health*, *19,* 1-7.

Darwish, A. M. (2010). Effectiveness of early intervention with florfenicol on a *Streptococcus iniae* infection in blue tilapia. *North American Journal of Aquaculture*, 72, 354-360.

Darwish, A. M. & Griffin, B. R. (2002, December). Study shows oxyetracycline controls *Streptococcus* in tilapia. *Global Aquaculture Advocate,* pp. 34-35.

Darwish, A. M. & Hobbs, M. S. (2005). Laboratory efficacy of amoxicillin for the control of *Streptococcus iniae* infection in blue tilapia. *Journal of Aquatic Animal Health, 17*, 197-202.

Duremdez, R., Al-Marzouk, A., Qasem, J. A., Al-Harbi, A., & Gharabally, A. (2004). Isolation of *Streptococcus agalactiae* from cultured silver pomfret, *Pampus argenteus* (Euphrasen), in Kuwait. *Journal of Fish Diseases*, *27*, 307-310.

Dvorak, G. (2009). Biosecurity for aquaculture facilities in the North Central Region. North Central Regional Aquaculture Center. *Fact Sheet Series #115*. Iowa State University, Ames, Iowa.

Eldar, A., Horovitcz, A., & Bercovier, H. (1997). Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. *Veterinary Immunology and Immunopathology, 56*, 175-183.

El-Sayed, A. -F. M. (2002). Effect of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. *Aquaculture Research*, *33*, 621-626.

El-Sayed, A. -F. M. (2006). *Tilapia Culture*. Oceanography Department, Faculty of Science, Alexandria University, Egypt. CABI Publishing.

Evans, J. J., Klesius, P. H., & Shoemaker, C. A. (2004). Efficacy of *Streptococcus agalactiae* (group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. *Vaccine, 22,* 3769-3773.

Evans, J. J., Klesius, P. H., Gilbert, P. M., Shoemaker, C. A., Al-Sarawi, M. A., Landsberg, J., Duremdez, R., Al-Marzouk, A., & Al-Zenki, S. (2002). Characterization of β-hemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and mullet, *Liza klunzingeri*

(Day), in Kuwait. *Journal of Fish Diseases, 25*, 505-513.

Evans, J. J., Klesius, P. H., Pasnik, D. J., & Shoemaker, C. A. (2007). Influence of natural *Trichodina* sp. parasitism on experimental *Streptococcus iniae* or *Streptococcus agalactiae* infection and survival of young channel catfish *Ictalurus punctatus* (Rafinesque). *Aquaculture Research*, *38*, 664-667.

Evans, J. J., Pasnik, D. J., Klesius, P. H., & Shoemaker, C. A. (2006). Identification and epidemiology of *Streptococcus iniae* and *Streptococcus agalactiae* in tilapia, *Oreochromis* spp. Proceeding of the 7th International Symposium on Tilapia in Aquaculture (pp. 25-52). 6th – 8th September 2006, Veracruz, Mexico.

Firdaus-Nawi, M., Noraini, O., Sabri, M. Y., Siti-Zahrah, A., Zamri-Saad, M., & Latifah, H. (2011). The effects of oral vaccination of *Streptococcus agalactiae* on stimulating gut-associated lymphoid tissues (GALTs) in tilapia (*Oreochromis* spp.) *Pertanika Journal of Tropical Agricultural Sciences*, *34*, 137-143.

Firdaus-Nawi, M., Yusoff, S. M., Yusof, H., Siti-Zahrah, A., & Zamri-Saad, M. (2012). Efficacy of feed-based adjuvant vaccine against *Streptococcus agalactiae* in *Oreochromis* spp. in Malaysia. *Aquaculture Research, 45*, 87-96.

Gaikowski, M. P., Wolf, J. C., Schleis, S. M., Tuomari, D., & Endris, R.G. (2013). Safety of florfenicol administered in feed to tilapia (*Oreochromis* sp.). *Toxicological Pathology*, *41*, 639-652.

Gaunt, P. S., Endris, R., McGinnis, A., Baumgartner, W., Camus, A., Steadman, J., Sweeney, D., & Sun, F. (2010). Determination of florfenicol dose rate in feed for control of mortality in Nile tilapia infected with *Streptococcus iniae. Journal of Aquatic Animal Health*, *22*, 158-166.

Geng, Y., Wang, K. Y., Huang, X. L., Chen, D. F., Li, C. W., Ren, S. Y., Liao, Y. T., Zhou, Z. Y., Liu, Q. F., Du, Z. J., & Lai, W. M. (2011). *Streptococcus agalactiae*, an emerging pathogen for cultured ya-fish, *Schizothorax prenanti*, in China. *Transboundary and Emerging Diseases*, *59*, 369-375.

Glibert, P. M., Landsberg, J., Evans, J. J., Al Sarawi, M. A., Faraj, M., Al-Jarallah, M. A., Haywood, A., Ibrahem, S., Klesius, P., Powell, C., & Shoemake, C. (2002). A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: the roles of disease, harmful algae, and eutrophication. *Harmful Algae*, *1*, 215-231.

Hernandez, E., Figueroa, J., & Iregui, C. (2009). Streptococcosis on a red tilapia, *Oreochromis* sp., farm: a case study. *Journal of Fish Diseases*, *32,* 247-252.

Huang, W. B. & Chiu, T. S. (1997). Effects of stocking density on survival, growth, size variation and productivity of *Tilapia* fry. *Aquaculture Research*, *28*, 165-173.

Hurvitz, A., Bercovier, H., & Van Rijn J. (1997). Effect of ammonia on the survival and the immune response of rainbow trout (*Oncorhynchus mykiss*, Walbaum) vaccinated against *Streptococcus iniae. Fish and Shellfish Immunology, 7*, 45-53.

Jensen, Ø., Dempster, T., Thorstad, E. B., Uglem, I., & Fredheim, A. (2010). Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. *Aquaculture Environment Interactions, 1*, 71-83.

Kim, J. H., Gomez, D. K., Choresca, C. H., & Park, S. C. (2007). Detection of major bacterial and viral pathogens in trash fish used to feed cultured flounder in Korea. *Aquaculture, 272,* 105-110.

Kitao, T., Aoki, T., & Iwata, K. (1979). Epidemiological study on streptococcicosis of cultured yellowtail (*Seriola quinquiradiata*) 1. Distribution of *Streptococcus* sp. in sea water and muds around yellowtail farm. *Bulletin of the Japanese Society of Scientific Fisheries*, *45,* 567-572.

Klesius, P. H., Shoemaker C. A., & Evans, J. J. (2008). *Streptococcus*: a worldwide fish health problem. Proceeding of the 8th International Symposium on Tilapia in Aquaculture, (Volume 1, p. 83-107). 12th - 14th October 2008, Cairo, Egypt.

Klesius, P. H., Shoemaker, C. A., & Evans, J. J. (2000a). Efficacy of single and combined *Streptococcus iniae* isolates vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). *Aquaculture, 188*, 237-246.

Klesius, P. H., Shoemaker, C. A., & Evans, J. J. (2000b). Vaccination: a health management practice for preventing diseases caused by *Streptococcus* in tilapia and other cultured fish. In Fitzsimmons, K., & Carvalho F. J. (Eds.), *Tilapia aquaculture in the 21st Century. Proceeding of the Fifth International Symposium on Tilapia Aquaculture, 2*, 558-564. 3rd - 7th September 2000. Rio de Janiero, Brazil.

Langston, A. L., Hoare, R., Stefansson, M., Fitzgerald, R., Wergeland, H., & Mulcahy, M. (2002). The effect of temperature on non-specific defence parameters of three strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). *Fish and Shellfish Immunology*, *12,* 61-76.

Le Breton A. D. (2009) Vaccines in Mediterranean aquaculture: practice and needs. *Options Méditerranéennes*, *86,* 147-154.

Le Morvan, C., Troutaud, D., & Deschaux, P. (1998). Differential effects of temperature on specific and nonspecific immune defences in fish. *The Journal of Experimental Biology*, *201*, 165-168.

Lombard, M., Pastoret, P. P., & Moulin, A. M. (2007). A brief history of vaccines and vaccination - a reviews. *International Office of Epizootics*, *26*, 29-48.

Merck (2013). *Merck Company. Animal Health*. http://aqua.merck-animal-health.com.

Mian, G. F., Godoy, D. T., Leal, C. A. G., Yuhara, T. Y., Costa, G. M., & Figueiredo, H. C. P. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary Microbiology, 136,* 180-183.

Milud, A., Hassan, D., Noordin, M., Khairani, S. B., Yasser, M., & Abuseliana, A. (2013). Environmental factors influencing the susceptibility of red hybrid tilapia (*Orechromis* sp.) to *Streptococcus agalactiae* infection. *Advanced Science Letters*, *19,* 3600-3604.

Muir, J. F., Van Rijn, J., & Hargreaves, J. (2000). *Production in intensive and recycle system*. In M. C. M. Beveridge & B.J. McAndrew (Eds.), *Tilapias: Biology and Exploitation*. Kluwer Academics Publishers. Dordrecht/Boston/London.

Najiah, M., Lee, S. W., Nadirah, M., Ruhil, H., Lee, K. L., Wendy, W., Amal, M. N. A., Basiriah, M. K., & Siti-Zahrah, A. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquaculture Research*, *40*, 630-632.

Nguyen, H. T., Kanai, K., & Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* isolated in cultured Japanese flounder, *Paralicthys olivaceus* using selective isolation procedure. *Aquaculture*, *205*, 7-17.

Noraini, O., Sabri, M. Y., & Siti-Zahrah, A. (2013). Efficacy of spray administration of formalin-killed *Streptococcus agalactiae* in hybrid red tilapia. *Journal of Aquatic Animal Health, 25,* 142-148.

Nur-Nazifah, M., Sabri, M. Y., Amal, M. N. A., Latifah, H., & Siti-Zahrah, A. (2009). Isolation and identification of *Streptococcus agalactiae* from *Oreochromis* spp. at Broga, Semenyih, Malaysia. Proceeding of the 21st Malaysian Veterinary Annual Conference, (p 360-362). 8th - 9th August 2009, Port Dickson, Malaysia.

Nur-Nazifah, M., Sabri, M.Y., & Siti-Zahrah, A. (2014). Development and efficacy of feed-based recombinant vaccine encoding the cell wall surface anchor family protein of *Streptococcus agalactiae* against streptococosis in *Oreochromis* sp. *Fish & Shellfish Immunology*, *37*, 193-200.

Serrano, P. H. (2005). *Responsible use of antibiotics in aquaculture*. FAO Fisheries Technical Paper 469, Food and Agriculture Organization of the United Nations. Rome. ftp://ftp.fao.org/docrep/fao/009/a0282e/a0282e00.pdf.

Shiomitsu, K., Kusuda, R., Osuga, H., & Munekiyo, M. (1980). Studies on chemotherapy of fish disease with erythromycin-II. *Fish Pathology, 15,* 17-23.

Shoemaker, C. A., Evans J. J., & Klesius, P. H. (2000). Density and doses: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). *Aquaculture, 188*, 229-235.

Shoemaker, C. A., Vandenberg, G. W., & Desormeaux, A. (2006). Efficacy of a *Streptococcus iniae* modified bacterin delivered using Oralject™ technology in Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 255,* 151-156.

Siddiqui, A. Q., Al-Harbi, A. H., & Al-Hafedh, Y. S. (1997). Effects of stocking density on patterns of reproduction and growth of hybrid tilapia in concrete tanks in Saudi Arabia. *Asian Fisheries Science, 10*, 41-49.

Sin, A. W., & Chiu, M. T. (1983). The intensive monoculture of the tilapias hybrid, *Sarotherodon nilotica* (males) x *S. mossambica* (females) in Hong Kong. In L. Fishelson & Z. Yaron (Eds.), *Proceeding of the International Symposium on Tilapia in Aquaculture* (p. 506-516). Tel Aviv University, Tel Aviv, Israel.

Sipauba-Tavares, L. H., Yoshida, C. E., & de Souza Braga, F. M. (2000). Effect of continuous water exchange on limnology of tilapia (*Oreochromis niloticus*) culture tanks. In K. Fitzsimmons & J.C. Filho (Eds.), *Tilapia culture in the 21st Century*. Proceeding from the Fifth International Symposium on Tilapia Aquaculture (pp. 279-287), Rio de Janeiro, Brazil. American Tilapia Association, Charles Town, West Virginia, and ICLARM, Penang, Malaysia.

Siti-Zahrah A., Padilah B., Azila A., Rimatulhana R., & Shahidan, H. (2005). Multiple streptococcal species infection in cage-cultured red tilapia, but showing similar clinical sign. In M. G. Bondad-Reantaso, C. V. Mohan, M. Crumlish, & R.P. Subasinghe (Eds.), *Disease in Asian Aquaculture VI*. Fish Health Section, Asian Fisheries Society, Manila, Philippines. Proceedings of the Sixth Symposium on Disease in Asian Aquaculture (pp. 332-339), Colombo, Sri Lanka.

Siti-Zahrah, A. & Rokiah, A. L. (1996). *Penyakit parasit ikan air tawar*. Buku Panduan. Jabatan Perikanan Malaysia.

Siti-Zahrah, A., Misri, S., Padilah, B., Zulkafli, R., Kua, B. C., Azila, A., & Rimatulhana, R. (2004). Pre-disposing factors associated with outbreak of Streptococcal infection in floating cage-cultured red tilapia in reservoirs. Abstracts of the 7th Asian Fisheries Forum 2004, The Triennial Meeting of The Asian Fisheries Society, (p. 129). 30th Nov - 4th Dec 2004, Penang, Malaysia.

Siti-Zahrah, A., Zamri-Saad, M., Zulkafli, A. R., Sabri, M. Y., Amal, M. N. A., Misri, S., Norazlan, G. W., & Nur-Nazifah, M. (2009). *Prevalence and significant of Streptococcosis in cage cultured red tilapia of West Malaysia*. Abstract of the Asian Pacific Aquaculture Conference, (p. 49). 4th - 6th November of 2009, PWTC Kuala Lumpur.

Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC) (2000). In. J.R. Arthur, C.R. Lavilla-Pitogo, & R.P. Subasinghe (Eds.), Proceedings of the meeting on the use of chemicals in aquaculture in Asia. 20-22 May 1996. Tigbauan, Phillipines.

Suanyuk, N., Kanghear, H., Khongpradit, R., & Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*). *Songklanakarin Journal of Science and Technology, 27,* 307–319.

Suanyuk, N., Kong, F., Ko, D., Gilbert, G. L., & Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand-relationship to human isolates? *Aquaculture*, *284,* 35-40.

Xu, D. H., Shoemaker, C. A., & Klesius, P. H. (2007). Evaluation of the link between gyrodactylosis and Streptococcosis of Nile tilapia *Oreochromis niloticus* (L.). *Journal of Fish Diseases, 30*, 230-238.

Yanong, R. P. E. & Floyd, F .R. (2002). *Streptococcal infections of fish*. Report from University of Florida. Series from the Department of Fisheries and Aquatic Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Zamri-Saad, M., Amal, M. N. A., & Siti-Zahrah, A. (2010). Pathological changes in red tilapias (*Oreochromis* spp.) naturally infected by *Streptococcus agalactiae*. *Journal of Comparative Pathology, 143,* 227-229.

Zhou, K., Cui, T. T., Li, P. L., Liang, N. J., Liu, S. C., Ma, C. W., & Peng, Z. H. (2008). Modelling and predicting the effect of temperature, water activity and pH on growth of *Streptococcus iniae* in Tilapia. *Journal of Applied Microbiology, 105,* 1956-1965.

Zulkafli, A. R. (2013). *Kod amalan akuakultur - Ternakan ikan tilapia dalam sangkar: Pengurusan makanan dan ternakan.* Retrieved from http://www.fri.gov.my/frigl/index.htm.

Zulkafli, A. R., Amal, M. N. A., & Misri, S. (2009). The effect of water quality and fish sizes on the susceptibility of red tilapia in floating net cages to *Streptococcus agalactiae* infection. Abstract of the Asian Pacific Aquaculture Conference, (p. 49). 4th-6th November of 2009, PWTC Kuala Lumpur.

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Performance of the Genetically Improved Farmed Tilapia (GIFT) Strain Over Ten Generations of Selection in Malaysia

Azhar Hamzah[1,2]*, Raul W. Ponzoni[3], Nguyen Hong Nguyen[3#], Hooi Ling Khaw[3], Hoong Yip Yee[3] and Siti Azizah Mohd Nor[1]

[1]School of Biological Science, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[2]National Prawn Fry Production and Research Centre, Kg. Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia
[3]World Fish Centre, 11960 Jalan Batu Maung, Penang, Malaysia

ABSTRACT

A selective breeding programme of Nile tilapia (*Oreochromis niloticus*) based on a fully pedigreed population of the GIFT (Genetically Improved Farmed Tilapia) strain has been carried out using Best Linear Unbiased Prediction (BLUP) method for genetic evaluation and selection. Two lines were created from the 2002 progeny; one selected based on high breeding values (selection line) and another one was selected for average breeding values (control line) for live weight (LW). The estimate of heritability for live weight at harvest was 0.24 ± 0.031, indicating that there is still abundant genetic variation and scope for further genetic improvement. The accumulated response was 107% in the latest generation of 2011, averaging 11.9% per generation. It can be concluded that although the selection programme in the nucleus of the GIFT strain in Malaysia resulted in significant improvement in harvest weight, there still exists an abundant genetic variation thus providing the scope for further enhancement in performance of this population.

Keywords: Nile tilapia, GIFT strain, selective breeding, BLUP, heritability, selection response

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E-mail addresses:
azhhas@yahoo.com (Azhar Hamzah),
r.ponzoni@cgiar.org (Raul W. Ponzoni),
nnguyen@usc.edu.au (Nguyen Hong Nguyen),
h.yee@cgiar.org (Hooi Ling Khaw),
h.khaw@cgiar.org (Hoong Yip Yee),
sazizah@usm.my (Siti Azizah Mohd Nor)
* Corresponding author
# Current author's affiliation
School of Science, Education and Engineering, University of the Sunshine Coast, Maroochydore, QLD 4558, Australia

INTRODUCTION

Genetic improvement has the potential to improve the productivity of cultured aquatic species (Gjedrem, 1998, 2000; Hulata, 2001). The Genetically Improved Farm Tilapia (GIFT) strain is an example where selective breeding has resulted in a

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high quality strain of fish for freshwater aquaculture. The strain was developed through a collaborative research programme between The WorldFish Centre, the Institute for Aquaculture Research, Norway (AKVAFORSK), Bureau of Fisheries and Aquatic Resources and Freshwater Aquaculture Centre (BFAR) of Central Luzon State University, Philippines in 1988 to 1997 (Bentsen *et al*., 1998; Eknath *et al*., 1993; Eknath & Acosta, 1998). A selection index combining information on individual, full sib and half sib live weights at harvest was used. The selection programme successfully resulted in an average response of 13% in growth rate and an accumulated response of 85% after six generations of selection (Eknath *et al*., 1998). Considering its fast growth and high yield, the GIFT strain was released in 1994 for an on-farm evaluation in Bangladesh, China, Thailand and Vietnam (ADB, 2005). In the Philippines, 70% of farmed tilapia is either GIFT strain or of GIFT- derived origin, whereas GIFT strain accounts for 46% of the total tilapia seed production in Thailand (ADB, 2005).

In Malaysia, tilapia (*Oreochromis* spp.) and catfish (*Clarias gariepinus*) are the major fish species for freshwater aquaculture. Aquaculture production of tilapia in Malaysia increased from 28,401 in 2005 to 38,642 tonnes in 2010, exhibiting 36% increase in its production during this period (DOF, 2005; 2010), valued approximately RM249 million. Due to the large-scale availability of diverse freshwater bodies such as lakes, reservoirs, ex-mining pools and irrigation canals, the potential for tilapia production in Malaysia is high. This species is widely cultured in ponds, cages and tanks, as well as in pen culture systems. However, most production is based on unimproved tilapia strains. Consequently, poor growth, high mortality, losses due to diseases and low economic return are quite common in tilapia grow-out farms. Therefore, in order to achieve sustainably high yields, a breeding programme to develop genetically improved tilapia strain seems imperative.

A selection programme using Best Linear Unbiased Prediction (BLUP) method for the estimation of genetic merit was implemented by the Department of Fisheries Malaysia (DOF) in collaboration with the WorldFish Centre. This collaborative programme provided opportunities for further improvement of the GIFT strain in Malaysia. To date, ten generations of a selection line (SL) and control line (CL) of GIFT strain have been produced and evaluated, and are maintained at the Aquaculture Extension Centre, DOF at Jitra, Kedah, Malaysia. The overall aim of the present study was to evaluate the performance of GIFT strain during the long-term selection programme in Malaysia. The specific objectives of the study were to: (i) examine the systematic fixed effects on growth performance traits, (ii) estimate genetic parameters for growth-related traits, and (iii) measure the direct response of the selection on harvest weight.

MATERIALS AND METHODS

The Genetic Lines

The initial population of the GIFT (Genetically Improved Farmed Tilapia) strain established in Malaysia was initiated using 63 full sib groups of 35 fish each, which were progenies from single pair-mated parents (i.e., 63 males each mated to a different female) provided by the GIFT Foundation International Inc., Philippines. They were used as the base population for the present genetic improvement programme. The fish were reared until they reached an average live weight of about 250 g before mating was initiated. A mating design to produce full and half sib groups of progeny was conducted by using hapas, where a male was allowed to mate with two different females in each mating hapa. The mating produced progenies in 2002. Two lines were formed from the progenies; one selected for high breeding value for live weight (selection line, SL), and another for average breeding values (control line, CL). The number of sires, the number of dams and the number of progeny harvested in each spawning season and line are shown in Table 1. Best Linear Unbiased Prediction (BLUP) procedures were used to estimate breeding values of all progeny in each generation. The full sib families and individuals within full sib families were then ranked on breeding values within each sex. Each male was

TABLE 1
Number of sires, dams and progeny, by spawning season and line.

Spawning Season	Line	Sire	Dam	Progeny
2002	Base Population	52	54	1684
2003	Selection	35	65	2560
	Control	19	19	1150
2004	Selection	54	84	3714
	Control	17	22	957
2005	Selection	42	76	1763
	Control	13	20	480
2006	Selection	49	88	3134
	Control	10	15	513
2007	Selection	41	71	4238
	Control	15	15	859
2008	Selection	52	76	2735
	Control	14	14	583
2009	Selection	51	69	2674
	Control	9	11	458
2010	Selection	52	70	2366
	Control	8	8	367
2011	Selection	55	66	3098
	Control	10	10	479
Total		598	853	33812

mated to two different females in the SL, whereas one male was mated to one female in the CL. The mate allocations in the SL were conducted by assigning the best available male from the best full sib family to mate with the best available female from the best family, and also the female from the second best family. As the intention was to keep low inbreeding rate (3% or less), the inbreeding coefficient of the potential progeny was checked. Matings resulting in greater inbreeding were rejected and another male and female combination was sought among families lower in rank. In each spawning season, mate allocation involved fifty or more sires. However, due to the death of females or failure to mate among some pairs, a few sires in the SL produced progeny from only one female. None of the parents used in each spawning season was reused in the next spawning seasons (i.e., generations were discrete).

Progeny Production and Performance Testing

Progeny Production

The production of families was conducted in one cubic meter breeding hapas installed in 0.05 ha pond according to the mating plan prepared for the SL (one male mated to two females) and CL (single pair mating) lines. Two weeks before mating, the male and female breeders were conditioned in separate cages (installed in breeding ponds). A total of 140 breeding hapas were used in each mating cycle. The female breeders were transferred into the breeding hapas before the males. Only the most 'ready to spawn' (Longalong *et al*., 1999) females were paired with the male in the hapa. After a week of mating, fertilized eggs were collected from the mouth of the female and immediately transferred to hatching jars. The date of spawning was recorded for each individual pair mated. The male was then paired to the second female in another hapa. The male and female breeders were mated again if they produced less than 200 fry. The breeders were not fed when the females were expected to spawn in order to prevent them from swallowing their eggs. The eggs that were collected from the female breeder's mouth were transferred into hatching jars made of fibreglass. The design and system of the jars acted as an artificial incubator (or artificial breeder's mouth) for the fertilized eggs with a constant flow through of filtered water to optimize the environment for the eggs. Meanwhile, the eggs from each female were stocked in the respective jar for three to five days until hatching. The hapa number was recorded on the jar for family identification. In order to ensure a good hatching rate, the water temperature was maintained in the range of 26^{o}C to 30^{o}C.

Rearing of Fry

The hatched fry from the incubators were transferred into the nursery hapas (1 m x 1 m x 1 m with 2 mm mesh size) according to their parents or family number at a density of 200 fry per cubic meter. The total live weight and quantity of fry were recorded before transferring them into the hapas. At least three replicates of nursery hapas

for each family were installed in the same pond to reduce environmental differences between families. They were reared for 21 days in the nursery hapas and then transferred into the bigger mesh size (8 mm) hapas (1 m x 1 m x 1 m) called B-net cages. The stocking density in the B-net was reduced to 120 fry per cubic meter. The purpose of using B-net was to allow better water circulation. Rearing in the B-net took another 21 days until the fry live weight reached 5 to 10 gm and were ready to be tagged. The complete procedure was repeated over ten generations. Fig.1 and Table 2 show the production summary and scheduled periods of reproduction over the generations.

Breeding Data

Data of body and reproduction traits were collected for each step of the breeding activity to estimate genetic parameters of the strain; beginning with the mating of breeders, egg collection, nursing of fry and tagging. The live weight of all breeders was recorded before and after mating. Recording was also done on the number of eggs per female breeder, number, total live weight and date of fry hatching, number of fry per nursery hapas and number of fry transferred and collected from B-net cages.

Progeny Identification (Tagging)

Accurate testing of the fish in farm environments requires individual or group identification. As the full and half sibs were placed in the various separate compartments until tagging time, maintenance of a fully pedigreed population was ensured. When the fingerlings reached an average weight of 5 g, twenty to one hundred individuals per family were randomly sampled, anesthetized using tricaine methanesulphonate (MS 222) solution (1 g per litre) and tagged.

The base population was identified using passive integrated transponder (PIT) tag. Twenty individuals per family were tagged before the culture trials. In the 2002 and 2003 spawning seasons, Floy® tags were used to tag 100 individuals per family. The third spawning season (2004) was marked with Floy® tags (100 individuals per family) and T-bar anchor tags (20 individuals per family). Due to the low retention rate of the Floy® and T-bar tag, PIT tags were used (70 individuals per family) in the fourth spawning season (2005) onwards. In all generations, the tag number, live weight (LW), body length (L), body depth (D) and body width (W) were recorded before stocking. The tagged fingerlings were pooled in a conditioning tank for two days without feeding before stocking in the test environments. Dead fingerlings were recorded and replaced by new ones from the respective family.

Testing Environments

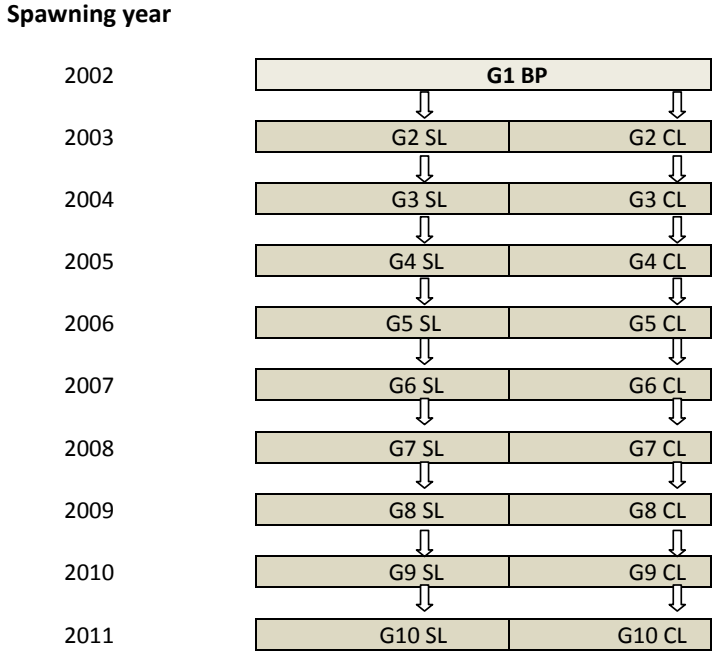
The tagged fish were grown either in cages or in earthen ponds. The cages were deployed in irrigation canal at Kodiang, Kedah, 22 km away from Jitra. Eight cages (3 m long by 3 m wide and 3 m depth) were positioned adjacent to each other, and the fish were assigned at random to

TABLE 2
Reproduction and management schedule

Activities	Spawning season									
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Mating	Feb - Mar 02	Jan - Feb 03	Nov 03 - Feb 04	Dec 04 - Feb 05	Nov 05 - Jan 06	Oct 06 - Mar 07*	Oct 07 - Feb 08*	Jan - Apr 09	Jan - Mar 10	Nov 10 - Apr 11
Nursing	Feb - Apr 02	Jan - Mar 03	Nov 03 - Feb 04	Dec 04 - Mar 05	Dec 05 - Feb 06	Nov 06 - Apr 07	Nov 07 - Mar 08	Feb - May 09	Jan - Apr 10	Dec 10 - May 11
Transfer to B-net	Mar - May 02	Feb - Apr 03	Dec 03 - Mar 04	Jan - Mar 05	Jan - Mar 06	Dec 06 - May 07	Dec 07 - Jun 08	Mar - Jun 09	Feb - May 10	Jan - Jun 11
Stocking	Apr - May 02	Mar - Apr 03	Feb - May 04	Mar - May 05	Mar - Apr 06	Feb - Jun 07	Mar - Jun 08	May - Jun 09	01-27 Jun 10	Mar - May 11
Grow-out	Apr - Nov 02	Mar - Sep 03	Feb - Sep 04	Mar - Sep 05	Mar - Sep 06	Feb - Aug 07	Mar - Nov 08#	May - Dec 09	Jun - Oct 10	Mar - Oct 11
Harvest	28 Oct - 13 Nov 02	18 Aug - 17 Sep 03	14 Aug - 22 Sep 04	18 Aug - 08 Sep 05	10 Aug - 04 Sep 06	14 Jun - 02 Aug 07	17 Sep - 05 Nov 08	10 Nov - 10 Dec 09	10-20 Oct 10	03 Aug - 10 Oct 11

** The prolonged mating period was due to insufficient families produced for the Control line and unfavourable climate.*
 # The prolonged grow out period and harvesting period were due to unfavourable weather condition, which affected the growth of the fish

them. The initial stocking density was 55 fish per square meter of surface water. The fish in both environments were fed an amount equivalent to 3 to 5% of their live weight on a commercial dry pelleted feed with 32% protein content twice a day (i.e. at 8.30 a.m and 5.00 p.m.). Water parameters (temperature, pH, dissolved oxygen) were monitored once a week. The culturing was conducted in cages and ponds in the spawning seasons of 2002, 2003 and 2004 whereas it was in earthen ponds only for 2005 and onward. The design was based on the findings of an earlier study which showed high genetic correlation (0.70 ± 0.113, Hamzah, 2006; Ponzoni *et al*., 2005 and 0.73 ± 0.092, Khaw *et al*., 2012) between environments leading to the conclusion that the live weight in ponds and cages were essentially the same trait. Earthen ponds (0.01 ha) located at the Aquaculture Extension Centre, DOF, Jitra, Kedah, were used for the experiments. The density in each pond was 3 fish per square meter of surface water. Water quality parameters (temperature, pH, dissolved oxygen and total ammonia) were also monitored once a week.



BP = base population, G = generation, SL = selection line, CL = control line

Fig.1: Schematic diagram summary of the selection and control lines produced in spawning season 2002 to 2011

Harvesting and Data Recording

Fish were harvested after 120 days of grow-out period. Those grown in cages were harvested by lifting up the net, transferred into aerated containers by using a scoop net, and later conditioned in brood stock tanks. A seine net was used for harvesting in the ponds by seining in three drags. The ponds were completely dried early the following morning. The fish were then transferred to the conditioning cages (3 m x 3 m x 1 m) installed in another pond. Data recording was done three days after conditioning. The individual tag numbers, sex, visual assessment of female sexual maturity, individual live weight (LW), body length (L), body width (W) and body depth (D) were recorded. Width and depth were measured at the mid-side of the fish, where they were the greatest. They were then transferred back to their respective conditioning cages and tanks until the estimation of their variance components and breeding values was completed. The age (in days) of each individual fish was computed based on the harvesting and hatching dates.

Statistical Analysis

Data Transformation and Standardization

The data were first examined using SAS (1990) to calculate simple statistics, remove anomalies (i.e. errors and outliers) and conduct a preliminary selection of the statistical models to be fitted. The procedure PROC MIXED (SAS Institute Inc., 1997) was used to estimate the fixed effects (spawning season, line, environment and sex) and the initial values of variance components, in which case sire (nested within spawning season and line) and dam (nested within sire, spawning season and line) were fitted as random effects. In a second phase, the computer programme ASReml was used (Gilmour *et al*., 2002). The models fitted included the fixed effects of spawning season (2002 to 2011), lines (SL and CL), environments (pond or cage) and sex, and their interactions.

Animal and dam (the non-genetic component) were fitted as random effects, whereas age of the fish was used as a covariate. The sub-set of effects fitted for different purposes varied and had been indicated in each particular case. This analysis enabled the estimation of breeding values (animal model) for all fishes, which were utilised in choosing the replacements for the SL and CL, and in estimating the genetic trend. The analysis also enabled the estimation of variance components, from which phenotypic and genetic parameters were calculated. Once the breeding values were estimated, all the fish in the respective family were ranked according to their estimated breeding values. Selection of brood stocks and mate allocation were based on the estimated breeding values of individuals and their relations to other animals in the pedigree.

Estimation of Phenotypic and Genetic Parameters

The ASReml programme (Gilmour *et al*., 2002) was used for variance component estimation. Spawning season, line, sex,

environment and their interactions were fitted as this was the model resulting in the greatest log likelihood value. Age at harvest was included as a covariate. The availability of a complete pedigree in the population enabled fitting a random animal model. Dam was fitted as another random effect, but solely accounting for the environmental effect on the progeny, without a genetic structure. In this case, the dam variance component (σ^2_D) is a combination of the maternal effect and the common environment (so $\sigma^2_D = \sigma^2_{M\_Ec}$) to which full sibs are exposed early in life (that is, while being hatched and while in the nursing and rearing hapas).

The animal variance component provided the estimate of the additive genetic variance (σ^2_A), whereas the phenotypic variance was estimated from the sum of all variance components ($\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_E$). The heritability ($h^2$) was computed as the ratio between the additive genetic and the phenotypic variances ($h^2 = \sigma^2_A/\sigma^2_P$). The maternal and common environmental effect (c^2) was calculated as the ratio between the dam variance component and the phenotypic variance ($c^2 = \sigma^2_D/\sigma^2_P$ or $\sigma^2_{M\_Ec}/\sigma^2_P$). The data on LW were transformed to square root in all analyses to improve the distribution of residuals.

Response to Selection

The progeny resulting from the 2002 spawning season were selected as parents of the next generation in two different ways, to create the SL and to continue the base population as the CL. The parents for the SL were selected from among those with the greatest breeding values whereas the parents of the CL were selected among those with breeding values as close as possible to the average of the population. Inbreeding was restricted by avoiding mating of full sibs, half sibs or cousins. This mating strategy was applied to ensure the least possible inbreeding coefficient in the progeny. Furthermore, the effective population size in each generation could be maintained at a satisfactory level for sustainability of the selection programme (Ponzoni *et al*., 2011). The same procedure was followed to produce the subsequent generations throughout the programme. Estimation of the genetic change in LW was calculated using two different methods: (i) comparing the estimated breeding values for LW between the progeny of the Selection line in two spawning seasons, and (ii) comparing the estimated breeding values of the SL and CL in progeny of the same spawning season.

RESULTS

Statistical Analysis

Statistical analyses were carried out using univariate model where the detailed analyses of selection response for LW at harvest of the ten generations bred in Malaysia from 2002 until 2011 are presented in Tables 3 through 6.

Descriptive Statistics

The fish were harvested at average age of 238 days with the mean weight of 214.9 g. Coefficient of variation in LW and age at harvesting were generally greater in the

earlier spawning seasons than in the later seasons (Fig.2 and Fig.3).

Fixed Effects

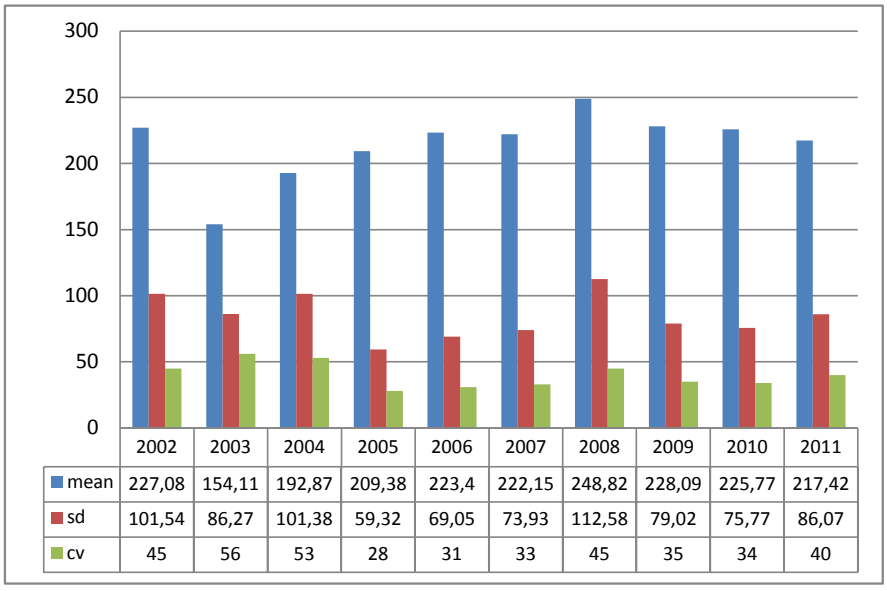
Table 3 shows the analysis of variance for $LW^{0.5}$. All effects fitted in the analysis of variance were statistically significant. The significant difference between lines suggests that there was response to selection. The significant spawning season by line by sex interaction (SS*L*S) can be explained by the fact that the between line difference in both males and females increased after each generation.

Heritability and Common Environmental Effects

Table 4 shows the estimates of variance components, heritability and maternal common environmental effect. The results indicate the presence of additive genetic variance and maternal common environmental effect in the population. The heritability for LW was moderate while the maternal common environmental effect was large.

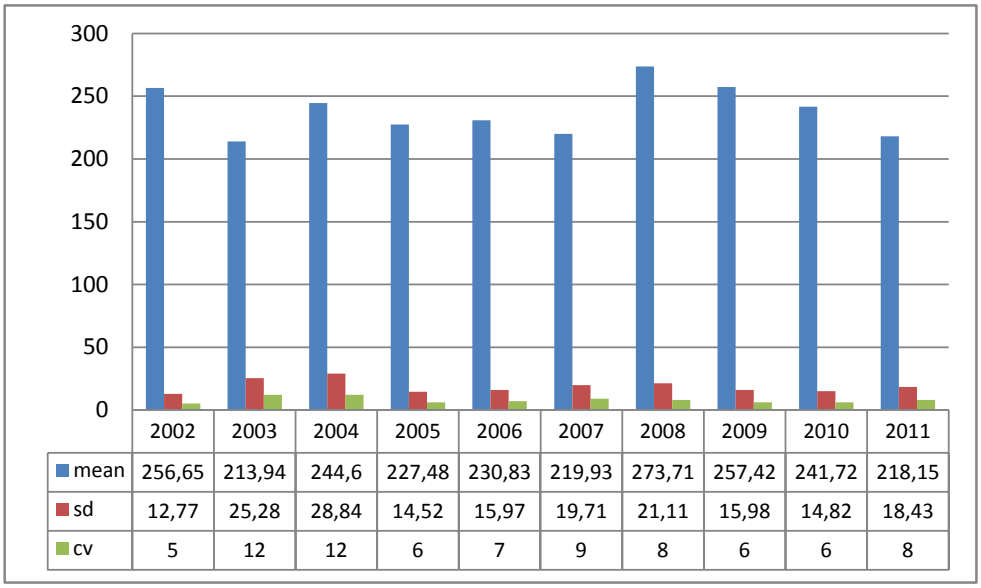
Response to Selection

The selection response in $LW^{0.5}$ during the ten generations was expressed in three different ways, namely, in actual units, as a percentage of the mean, and in genetic standard deviation units (Tables 5 and 6). Response to selection was estimated by using two methods. In the first method, the estimated breeding values were compared in consecutive generations. The second method involves the comparison of the estimated breeding values between the SL and CL in each spawning season. There was continued



	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
mean	227,08	154,11	192,87	209,38	223,4	222,15	248,82	228,09	225,77	217,42
sd	101,54	86,27	101,38	59,32	69,05	73,93	112,58	79,02	75,77	86,07
cv	45	56	53	28	31	33	45	35	34	40

Fig.2: Mean, standard deviation and coefficient of variation of LW (g) at harvesting



	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
mean	256,65	213,94	244,6	227,48	230,83	219,93	273,71	257,42	241,72	218,15
sd	12,77	25,28	28,84	14,52	15,97	19,71	21,11	15,98	14,82	18,43
cv	5	12	12	6	7	9	8	6	6	8

Fig.3: Mean, standard deviation and coefficient of variation of age at harvesting

TABLE 3
Analysis of variance for $LW^{0.5}$: Tests of fixed effects using PROC MIXED.

Effects	F Value	Prob. > F
Spawning Season (SS)	29.22	< 0.0001
Line (L)	22.34	<0.0001
Sex (S)	45.28	< 0.0001
Environment (E)	7.80	0.0052
SS*L*S	3.86	<0.0001
Age (SS, S, E)	152.32	< 0.0001
Residual Variance	2.8313	

***Remark**: This is the model with the best fit statistic (BIC: 84709.7). The random effects fitted were sire within spawning season and line, and dam within sire, spawning season and line.*

TABLE 4
Variance components, heritability and maternal common environment effect for $LW^{0.5}$.

Parameter	REML Estimate
Additive genetic variance (σ^2_A)	1.60
Maternal and common environmental variance ($\sigma^2_D = \sigma_{M\_Ec}$)	2.71
Phenotypic variance (σ^2_P)	6.58
Heritability (standard error) [h^2 (s.e.)]	0.24 (0.0311)
Maternal common environment (standard error) [c^2 (s.e.)]	0.41 (0.0169)

TABLE 5
Response to selection estimated by comparing the estimated breeding value for live weight (LW) between progeny from selection line of two subsequent spawning seasons

Method (between spawning season)	Model (effects)	Selection Response ($LW^{0.5}$)[A]		
		Actual units ($g^{0.5}$)	%	Genetic Standard Deviation Units (Actual/σ_A)
2002 and 2003	Fixed: SSxLxSxE	1.060	8.09	0.838
2003 and 2004		0.954	8.08	0.754
2004 and 2005	Covariate: Age at harvest (SS, L, S, E)	0.852	7.22	0.673
2005 and 2006		0.647	5.48	0.512
2006 and 2007	Random: spline(age_hv), uni(sex,2), animal and DAM	0.812	6.88	0.642
2007 and 2008		0.518	4.39	0.409
2008 and 2009		0.587	4.97	0.464
2009 and 2010		0.559	4.73	0.442
2010 and 2011		0.604	5.12	0.478

[A] Actual units are $LW^{0.5}$ difference in mean breeding values for methods (i) and (ii); percentage refers to actual units, in relation to the least squares means of $LW^{0.5}$ for the control population ($11.8023 g^{0.5}$); Genetic standard deviation equals the square root of the additive genetic variance in Table 4 ($_A$ = $1.2649 g^{0.5}$).

response over the period examined, as well as good agreement between the two methods used (55.85% vs 53.77% for the first and second methods, respectively).

DISCUSSION

Heritability

The heritability for $LW^{0.5}$ (0.24) was moderate. This estimate is in good agreement with those reported by Charo-Karisa *et al*. (2005), Gall and Bakar (2002), Maluwa *et al.* (2006), Ponzoni *et al*. (2005), and Rutten *et al*. (2005) fitting an animal model to the data. However, higher values have been reported in studies where the heritability estimate was based on full sib analysis. For instance, Kronert *et al*. (1989) and Oldorf *et al*. (1989) report heritabilities of 0.65 and 0.51, respectively, from full sib analyses. The greater values are likely to be biased upwards due to effects common to full sibs other than additive genetic effects (e.g., environmental effects due to the separate rearing of the families in hapas until tagging, maternal effects and components of non-additive genetic effects common to full-sibs). Similarly, Bolivar and Newkirk (2002) also report a high heritability (0.56) in Nile tilapia selected for growth rate over twelve generations, possibly due to the fact that the maternal and common environmental effect (c^2) was not accounted for in the model fitted.

In other aquaculture species, heritability estimates have also been mostly reported for LW. Hetzel *et al*. (2000) reported that the average realized heritability for weight at six months of age of *Penaeus japonicus* was 0.24 whereas that of the common carp

TABLE 6
Response to selection estimated by comparing the estimated breeding value for live weight (LW) between progeny from control line and selection line of the same spawning season

Method (within spawning season)	Model (effects)	Selection Response ($LW^{0.5}$)[A]		
		Actual units ($g^{0.5}$)	%	Genetic Standard Deviation Units (Actual/σ_A)
2003	Fixed: SSxLxSxE	1.387	11.75	1.096
2004		2.395	20.29	1.893
2005	Covariate: Age at harvest (SS, L, S, E)	3.044	25.79	2.406
2006		3.548	30.06	2.805
2007		4.354	36.89	3.442
2008	Random: spline(age_hv), uni(sex,2), animal and DAM	4.826	40.89	3.815
2009		5.430	46.00	4.293
2010		5.904	50.02	4.667
2011		6.346	53.77	5.017

[A] Actual units are $LW^{0.5}$ difference in mean breeding values for methods (i) and (ii); percentage refers to actual units, in relation to the least squares means of $LW^{0.5}$ for the control population ($11.8023g^{0.5}$); Genetic standard deviation equals the square root of the additive genetic variance in Table 4 ($_A$ = $1.2649g^{0.5}$).

(*Cyprinus carpio* L.) was in the range of 0.31 to 0.41 (Vandeputte *et al*., 2008). In redclaw crayfish (*Cherax quadricarinatus*), estimates of realized heritibilities for harvest weight varied from 0.13 to 0.38 (McPhee *et al*., 2004). In addition to its great importance in the breeding objective, the high heritability for LW across species had justified its selection as the sole criterion in many breeding programmes. The focus on LW is also related to the current market practice that is based on whole live fish weight. Compared to other body trait measurements (length, depth and width), harvest weight is the most efficient criterion to improve overall performance of the fish (Nguyen *et al*., 2007), and the best predictor of fillet weight, a carcass trait of great importance in fish (Nguyen *et al*., 2010).

Maternal and Common Environmental Effects

The maternal and common environment effects (c^2) estimated from the dam variance component (0.41) was larger than other estimates reported in the literature. Ponzoni *et al*. (2005) reported a c^2 value of 0.15 whereas Rutten *et al*. (2005) and Maluwa *et al*. (2006) found c^2 value of 0.09 and 0.21, respectively. The lower c^2 reported by Rutten *et al*. (2005) could be attributed to the larger LW at harvest compared to this current study (609 vs. 215 g). The smaller size of fish at harvest in this study (200 to 400 g) is the mature size for mating and preferred by consumers in Malaysia. As the harvested size was small, the c^2 remains an important consideration in order to obtain unbiased estimation of parameters. Nguyen *et al*. (2010) noted that the maternal and

common environmental effects diminished with a longer grow out period and greater weight at harvest. Charo-Karisa *et al*. (2005) reported a high c^2 (0.61) for LW at 49 days. Vandeputte *et al.* (2002) also noted that estimates of c^2 effects in traits measured at early development stages often include large maternal effects. As the estimate of c^2 in the current GIFT breeding programme was high, this effect was included in the statistical model to estimate the genetic parameters. The high estimate of c^2 in the GIFT selection programme in Malaysia is related to keeping the full sibs together in their respective nursery hapas until reaching size for safe tagging (at 5 g on average). By then, they have been maintained in hapas for 60 days in order to record full pedigree information. However, practical attempts to reduce the c^2 value have been carried out by transferring the fry to larger mesh size hapas after a month of rearing period as well as by reducing the stocking density from 200 to 120 fry per hapa. This technique had abbreviated the rearing period before tagging and produced a uniform size fry. Therefore, the c^2 effect can be reduced by better management techniques, but the maternal effects could still remain due to the egg size and the mouth brooding nature of Nile tilapia (Khaw *et al.*, 2008). In general, genetic evaluation of growth related traits should account for common effects other than additive genetic effects in the statistical model (Johansson *et al*., 1993; Nguyen & McPhee, 2005; Roehe & Kennedy 1993).

A more uniform nursing environment provided to the fry before they reach the tagging size or by genotyping the fingerlings to ascertain parentage could also reduce c^2 (Fjalestad *et al*., 2003). Therefore, microsatelite marker techniques for genetic identification of parentage is one of the options since all the fry from different families can be cultured together in one pond until harvest. Thus, nursing at the fry stage in separate hapas could be eliminated. Ninh (2009) reported that maternal and common environmental effects were close to zero in communal rearing of common carp fry (*Cyprinus carpio*) using seven microsatellite loci for parentage assignment. Although genetic tagging could reduce the c^2, the application of this technique in fish improvement programmes should be weighed against the costs and its benefits.

Selection Response

The GIFT selection programme in Malaysia yielded about 55% of selection response. James (2007) showed that the selection response expressed as a percentage after square root transformation is a fraction 0.501 of that in actual units. This means that in actual units the response was of the order of 107% (an average about 11% genetic gain per generation). The responses were large enough to suggest that genetic change was being achieved and in the intended direction. This response to selection is comparable to the estimate reported by Eknath *et al*. (1998) for Nile tilapia and for Atlantic salmon (Kinghorn, 1983). For instance, the gain obtained in the Egyptian Nile Tilapia was 5.8% (Rezk *et al*., 2009), 6.6% in *Oreochromis shiranus* (Maluwa & Gjerde,

2007), 12.45%, 3% and 13.3% in Nile tilapia reported by Bolivar and Newkirk (2002), Basiao and Doyle (1999) and Gall and Bakar (2002), respectively. The genetic gain per generation achieved in the current breeding programme of GIFT in Malaysia was in line with Gjedrem's (2000) estimation of 10% to 20% genetic gain per generation in aquatic animals in general.

To date, there has been no evidence of any slowing down of the rate genetic gain in the GIFT population. This may be partly explained by the genetic variation assembled in the base population (sample of eight different Nile tilapia strains (Eknath *et al*., 2007) and the mating strategy to constrain the accumulation of inbreeding and maintain a relatively high effective population size (Ponzoni *et al*., 2010). The selection and mate allocation of fish can affect the genetic variance and consequently the genetic gain. The level of inbreeding in the latest generation of GIFT in Malaysia was 2.14%. FAO (1998) and Hall (2004) suggest a minimum effective population number of 50 whereas a range of 100 to 150 was proposed by Smitherman and Tave (1987). Bijma (2000) suggested values of 50 to 100, and added that with these values inbreeding can be contained and heritabilities maintained. The effective population in the GIFT population size in Malaysia was 88 (Ponzoni *et al*., 2010), above the critical number for maintaining the genetic variation, auguring well for selection response in future generations.

Results of this breeding programme reflected that a sustained improvement of harvest weight was achieved. Thus, the methodology adopted by GIFT breeding programme could be used as a guideline to initiate similar genetic improvement programmes for other important aquaculture species in Malaysia.

Since the gain achieved by selective breeding is permanent, the improved GIFT strain must be managed and disseminated for sustainable benefits. The estimated per capita annual fish consumption in Malaysia was about 56 kg in 2010 (Dasar Pertanian Negara ke-3, 2006) and the total consumption is increasing in concomitant with the growing population. Meanwhile, the declining fish catch from capture fisheries and overfishing has increased the gap between fish demand and supply. Hence, the use of genetically improved strains in aquaculture gains significance in order to bridge this gap and to supply the cheap protein food. As the GIFT strain performs well in both pond and cage culture environments (the main culture systems for tilapia) thus providing an attractive option for the Malaysian aquaculture industry.

CONCLUSION

Analyses of the GIFT breeding programme data collected over 10 years (2002-2011) in Malaysia indicated that there has been significant genetic improvement in harvest weight in this population. The GIFT strain is thus a valuable genetic resource for the aquaculture industry. Therefore, a systematic approach of brood stock management and dissemination should be implemented to ensure an effective use and

sustainability of this strain. Furthermore, the strain offers ample scope for further genetic improvement.

REFERENCES

Asian Development Bank (ADB). (2005). *An Impact Evaluation of the Development of Genetically Improved Farmed Tilapia.* Operations Evaluation Department, ADB, Manila, Philippines. 124 pp.

Basiao, Z. U., & Doyle, R. W. (1999). Test of size-specific mass selection for Nile tilapia, *Oreochromis niloticus,* L., cage farming in the Philippines. *Aquaculture Research, 30*, 373-378.

Bentsen, H. H., Eknath, A. E., Palada-de Vera, M. S., Danting, J. C., Bolivar, H. L., Reyes, R. A., Dionisio, E. E., Longalong, F. M., Circa, A. V., Tayamen, M. M., & Gjerde, B. (1998). Genetic improvement of farmed tilapias: growth performance in a complete diallel cross experiment with eight strains of *Oreochromis niloticus*. *Aquaculture, 160*, 145–173.

Bijma, P. (2000). *Long-term genetic contributions: prediction of rates of inbreeding and genetic gain in selected populations*. (Ph.D. Thesis). Animal Breeding and Genetics Group, Wageningen University, Wageningen, the Netherlands.

Bolivar, R. B., & Newkirk, G. F. (2002). Response to within family selection for live weight in Nile tilapia (*Oreochromis niloticus*) using a single-trait animal model. *Aquaculture, 204*, 371–381.

Charo-Karisa, H., Komen, H., Rezk, M. A., Ponzoni, R. W., van Arendonk, J. A. M., & Bovenhuis, H. (2006). Heritability estimates and response to selection for growth of Nile tilapia (*Oreochromis niloticus*) in low-input earthen ponds. *Aquaculture, 261*, 479–486.

Charo-Karisa, H., Rezk, M. A., Bovenhuis, H., & Komen, H. (2005). Heritability of cold tolerance in Nile tilapia, *Oreochromis niloticus*, juveniles. *Aquaculture 249*, 115–123.

Department of Fisheries (DOF), Malaysia. (2005). *Annual Fisheries Statistics.* Retrieved 13 May 2012, from http://www.dof.gov.my/59.

Department of Fisheries (DOF), Malaysia (2010). *Annual Fisheries Statistics.* Retrieved 13 May 2012, from http://www.dof.gov.my/59.

Department of Fisheries (DOF), Malaysia. (2010). Kementerian Pertanian dan Industri Asas Tani, Pusat Pentadbiran Kerajaan Persekutuan, Wilayah Persekutuan Putrajaya, Malaysia. *Dasar Pertanian Negara ke-3.*

Eknath, A. E., & Acosta, B. O. (1998). *Genetic Improvement of Farmed Tilapias (GIFT) Project: Final report, March 1988 to December 1997.* International Center for Living Aquatic Resources Management, Makati City, the Philippines.

Eknath, A. E., Bentsen, H. B., Ponzoni, R. W., Rye, M., Nguyen, N. H., Thodesen, J., & Gjerde. B. (2007). Genetic improvement of farmed tilapias: Composition and genetic parameters of a synthetic base population of *Oreochromis niloticus* for selective breeding. *Aquaculture, 273*, 1-14.

Eknath, A. E., Dey, M. M., Rye, M., Gjerde, B., Abella, T. A., Sevilleja, R. A., Tayamen, M. M., Reyes, R. A., & Bentsen, H. B. (1998). Selective breeding of Nile tilapia for Asia. Proceedings of the 6th World Congress on Genetics. *Applied to Livestock Production Armidale, Australia, 27*, 89–96.

Eknath, A. E., Tayamen, M. M., Palada-de Vera, M. S., Danting, J. C., Reyes, R. A., Dionisio, E. E.,Capili, J. B., Bolivar, H. L., Abella, T. A., Circa, A. V., Bentsen, H. B., Gjerde, B., Gjedrem, T., & Pullin, R. S. V. (1993). Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. *Aquaculture, 111*, 171–188.

Fjalestad, K. T., Moen, T., & Raya, L. G. (2003). Prospects for genetic technology in salmon breeding programmes. *Aquaculture Research, 34*, 397-406.

Food and Agriculture Organization (FAO). (1998). *Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans*. FAO, Rome, Italy. 215pp.

Gall, G. A. E., & Bakar, Y. (2002). Application of mixed-model techniques to fish breed improvement: analysis of breeding-value selection to increase 98-day live weight in tilapia. *Aquaculture, 212*, 93–113.

Gilmour, A. R., Cullis, B. R.,Welham, S. J., & Thompson, R. (2002). *Asreml reference manual. NSW Agriculture Biometric Bulletin No.3*. Orange Agricultural Institute, Forest Road, Orange 2800 NSW Australia.

Gjedrem, T. (1998). Developments in fish breeding and genetics. Acta Agriculturae Scandinavica. *Section Animal Science Supplement, 28*, 19–26.

Gjedrem, T. (2000). Genetic improvement of cold-water species. *Aquaculture Research, 31*, 25–33.

Gupta, M. V., & Acosta, B. O. (2004). From drawing board to dining table: the success story of the GIFT project. *NAGA, World Fish Center Quarterly, 27*(3-4), 4-14.

Hall, S. J. G. (2004). *Livestock Biodiversity: Genetic Resources for the Farming of the Future*. Blackwell Science Ltd., Oxford, UK. 269pp.

Hamzah, A. (2006). *Genetic improvement of tilapia (Oreochromis niloticus) through selective breeding and crossbreeding*. MSc thesis Universiti Sains Malaysia, Penang, Malaysia. 77pp.

Hetzel, D. J., Crocos, P. J., Davis, G. P., Moore, S. S., & Preston, N. C. (2000). Reponse to selection and heritability for growth in Kuruma prawn, *Penaeus japonicus. Aquaculture, 181*, 215-223.

Huang, C. M., & Liao, I. C. (1990). Response to mass selection for growth rate in *O. niloticus. Aquaculture, 85***,** 199–205.

Hulata, G. (2001). Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. *Genetica, 111*, 155–173.

Hulata, G., Wohlfarth, G. W., & Halevy, A. (1986). Mass selection for growth rate in the Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 57*, 177–184.

Hussain, M. G., Islam, M. S., Hossain, M. A., Wahid, M. I., Kohinoor, A. H. M., Dey, M. M., & Mazid, M. A. (2002). Stock improvement of silver barb (*Barbodes gonionotus* Bleeker) through several generations of genetic selection. *Aquaculture, 204*, 469 – 480.

James, J. W. (2007). Transformations and response to selection. *Australian Association for the Advancement of Animal Breeding and Genetics, 7*, 150-153.

Johansson, K., Kennedy, B. W., & Quinton, M. (1993). Prediction of breeding values and dominance effects from mixed models with approximations of the dominance relationship matrix. *Livest. Prod. Sci., 33*, 213-223.

Kause, A., Ritola, O., Paananen, T., Wahlroos, H., & Mäntysaari, E. A. (2005). Genetic trends in growth, sexual maturity and skeletal deformations, and rate of inbreeding in a breeding programme for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture, 247*, 177–187.

Khaw, H. L., Ponzoni, R. W., & Danting, M. J. C. (2008). Estimation of genetic change in the GIFT strain of Nile tilapia (*Oreochromis niloticus*) by comparing contemporary progeny produced by males born in 1991 or in 2003. *Aquaculture, 275,* 64–69.

Khaw, H. L., Ponzoni, R. W., Hamzah, A., Abu-Bakar, K. R., & Bijma, P. (2012). Genotype by

production environment interaction in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 326-329*, 53–60.

Kinghorn, B. P. (1983). A review of quantitative genetics in fish breeding. *Aquaculture, 31*, 283-304.

Kronert, U., Horstgen-Schwark, G., & Langholz, H.J. (1989). Prospects of selecting for late maturity in Tilapia (*Oreochromis niloticus*): I. Family studies under laboratory conditions. *Aquaculture, 77,* 113 – 121.

Longalong, F. M., Eknath, A. E., & Bentsen, H. B. (1999). Response to bidirectional selection for frequency of early maturing females in Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 178*, 13–25.

Maluwa, A. O., Gjerde, B., & Ponzoni, R. W. (2006). Estimates of heritability and genotype by environment interaction for harvest live weight of *Oreochromis shiranus* in three different test environments. *Aquaculture, 259*, 47-55.

Maluwa, A. O., & Gjerde, B. (2007). Response to selection for harvest live weight of *Oreochromis shiranus. Aquaculture, 273*, 33-41.

Mambrini, M., Labbe, L., Randriamanantsoa, F., & Boujard, T. (2006). Response of growth-selected brown trout (*Salmo trutta*) to challenging feeding conditions. *Aquaculture, 252*, 429-440.

McPhee, C. P., Jones, C. M., & Shanks, S. A. (2004). Selection for increased weight at 9 months in redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture, 237*, 131-140.

Moav, R., & Wohlfarth, G. W. (1976). Two way selection for growth rate in the common carp (*Cyprinus carpio*). *Genetics, 82*, 83-101.

Neira, R., Diaz, N. F., Gall, A. E., Gallardo, J. A., Lhorente, J. P., & Manterola, R. (2006). Genetic improvement in Coho salmon (*Oncorhynchus kisutch*). I: Selection response and inbreeding depression on harvest weight. *Aquaculture, 257*, 9-17.

Nguyen, N. H., Khaw, H. L., Ponzoni, R. W., Hamzah, A., & Kamaruzzaman, N. (2007). Can sexual dimorphism and body shape be altered in Nile tilapia (*Oreochromis niloticus*) by genetic means? *Aquaculture, 272*, 38-46.

Nguyen, N. H., & McPhee, C. P. (2005). Genetic parameters and responses in performance and body composition traits in pigs selected for high and low growth rate on a fixed ration over a set time. *Genetics Selection Evolution, 37,* 199–213.

Nguyen, N. H., Ponzoni, R. W., Abu-Bakar, K. R., Hamzah, A., Khaw, H. L., & Yee, H. Y. (2010). Correlated response in fillet weight and yield to selection for increased harvest weight in genetically improved farmed tilapia (GIFT strain), *Oreochromis niloticus*. *Aquaculture, 305*, 1-5.

Ninh (2009). *Communal or separate rearing of families in selective breeding of common carp (Cyprinus carpio)*. (PhD thesis). University of Sterling, United Kingdom.

O'Flynn, F.M., Bailey, J. K., & Friars, G. W. (1999). Responses to two generations of index selection in Atlantic salmon (*Salmo salar*). *Aquaculture, 173*, 143-147.

Oldorf, W., Kronert, U., Balarin, J., Haller, R., Horstgen-Schwark, G., & Langholz. H. J. (1989). Prospects of selecting for late maturity in Tilapia (*Oreochromis niloticus*): II. Strain comparisons under laboratory and field conditions. *Aquaculture, 77*, 123 – 133.

Ponzoni, R. W., Hamzah, A., Tan, S., & Kamaruzzaman, N. (2005). Genetic parameters and response to selection for live weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 247*, 203–210.

Ponzoni, R. W., Khaw, H. L., Nguyen, N. H., & Hamzah, A. (2010). Inbreeding and effective population size in the Malaysian nucleus of the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 302*, 42-48.

Ponzoni, R. W., Nguyen, N. H., Khaw, H. L., & Hamzah, A. (2011). Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish Center with the GIFT strain. *Reviews in Aquaculture, 3*, 27-41.

Rezk, M. A., Ponzoni, R. W., Khaw, H. L., Kamel, E.A., Dawood, T. I., & John, G. (2009). Selective breeding for increased live weight in a synthetic breed of Egyptian Nile tilapia, *Oreochromis niloticus*: Response to selection and genetic parameters. *Aquaculture, 293*, 187-194.

Roehe, R., & Kennedy, B. W. (1993). The influence of maternal effects on accuracy of evaluation of litter size in swine. *Journal of Animal Science, 71*, 2353–2364.

Rutten, M. J. M., Komen, H., & Bovenhuis, H. (2005). Longitudinal genetic analysis of Nile tilapia (*Oreochromis niloticus* L.) live weight using a random regression model. *Aquaculture, 246*, 101–113.

SAS Institute Inc. (1990). *SAS/STAT® User's Guide*, Version 6 (4th Edition), Volumes 1 and 2. Cary, NC, USA. 1686pp.

SAS Institute Inc. (1997). *SAS/STAT® Software: Changes and Enhancements through Release 6.12*. Cary, NC, USA. 1167pp.

Smitherman, R. O., & Tave, D. (1987). Maintenance of genetic quality in cultured tilapia. *Asian Fisheries Science, 1*, 75–82.

Teichert-Coddington, D. R., & Smitherman, R. O. (1988). Lack of response by *T. nilotica* to mass selection for rapid early growth. *Trans. Am. Fish. Soc. 117,* 297–300.

Vandeputte, M., Quillet, E., & Chevassus, B. (2002). Early development and survival in brown trout (*Salmo trutta fario* L.): indirect effects of selection for growth rate and estimation of genetic parameters. *Aquaculture, 204*, 435–445.

Vandeputte, M., Kocour, M., Mauger, S., Rodina, M., Launay, A., Gela, D., Dupont-Nivet, M., Hulak, M., & Linhart, O. (2008). Genetic variation for growth at one and two summers of age in the common carp (*Cyprinus carpio*) heritability estimates and response to selection. *Aquaculture, 277*, 7–13.

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Attaining Sufficient Yield of Sugarcane in Bangladesh: An Empirical Approach

Rana, M. S.[1]*, Hossain, F.[2] and Roy, S. S.[3]

[1]School of Spatial Planning, Technical University of Dortmund, Germany
[2]Development Studies-specializing in Geography, Norwegian University of Science and Technology (NTNU), Norway
[3]Department of Urban and Regional Planning, Bangladesh University of Engineering and Technology (BUET), Bangladesh

ABSTRACT

The paper contains an empirical research approach on the demand and supply analysis for sugarcane in Bangladesh. In be more specific, how sufficient yield of sugarcane could be achieved, as well as sustained in the country, is discussed in the paper based on the analysis, along with exploring its strengths, weaknesses, opportunities and threats. Bangladesh imports around 1.26 million metric ton of sugar each year to meet its national demand deficit. The research shows that local growing of sugarcane rather imports of sugar would save around BDT8,937.94 million per year. In addition, local production of sugarcane and its processing to sugar would result in the by-production of molasses, *chobra* and spirit/ alcohol at massive scale that might considerably contribute to the national economy. The paper, in this regard, proposes eight contiguous districts of Bangladesh with high sugarcane productivity to form a regional belt where deficit amount of sugarcane could be grown every year. In addition, several policy recommendations are made with a view to sustaining this yield of sugarcane in the country.

Keywords: Demand Deficit, *Gur*, Import, Market Area, Sugarcane Belt, Sufficient Yield

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E-mail addresses:
srana.buet@gmail.com (Rana, M. S.),
fahim.bueturp06@gmail.com (Hossain, F.),
ssr.buet@gmail.com (Roy, S. S.)
* Corresponding author

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INTRODUCTION

Bangladesh has a yearly production of 6.00 million metric tons of sugarcane that comes from the cultivation of 0.35 million acres land (BBS, 2007). However, the amount of production seems merely sufficient to meet the increasing demand for sugar and *gur* (jaggary) at the national level.

The average yearly demand for sugar in Bangladesh is 1.40 million metric ton, while the figure is only 0.30 million metric ton for *gur* (BSFIC, 2013a). On average, 0.17 million metric ton of sugar is processed from 2.31 million metric tons of sugarcane in the country each year (BSRI, 2013a). The production amount points to the yearly demand deficit of sugar by 1.23 million metric ton. As a result, the country needs to import 1.26 million metric ton of sugar on average to overcome this national demand deficit each year (Khan, 2009).

On an average, 11 kg sugar is produced from 100 kg of good quality of sugarcane (Russel, 2002). However, the local rate of sugar processing from sugarcane in Bangladesh is below the international standard. In Bangladesh, 1 kg of sugar is processed from 13.20 kg of sugarcane (BSRI, 2013a). Considering the local standard, 16.63 million metric tons of sugarcane is needed to produce the required amount, in addition to the current production in our country to fulfill the total national demand for sugar each year.

The average sugarcane production rate in Bangladesh is 17.26 metric tons/acre (BBS, 2007) which points to the requirement of additional 0.96 million acres land under sugarcane cultivation scheme in order to meet the demand deficit for sugar. The paper, in this regard, deals with exploring rationale of local yield of sugarcane and its monetary benefits against the import of sugar in Bangladesh. Moreover, the research aims at formulating policies with view to sustaining the local yield in the long run.

MATERIALS AND METHODS

Selection of the Study Area

Two districts were selected as study areas for conducting the survey. The selection of the study areas were based on the following criteria:

i. District's average sugarcane production rate (metric ton/acre), i.e. the district with the highest sugarcane production rate figure is considered with priority.

ii. Mill-zone/Non-mill zone area: Out of two study areas one should be located at mill-zone and another at non-mill zone area so that the variation in crop yield as well as the production cost rate data is captured well.

iii. Proximity to Dhaka: Distance of districts (in kilometer) from Dhaka is also considered for the convenience of the field survey.

iv. Regional variation: In case of contiguous districts, only one district fulfilling the above mentioned criteria better than other(s) is considered in order to ensure data authenticity.

The average sugarcane production rate in Bangladesh is 17.26 metric tons/acre aforementioned (BBS, 2007). Initially, the districts with sugarcane production rate more than the country average were considered in order to ensure data quality. Table 1 shows that the sugarcane production rate of Manikganj district is 193.22 metric tons/acre, and this is the highest figure compared to the rest. Above all, the district is the closest to Dhaka. Considering the

criteria aforementioned and putting priority on the first and third, therefore, Manikganj District was selected as the first study area.

All the criteria were attempted to be met when selecting the second study area. Accordingly, the Chuadanga District was chosen as it includes a considerable production rate, feasible distance from Dhaka and regional variation from the Manikganj District. Moreover, the area is also located within the Carew & Co. Sugar Mill Zone area.

Data Collection

Data on cultivation costs and earning from sugarcane were extensively collected from the survey on sugarcane farmers. The total number of farmers in the Manikganj District is 171,068, and there are 173,698 farmers in the Chuadanga District (BBS, 2010). At 95% confidence level and confidence interval of 5, a total of 384 farmers were sampled for both districts. The whole sample size was then divided into two according to the district's percentage share to total farmers. Accordingly, 191 farmers were sampled to be surveyed in the Manikganj and 193 in Chuadanga.

RESULTS AND DISCUSSION

Whether to Import or to Produce Locally?

The amount of average yearly import of sugar in Bangladesh is 1.26 million metric ton (Khan, 2009), which costs around BDT 77,450.35 million in total as per sugar purchasing rate of BDT 61,468.53 /ton

TABLE 1
Key information of the initially selected study districts

Districts	Sugar Cane Production		Production Rate (metric tons/ acre)	Name of Sugar Mill Located within the District††	Distance of Districts from Dhaka (km) †††
	Area (acres) †	Production (metric ton) †			
Manikganj	7,705	1488,789	193.22	-	63
Thakurgaon	1,525	245,455	160.95	Thakurgaon Sugar Mill	407
Khagrachari	1,489	75,188	50.49	-	259
Barguna	270	7,044	26.09	-	247
Netrokona	204	5,296	25.96	-	158
Chandpur	1,852	47,120	25.44	-	115
Chuadanga	32,088	801,736	24.98	Carew & Co. Sugar Mill	215
Sherpur	531	13,242	24.94	-	188
Gaibandha	8,152	187,802	23.04	-	268
Patuakhali	109	2,445	22.43	-	204
Nilphamari	551	11,321	20.55	-	359
Tangail	9,009	181,203	20.11	-	92
Jhenaidah	13,207	262,735	19.89	Mobarakganj Sugar Mill	178
Joypurhat	3,665	69,387	18.93	Joypurhat Sugar Mill	249
Kushtia	34,656	619,246	17.87	Kushtia Sugar Mill	183

†Source: BBS, 2007; †† Source: BSFIC, 2012; ††† Source: RHD, 2007

(including all types of duties and freight costs) (Sugaronline, 2012).

Thus, as mentioned earlier, in order to avoid the import of 1.26 million metric ton of sugar, the amount of sugarcane required to produce locally is 16.63 million metric tons from cultivating 0.96 million acres of land a year. Per acre sugarcane production cost is BDT 13,380.00 for a single crop season (see Fig.1).

Therefore, to produce 16.63 million metric tons of sugarcane, the production cost required is BDT 12,894.67 million. In sugar mill, the cost for 1 metric ton sugarcane processing is BDT 3,344.02 (including packing and other raw materials cost, salary and wage at factory, repairing and maintenance, insurance, VAT, electricity and fuel costs and other costs) at 7.17% of recovery performance (BSFIC, 2013b). Thus, to process a total of 16.63 million metric tons amount of sugarcane, processing cost required is BDT 55,617.74 million. Hence, the total cost required for producing and processing the required amount of sugar avoiding import is BDT 68,512.41 million per year. This indicates that the local production of sugarcane would save around BDT 8,937.94 million per year.

In addition, the production of sugar results in some by-products like molasses, *chobra* (cane tops and bagasse) and spirit/ alcohol which may contribute to the national GDP. In the 2009-10 fiscal year, 32,716.69 metric tons of molasses, 313,527.00 metric tons of *chobra*, and 4352,000.00 litres of spirit/alcohol were produced as by-products from the processing of 866,573.00 metric tons of sugarcane at average 7.17% recovery rate of sugar (BSFIC, 2013c). The statistics

Costing per *Bigha*†	**Earning per *Bigha***
Land Preparation & Plantation Cost: BDT 2,246 **Irrigation Cost**: BDT 720 *(Irrigation for 2-3 times)* **Fertilizer Cost**: BDT 534 (20 kg Urea for 2 times, 1.5 kg of Nitrogen, 0.3 kg of Phosphorus and 3.25 kg of Potassium for single time) **Labor Cost**: BDT 960 *(8 laborers in total)* **Total Production Cost**: BDT 4,460	**Growing Duration of Sugarcane**: 12 months **Average Price per kg of Sugarcane**: BDT 2.14 **Average Total Earning from the Cultivation of 1 *Bigha***: BDT 23,417 **Net Profit from the Cultivation of 1 *Bigha***: BDT (23417-4460) = BDT 18,957
Total Production Cost per Acre: BDT (3*4460) = BDT 13,380	**Total Net Profit from 1 Acre**: BDT (3*18957) = BDT 56,871

† 1 Bigha= 1/3 acre
Source: Field Survey, 2012

Fig.1: Costing and earning details for per *Bigha* sugarcane cultivation in a single season

indicates that on average, 0.04 metric ton of molasses, 0.36 metric ton of *chobra*, and 5.02 liters of spirit/alcohol are produced as by-products from the processing of one metric ton of sugarcane. Therefore, the additional production of 16.63 million metric tons of sugarcane towards meeting the national demand deficit may result to the by-production of around 0.63 million metric tons of molasses, 6.02 million metric tons of *chobra* and 83.53 million litres of spirit/alcohol. In Bangladesh, the wholesale market price of molasses and spirit/alcohol are BDT 18,496.69 per metric ton and BDT 59.32 per litre, respectively, as recorded at 2009-10 fiscal year (BSFIC, 2013d). This indicates that local production of 16.63 million metric tons of sugarcane has the potentials to generate a rough additional income of BDT 11,690.20 million from molasses and BDT 4,955.09 million from spirit/alcohol. Moreover, 6.02 million metric tons of *chobra* produced as by-product would contribute to this additional income by considerable percentage. In the fiscal year of 2003-04, these by-products of sugarcane contributed 4.23% GDP within the sugarcane economy which eventually, resulted in 0.03% GDP contribution to the national economy (BSRI, 2013b).

The study findings henceforth recommend for sustainable local production of sugarcane than importing since import involves wastage of huge amount of money both in terms of loss of capital and opportunities. The study, therefore, is forwarded to the policy formulations with a view to attaining sufficient as well as sustainable local yield.

The Concept of Sugarcane-Belt

Application of belt concept in sugarcane production could counter-act to the disadvantages of haphazard cultivation of the crop. In this concept, high sugarcane yielding areas or the areas having the potentials to contribute to both local and national sugarcane economy could form a belt from where additional quantities of sugarcane required to meet the national demand deficit would be cultivated. The belt should have self-sufficiency, as well in terms of provision of labour forces and lands required to cultivate the given amount. The belt concept could be implemented by patronizing sugarcane cultivation within the belt through ensuring availability of good quality seeds, inputs and other technical supports.

This study propose the Sugarcane-Belt covering Gaibandha, Sherpur, Jamalpur, Tangail, Pabna, Manikganj, Rajbari, and Kushtia districts, as shown in Fig.3. In total, 98,013 acres of agricultural lands in these eight districts are currently allocated for sugarcane cultivation and this proposed belt has current production of 2.97 million metric tons of sugarcane in a year (BBS, 2007).

Basis for Selecting the Belt Districts

Location Quotient (LQ)

Gibson *et al.* (1991) stated that Location Quotient (LQ) yields a coefficient or a simple expression of how well represented a particular industry is in a given study region. Isard (1960) defines Location Quotient (LQ) as a related device for comparing a

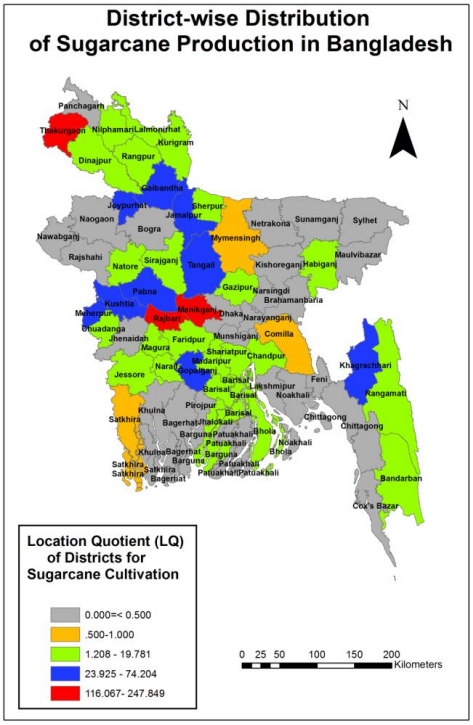

Fig.2: Overall district-wise distribution of sugarcane production in Bangladesh

region's percentage share of a particular activity with its percentage share of some basic aggregate.

The Location Quotient (LQ) value of a district for sugarcane production points to the district's contribution to the national sugarcane production. The average LQ of Bangladesh for sugarcane production is 19.77, as calculated from the district wise agricultural production data provided in Bangladesh Bureau of Statistics (BBS, 2007). The formulae used to calculate LQ for a district for sugarcane production is as following:

$$\text{LQ for District "}X\text{"} = \frac{\text{Quantity of Sugarcane Produced in District "}X\text{"}}{\text{Quantity of Total Agricultural Products Produced in District "}X\text{"}} \Big/ \frac{\text{Total Quantity of Sugacane Produced in the Whole Country}}{\text{Total Quantity of Agricultural Products Produced in the Whole Country}}$$

Fig.2 shows the comparative LQ values of all districts of Bangladesh for sugarcane production. The districts having Location Quotient (LQ) value of more than the country average are considered initially to form the belt. Table 2 presents the list of eleven districts fulfilling the criterion.

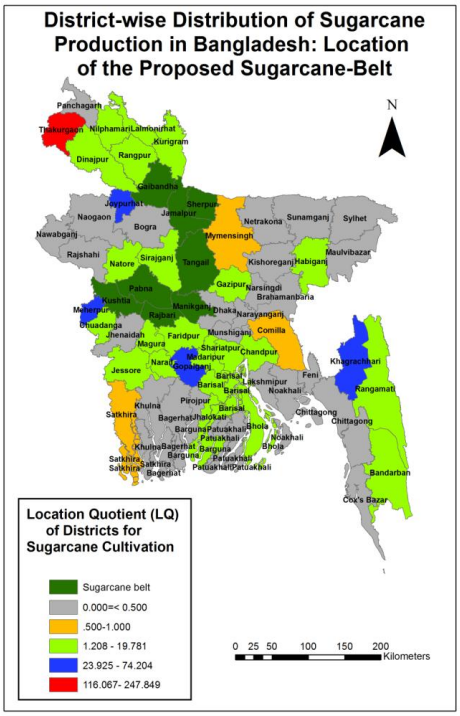

Fig.3: Location of the proposed Sugarcane-belt

TABLE 2
LQ value and Production Rate of districts for sugarcane production

Districts	Location Quotient (LQ) Value	Production Rate (tons/acre)
Manikgonj	247.74	193.22
Thakurgaon	143.15	160.95
Rajbari	116.01	14.15
Meherpur	74.17	13.39
Tangail	53.67	20.11
Pabna	48.49	3.91
Gaibandha	45.36	23.04
Jamalpur	34.88	14.16
Kushtia	32.94	17.87
Khagrachari	30.47	50.49
Gopalganj	29.64	13.11
Sherpur	6.19	24.94

Source: BBS, 2007

However, the LQ value of a district cannot sufficiently elucidate its productivity as this tool is concerned with the district's total output but the production rate. A district with larger cultivable land area can produce higher quantity and may therefore hold bigger LQ value. In transposition, the quantity of output produced by a small district may seem off-set to the national level production though the district might have greater productivity in terms of production per acre than the larger district. While selecting districts to form a special crop-belt, therefore, per acre production of district should also be considered to ensure sufficient yield of that crop from minimal utilization of lands.

Production Rate (metric tons/acre)

Production rate of a district indicates how prolific or productive the district is for cultivating the given type of crops. The country average production rate is 16.95 metric tons/acre for sugarcane cultivation (BBS, 2007). The districts with production rate greater than the country average are initially considered and then validated by the LQ values of those districts to be finally selected for the belt. Other districts with considerable LQ values but lower production rates (and vice-versa) are also considered based upon the location factor or geographical contiguity to the belt areas.

Location Factor

Manikganj, Rajbari, Tangail, Gaibandha, Jamalpur and Kushtia with both the LQ values and Production Rates greater than the country averages are selected to form the sugarcane belt. Pabna holds considerable LQ value but very low production rate, as shown in Table 2. Nevertheless, Pabna district is considered to form the belt as it is geographically contiguous to the primarily selected sugarcane belt. Accordingly, Sherpur district with lower LQ value but higher production rate than the country's average is considered for its geographical contiguity.

On the other hand, Thakurgaon, Meherpur, Khagrachari and Gopalgonj districts have considerable productivity but they are geographically isolated. Therefore, these districts are not considered in forming the belt area.

Availability of Lands under Crop Cultivation

The proposal of this research is to produce the additional 16.63 million metric tons of sugarcane required to meet the national demand deficit per year in the proposed sugarcane-belt. In order to produce this huge amount of sugarcane, the land area required is 0.96 million acres. Manikganj, Rajbari, Tangail, Gaibandha, Jamalpur, Kushtia, Sherpur and Pabna are the eight districts selected with a total of 2.48 million acres of agricultural lands under crop cultivation (BBS, 2010).

Manikganj, Rajbari, Gaibandha, Kushtia and Pabna districts are, in addition located on the bank of three major rivers of Bangladesh-Padma, Jamuna and Brahmaputra, which makes these districts to have huge amount

of lands in the '*char'* (river side sandy land) area. It is worth mentioning that *char* lands are highly suitable for sugarcane cultivation (BSS, 2012).

Key Information on the Proposed Sugarcane-Belt

Sugarcane-belt consists of eight districts of Bangladesh, namely, Manikganj, Rajbari, Tangail, Gaibandha, Jamalpur, Kushtia, Sherpur and Pabna (see Table 3 and Fig.3). These districts are characterized by high sugarcane productivity as well as significant contribution to the national sugarcane production.

The average sugarcane production rate of the selected belt is 38.93 metric tons/acre, whereas the country average is 16.95 metric tons/acre. In collective manner, the Location Quotient value of the belt area is 73.16. Information in relation to other aspects such as area under sugarcane cultivation, production amount, number of agricultural labour, amount of lands under crop cultivation and culturable waste and fallow lands associated with the belt area are given in Table 3.

Policy Recommendations

Emphasizing *Char* Area Production

River-side sandy soil or *char* areas are highly suitable for sugarcane cultivation. Tangail, Pabna, Jamalpur and Kushtia are the four districts of the selected sugarcane-belt that have around 153,000 acres of fallow and 23,000 acres of culturable waste lands, as shown in Table 3 (Yearbook of Agricultural Statistics of Bangladesh, 2011).

TABLE 3
Information on the proposed sugarcane-belt

Districts forming Sugarcane-Belt	Area under Sugarcane Cultivation (acres) †	Production Amount of Sugarcane (metric tons)†	Sugarcane Production Rate (metric tons/acre)	Number of agricultural Labour††	Amount of Area under Crop Cultivation (acres) †††	Culturable Waste (acres) ††††	Fallow Land (acres) ††††
Manikganj	7,705	1488,789	193.22	81,957	186,696	-	-
Jamalpur	29,576	418,872	14.16	212,634	391,038	8,000	38,000
Tangail	9,009	181,203	20.11	213,685	498,146	4,000	57,000
Rajbari	12,384	175,254	14.15	76,716	149,109	-	-
Kushtia	34,656	619,246	17.87	150,564	259,317	3,000	2,000
Pabna	35,295	138,170	3.91	198,378	387,463	8,000	56,000
Sherpur	531	13,242	24.94	142,024	248,681	-	-
Gaibandha	8,152	187,802	23.04	280,222	358,717	-	-
Total	137,308	3222,578	(average) 38.93	1356,180	2479,167	23,000	153,000

†Source: BBS, 2007; ††Source: Agricultural Census, 2008; †††Source: BBS, 2010; ††††Source: Yearbook of Agricultural Statistics of Bangladesh, 2011
(Note: '-' sign indicates that the data for the district is not available)

Moreover, as they are situated on the bank of a river, Gaibandha, Pabna, Manikganj, Kushtia and Rajbari districts have huge amount of cultivable or cultivable fallow lands in the *char* area. These *char* areas, along with other fallow lands and culturable waste lands, could be brought under the sugarcane cultivation scheme which would ensure maximum utilization of *char* lands and thus higher production possibility of sugarcane. Proper patronization (e.g., extension of irrigation, provision of seeds, inputs and training, etc.) from the Ministry of Agriculture (MoA) and/or Bangladesh Sugarcane Research Institute (BSRI) would help in this regard in bringing those lands under sugarcane cultivation.

Agricultural Labour Policy

On an average, 24 labour-days are required to cultivate 1 acre of sugarcane (Field Survey, 2012). As mentioned earlier, 0.96 million acres of land are required to be cultivated with sugarcane within the selected belt area in order to meet the national demand deficit for sugar. Therefore, a total of 23.13 million labour-days are required to cultivate the required amount in a season. The statistics estimates that 110,140 labour-days are required per day in the sugarcane field within the belt area. However, as shown in Table 3, the selected sugarcane-belt area has a supply of around 1356,180 agricultural labours (Note: agriculture labour is defined as labour exchanged for wages in cash or kind or both for agricultural activities on land operated by other households) (Agricultural Census, 2008). It is important to mention here that the number of actual agricultural labours available within the belt area could be bigger since land-owners have also been found to giving labour to their own fields at times.

Land Renting Policy

The study shows that the average agricultural (sugarcane) labour charge in the rural parts of Bangladesh is BDT 120 per day (Field Survey, 2012). Therefore, considering 25 working days in a month, a sugarcane labourer can earn BDT 3000 monthly. However, a rural family with an average of 4 family members would need BDT 3000-3500 per month to be run properly (Field Survey, 2012). Therefore, the monthly income of BDT 3000 of a labourer is merely sufficient to run his family since there might appear some unexpected costs at times.

Considering the issue, land renting policy is proposed here with a view to enhancing the earning capability of the labourers. Under this policy, all agricultural labourers within the belt area are supposed to cultivate at least 1 *Bigha* (Note: 1 *Bigha*= 1/3 acre) land with sugarcane on land renting basis each year. A local body for sugarcane cultivation is recommended to be built up within the belt area, and in this regard, that one body would take care of implementing the proposed land renting policy.

Cultivation of sugarcane in 1 *Bigha* land generates seasonal profit of BDT 18,957, as calculated and presented in Fig.1. Therefore, a labourer with 1 *Bigha* rented land cultivated with sugarcane would be able to earn an additional income of BDT

1,579.75 per month. This additional earning would help the labourers to have savings of a reasonable amount per month. Moreover, other crops like pulses could be cultivated in the same land in parallel to the sugarcane. This might allow them to earn more and improve their quality of life.

Industrial Policy

Out of 15 government-run sugar mills in Bangladesh, three (Zeal Bangla Sugar Mill, Pabna Sugar Mill and Kushtia Sugar Mill) are located within the proposed sugarcane-belt. Nine other sugar mills are geographically located in the adjacent districts to the proposed belt area. All these mills should have maximum access to the belt area, especially for raw materials (i.e., sugarcane). The market area for each of sugarcane mills should be geographically defined to ensure systematic and effective production of sugarcane in fields. Improved transportation networks should also be established between these mills and the corresponding parts of the belt area.

SWOT Analysis

Based on the findings of the study, the strengths, weaknesses, opportunities and threats for the local production of sugarcane within the proposed belt area are as follows:

Strengths

Char area: Availability of large amount of '*char* lands' within the proposed belt area, which is highly suitable for sugarcane cultivation.

Favourable environment: Most of the districts within the proposed belt area have favourable environment in terms of temperature, relative humidity and soil quality for sugarcane cultivation (Hoque, 2001).

NGO Activities: Most of the districts within the proposed sugarcane-belt area are characterized by adequate concentration of NGO activities (NGO Affairs Bureau, 2013). Some of these NGOs can offer financial help in the form of micro-credit to the farmers to promote the cultivation of sugarcane.

Availability of Labourers: There is sufficient supply of agricultural labourers within the proposed belt.

Optimum Distance of the Belt Area from Other Parts of the Country: The proposed sugarcane-belt is located almost at the central part of the country. This location factor would reduce the transportation cost of sugar as well as sugarcane throughout the whole country.

Weaknesses

Longer Growing Duration: Cultivation of sugarcane requires 12 months from plantation to harvesting time and this might discourage farmers to grow this particular crop.

Illegal Ownership of Char Lands: Most of the char lands in Bangladesh are occupied illegally by local influential people. They would locally try to resist any kind of intervention in their illegally occupied lands.

This issue might reduce the production possibility of sugarcane in those char areas.

Opportunities

Fuel: Cane tops and bagasse could be used as cheap fuel within and surrounding the belt area.

Absorption of Local Agricultural Labourers: Agricultural labourers would be required in sugarcane field on regular basis. This could ensure their income certainty throughout the year.

Threats

Aggression of Tobacco: Nowadays, farmers in Kushtia are inclined to tobacco cultivation instead of sugarcane for more profit (Akhter, 2011).

Flood Prone Area: Char lands lie within the flood prone area and therefore, the production might get damaged on sudden basis.

CONCLUSION

Local production of sugarcane in Bangladesh would save wastage of BDT 8,937.94 million per year. Moreover, a huge amount of money could be earned in this sector through the by-production of molasses, spirit/alcohol and *chobra*. These two issues point to the logical reasoning behind going for sustainable local growing of sugarcane. However, the belt concept would help to promote production of sugarcane since regionalization, especially in the case of agricultural activities, results to the efficient outcomes always

In this study, eight districts were selected to form the sugarcane-belt based on their location quotient value for sugarcane production, production rate, location factor and land supply ability. Policy recommendations were also made in the research to attain enhanced production and improved quality of life for agricultural labourers within the proposed belt area.

REFERENCES

Agricultural Census. (2008). *Preliminary Report of Agricultural Census-2008*: *Table 2- Number and Percentage Distribution of Households by Type and by District and Division*, Bangladesh Bureau of Statistics, pp 22-23 [Retrieved from: http://www.bbs.gov.bd/WebTestApplication/userfiles/Image/AgricultureCensus/ag_pre_08.pdf?page=/PageReportLists.aspx?PARENTKEY=44, Retrieved on: September 3, 2012].

Akhter, F. (2011). Tobacco Cultivation and Its Impact on Food Production in Bangladesh. *UBINIG*. [Retrieved from: http://www.fairtradetobacco.org/wp-content/uploads/2011/07/Farida-Akhter_Tobacco-to-Food-Production.pdf, Retrieved on: May 10, 2013]

BBS, (2007). *Zilla Profile-Agriculture Product,* Bangladesh Bureau of Statistics. Retrieved from http://www.bbs.gov.bd/RptZillaProfile.aspx, Retrieved on: August 10, 2012]

BBS (2010). *Chapter 4: Agriculture: Crops, Livestock, Forestry and Fishery*. Bangladesh Bureau of Statistics, pp. 133-157, [Retrieved from: http://www.bbs.gov.bd/webtestapplication/userfiles/image/SY2010/Chapter-04.pdf, Retrieved on: September 5, 2012]

BSFIC (2012). *Mills/Factories under BSFIC,* Bangladesh Sugar and Food Industry Corporation. Retrieved from: http://www.bsfic.gov.bd/index.php/mills-factories-under-bsfic, Retrieved on: August 14, 2012]

BSFIC (2013a). *Annual Report (2009-10): Annual Demand for Sugar in the Country and Production Capacity of Sugar Mi*lls, MIS and ICT Department, Bangladesh Sugar and Food Industry Corporation, pp 2-3, March 25, [Retrieved from: http://www.bsfic.gov.bd/index.php/report/bsfic-download-center/item/annual-report, Retrieved April 10, 2013.

BSFIC. (2013b). *Annual Report (2009-10): Estimation of Profit/Loss of Sugar Mills* on the Date June 30, 2010, MIS and ICT Department, Bangladesh Sugar and Food Industry Corporation, March 25, [Retrieved from: http://www.bsfic.gov.bd/index.php/report/bsfic-download-center/item/annual-report, Retrieved on: April 10, 2013.

BSFIC. (2013c). *Annual Report (2009-10): Production Activities,* MIS and ICT Department, Bangladesh Sugar and Food Industry Corporation, pp 7-9, March 25, [Retrieved from: http://www.bsfic.gov.bd/index.php/report/bsfic-download-center/item/annual-report, Retrieved on: April 10, 2013]

BSFIC. (2013d). *Annual Report (2009-10): Selling Activities,* MIS and ICT Department, Bangladesh Sugar and Food Industry Corporation, pp 9-10, March 25, [Retrieved from: http://www.bsfic.gov.bd/index.php/report/bsfic-download-center/item/annual-report, Retrieved on: April 10, 2013]

BSRI (2013a). *Sugar Statistics,* Bangladesh Sugarcane Research Institute. Retrieved April 10 from: http://www.bsri.gov.bd/sugar.php, Retrieved on: April 12, 2013]

BSRI (2013b). *Sugar Statistics: GDP contribution to sugarcane economy (2003-04),* Bangladesh Sugarcane Research Institute, April 10 [Retrieved from: http://www.bsri.gov.bd/sugar.php, Retrieved on: April 12, 2013]

BSS (2012). Sugarcane Cultivation in Fallow Char lands Can Bring Prosperity. *Bangladesh Sangbad Sangstha* (National News Agency of Bangladesh), June 1, [Retrieved from: http://www1.bssnews.net/newsDetails.php?cat=4&id=253449&date=2012-06-01, Retrieved on: September 10, 2012]

Gibson, James L., Miller, M. M., & Wright, N. G. (1991). Location Quotient: A Basic Tool for Economic Development Analysis, *Economic Development Review*, *9*(2) (Spring 1991), pp. 65-68.

Hoque, M. E. (2001). Crop Diversification in Bangladesh. In *Crop Diversification in the Asia-Pacific Region, Food and Agriculture Organization of The United Nations, Regional Office for Asia and The Pacific, Bangkok, Thailand, April, pp 13-14 [Retrieved from:* ftp://ftp.fao.org/docrep/fao/003/x6906e/x6906e00.pdf, Retrieved May 4, 2013.

Isard, W. (1960). *Methods of Regional Analysis: an Introduction to Regional Science*. Massachusetts: The M.I.T Press.

Khan, S. (2009 September 13). When Sugar Turns 'Sour'. *The Daily Financial Express*. Retrieved from: http://www.thefinancialexpress-bd.com/2009/09/13/78829.html, Retrieved on: August 12, 2012]

NGO Affairs Bureau (2013). List of NGOs as on 30 April, 2013. Retrieved from http://www.ngoab.gov.bd/Files/NGO_LIST.pdf, Retrieved on: 10 May, 2013]

RHD (2007). *Distance Matrix (From District HQ to District HQ)*, RHD GIS Unit, HDM Circle, Roads & Highways Department, Bangladesh, March [Retrieved from: http://www.discoverbangla.com/distancechart.pdf, Retrieved on: August 8, 2012]

Russel, A. (2002). Sugar Production from Sugarcane. *Practical Action*, The Schumacher Centre, Bourton on Dunsmore, Rugby, Warwickshire, CV23 9QZ, UK, p-2, February 2, [Retrieved from: http://practicalaction.org/print/docs/technical_information_service/sugar_production_from_cane.pdf, Retrieved on: August 10, 2012

Sugaronline (2012 February 11). *Bangladesh: Raw Sugar Imports Soar*. Retrieved from http://www.sugaronline.com/news/website_contents/view/1203332, Retrieved on: August 26, 2012)

Yearbook of Agricultural Statistics of Bangladesh (2011). *Chapter-10: Land-use Statistics,* Bangladesh Bureau of Statistics, p-305, [Retrieved from: http://www.bbs.gov.bd/WebTestApplication/userfiles/Image/ArgYearBook11/Chapter-10.pdf, Retrieved on: September 5, 2012]

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Occurrence of *Fusarium* spp. on Vegetable Crops and Assessment of Their Pathogenicity

Nurul Huda Mohamad Saseetharan and Latiffah Zakaria*

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

ABSTRACT

Fusarium are among the fungal genera that can cause contamination or spoilage on vegetable crops. Therefore, it is important to identify the occurrence of *Fusarium* species on these commodities as some species are plant pathogen and some other are toxigenic. In the present study, 83 *Fusarium* isolates were recovered from rotting tissues of nine vegetable crops, namely, cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), okra (*Hibiscus esculentus)*, loofah (*Luffa acutangula*), bitter gourd (*Momordica charantia*), moringa *(Moringa olifel)*, brinjal (*Solanum melongena*), long bean (*Vigna sesquipedalis*) and red chilli (*Capsicum annuum*). The species identified were *F. oxysporum* (22 isolates), *F. semitectum* (19 isolates), *F. solani* (19 isolates), *F. proliferatum* (14 isolates), *F. pseudocircinatum* (four isolates), *F. sacchari* (two isolates), *F. equiseti* (two isolates) and *F. verticillioides* (one isolate). From pathogenicity test, only 21 isolates were found to be pathogenic, causing vegetable rot on their host. The present study showed that *Fusarium* species are prevalent on vegetable crops and the species might be pathogenic or epiphytic .

Keywords: Fusarium, vegetable crops, pathogenicity

INTRODUCTION

Many *Fusarium* species are plant pathogen and cause vascular wilts, root and fruit rots diseases on various types of vegetable crops. There are also opportunistic species or weak pathogen which colonize plant tissues after the plants have become stressed, especially the species associated with spoilage or postharvest disease on vegetables crops. After harvest, vegetables contain relatively high microorganism which includes spoilage and plant pathogenic fungi that can cause deterioration and reduction in quality, texture and loss of nutrients (Barth *et al*.,

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E-mail addresses:
nurulhuda_ms@yahoo.com (Nurul Huda Mohamad Saseetharan),
lfah@usm.my, latiffahz@yahoo.com (Latiffah Zakaria)
* Corresponding author

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2009). It can also reduce shelf-life and the acceptability of the produce. Among the fungal pathogens, the *Fusarium* species is commonly found to be associated with losses caused due to rotting and spoilage of several types of vegetable crops. Theses include vegetables mainly belonging to the solanaceae and cucurbitaceae families (Snowdon, 1990; Tournas, 2005a, d, b; Naureen *et al*., 2009).

In Malaysia, occurrences of *Fusarium* species on vegetable crops have not been given much attention compared to other agricultural crops. Therefore, the present study was conducted to evaluate the occurrences of *Fusarium* species on several vegetable crops and determine if the isolates were pathogenic and caused vegetable rot.

MATERIALS AND METHODS

Isolation and Identification of the Fusarium species

Fusarium isolates were isolated from rotting tissues of nine vegetable crops, namely, cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), okra (*Hibiscus esculentus)*, loofah (*Luffa acutangula*), bitter gourd (*Momordica charantia*), moringa *(Moringa olifel)*, brinjal (*Solanum melongena*), long bean (*Vigna sesquipedalis*) and red chilli (*Capsicum annuum*) obtained from several markets and hypermarkets in Penang Island, Malaysia. The mycelium grow on the vegetable was transferred onto Peptone Pentachloronitrobenzene Agar, a semi-selective medium for isolation of *Fusarium*. The medium was incubated at 27±1°C for 4-5 days or until the mycelia growth were observed. The mycelia were then subcultured onto potato sucrose agar (PSA).

For identification, the procedures in The *Fusarium* Laboratory Manual (Leslie & Summerell, 2006) were adopted and single spore culture was used. Each isolate was cultured on Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA). The CLA was used to determine the shapes of microconidia and macroconidia, the number of septa and the shapes of the apical and basal cells of the macroconidia, formation of conidiogenous cell, the presence and the colour of sporodochia, and presence of chlamydospore. The cultures plated on CLA were incubated at 27±1°C for 4-5 days. On PDA, cultural characteristics and pigmentation were determined, in which the observations were made after 3 days of incubation at 27±1°C. Species descriptions in The *Fusarium* Laboratory Manual (Leslie & Summerell, 2006) were adapted for the identification of the *Fusarium* isolates to the species level.

Pathogenicity Test

All the isolates of *Fusarium* successfully isolated from the nine vegetable crops were used in the pathogenicity test. Different types of healthy vegetables, namely, cucumber, tomato, okra loofah, bitter gourd, moringa, brinjal, long bean and red chilli were washed with running tap water, disinfected with sodium hypochloride (10%), rinsed with distilled water, and dried at 27 ± 1°C. Inoculations were performed on wounded and unwounded vegetables with three

replicates for each isolate. Mycelial plug (6 mm) was prepared from 5-day old culture and used as inoculum. Three replicates were made for each vegetable and the test was repeated twice. Uninoculated vegetable served as a control. Inoculated vegetables were incubated in a sterile plastic container (40 cm x 30 cm) at 27 ±1°C and disease symptoms were assessed daily through visual examination. Disease symptoms were recorded based on the following scale which was adapted (with some modifications) from Benyon *et al*. (1996): 1 = 20% diseased area, 2 = 50% diseased area, 3 = 80% diseased area, 4 = 100% diseased area. Based on the scale, the percentage of rotted areas was estimated. Reisolation of the fungi was made by direct isolation from the mycelia developed on the rotting tissues, and plated on PSA.

RESULTS AND DISCUSSION

Eighty three *Fusarium* isolates were isolated from rotting tissues of all the vegetable crops, in which 22 isolates were recovered from okra, 13 from tomato and bitter gourd, seven from brinjal, four from cucumber, three from moringa and loofa, and one from long bean. The *Fusarium* isolates were identified as *F. oxysporum* (22 isolates), *F. semitectum* (19 isolates), *F. solani* (19 isolates), *F. proliferatum* (14 isolates), *F. pseudocircinatum* (four isolates), *F. sacchari* (two isolates), *F. equiseti* (two isolates) and *F. verticillioides* (one isolate) (Table 1). The morphological characteristics of each species are presented in Table 2 and Fig.1.

TABLE 1
Fusarium species isolated from the rotting symptom of vegetable crops

Host	*Fusarium* species / number of isolates
Solanaceae	
Tomato	*F. oxysporum* (7)
	F. solani (5)
	F. proliferatum (1)
Chilli	*F. solani* (4)
	F. pseudocircinatum (2)
	F. proliferatum (2)
	F. sacchari (1)
	F. semitectum (1)
Brinjal	*F. proliferatum* (3)
	F. solani (2)
	F. equiseti (1)
	F. pseudocircinatum (1)
Cucurbitaceae	*F. semitectum* (2)
Cucumber	*F. solani* (1)
	F. oxysporum (1)
Loofa	*F. semitectum* (3)
Bitter gourd	*F. oxysporum* (6)
	F. solani (3)
	F. semitectum (2)
	F. proliferatum (2)
Malvaceae	*F. semitectum* (11)
Okra	*F. oxysporum* (7)
	F. proliferatum (5)
	F. solani (2)
	F. pseudocircinatum (2)
	F. verticillioides (1)
Moringaceae	*F. oxysporum* (1)
Moringa	*F. solani* (2)
Fabaceae	*F. semitectum* (1)
Long bean	

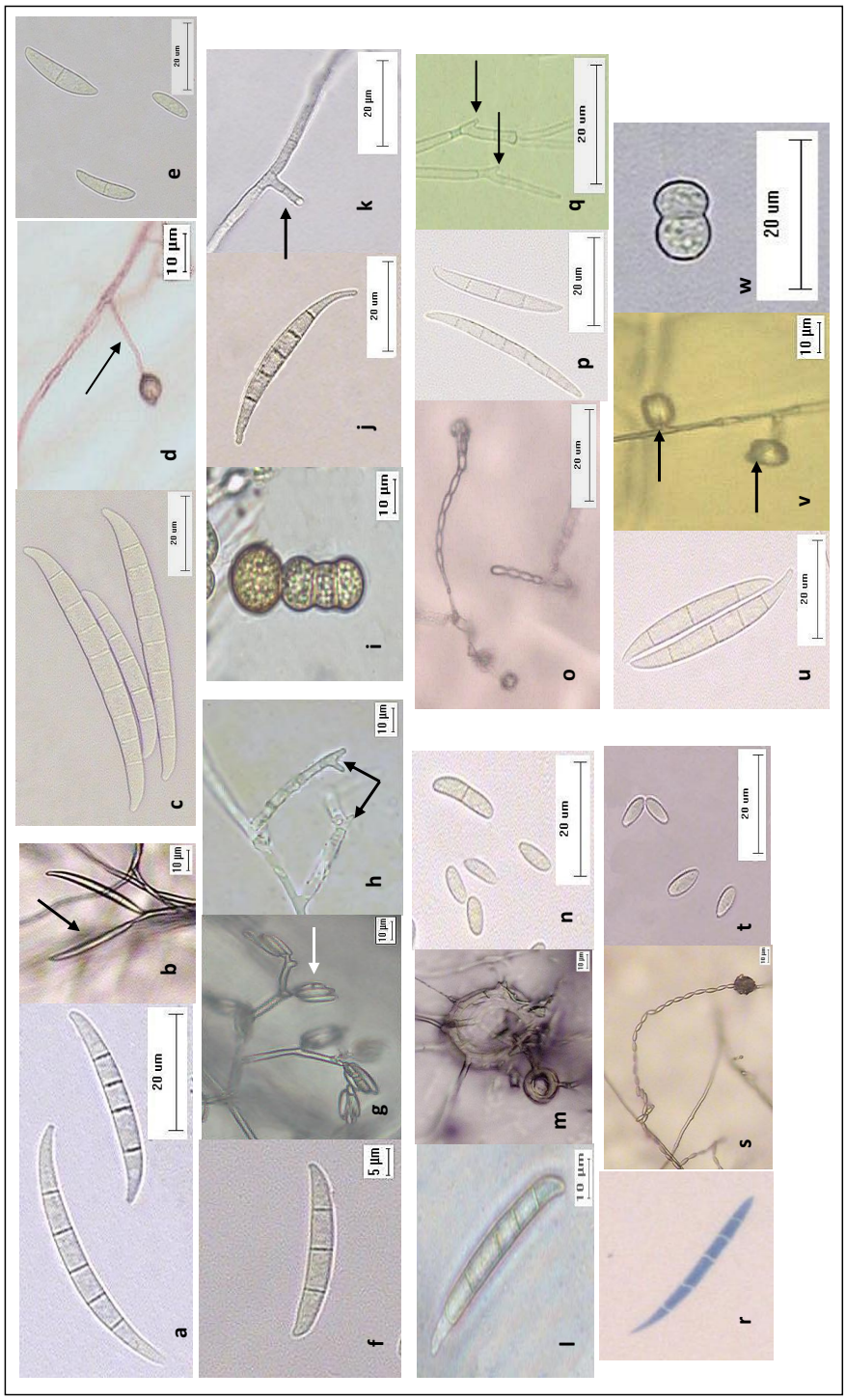

Fig.1: Morphological characteristics of *Fusarium* spp. isolated from rotting tissues of vegetable crops. **(a - b)** : *F. semitectum*, a: macroconidia, b: rabbit ear. **(c – e)** : *F. solani* , c: macroconidia, **d**: long moniphialide, e: microconidia. **(f – h)** : *F. sacchari*, f: macroconidia, g: mesoconidia, h:polyphiliades. **(i – k)** : *F. equiseti*, **i**: chlymadospores, j: macroconidia, **k**: monophialide. **(l – n)** : *F. pseudocircinatum*, **l**: macoconidia, **m**: coiled hyphae, **n**: microconidia. **(o – q)** *F. verticillioides*, **o**: conidia in chain, **p**: macroconidia, **q**: polyphialides. **(r – t)** : *F. verticillioides*, r: macroconidia, **s**: conidia in chain, **t**: microconidia. **(u – w)** : *F. oxysporum*, **u**: macroconidia, **v**: false heads, **w**: chlamydospores

TABLE 2
The morphological characteristics of *Fusarium* species isolated from nine vegetable crops

	Species			
Characteristics	*F. oxysporum*	*F. semitectum*	*F. solani*	*F. sacchari*
Microconidia	Abundant , formed in aerial mycelia, oval to kidney-shaped produced in false head	Scarce, presence of fusoid mesoconidia in aerial mycelia, rabbit-ear appearance.	Abundant, oval to kidney-shaped produced in the agar and carnation leaf.	Abundant, oval, produced only in false head. Presence of mesoconidia in false head.
Macroconidia	Abundant in sporodochia, slightly sickle-shaped, thin walled, tapered apical cell, foot-shaped basal cell	Relatively slender, curved apical cell and foot-shaped basal cell	Abundant, stout, cylindrical, blunt apical cell, distinct and rounded foot-shaped basal cell.	Abundant, slightly sickle-shaped to almost straight, curved apical cell and poorly developed basal cell.
Conidiogenous cell	monophailides	monophailides and polyphialides	long monophailides	polyphailides and monophailides.
Chlamydospore	Present, singly or in pairs.	Present	Present singly or in pairs.	Absent
Pigmentation	White to purple.	Beige to brown	Cream to white	White to purple.

	Species			
Characteristics	*F. proliferatum*	*F. pseudocircinatum*	*F. verticillioides*	*F. equiseti*
Microconidia	Abundant, club shape with flattened base, in chain (10- 15 conidia) and false head.	Abundant, formed in aerial mycelia, oval, produced in false head and in short chain (5 – 10 conidia).	Abundant, formed in aerial mycelia, oval to club shaped, produced in long chain (more than15 conidia).	Absent
Macroconidia	Abundant, slender, almost straight, curved apical cell and poorly developed foot-shaped basal cell.	Scarce, slightly sickle-shaped to almost straight, curved apical cell and poorly developed basal cell.	Scarce, slightly sickle-shaped to almost straight, curved, tapered to a point apical cell and foot shaped basal cell.	Abundan, formed in aerial mycelia, long and quite slender, elongated apical cell and obvious foot shape basal cell.
Conidiogenous cell	polyphailides and monophailides	Usually monophailides. Presence of coiled hyphae	monophailides.	Monophailides
Chlamydospore	Absent	Absent	Absent	present, formed in clumps or chains.
Pigmentation	White to purple	White to light purple.	White to light purple.	Brown to dark brown

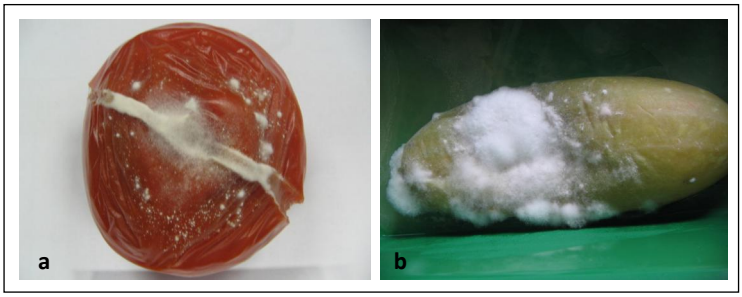

Fig.2: (a) Symptom on tomato inoculated with *F. proliferatum* (TMTC1) on non-wounded treatment
(b) Symptom on cucumber inoculated with *F. solani* (TMN S1b) on wounded treatment

From the pathogenicity test, only 21 isolates were pathogenic to their host (Table 3) as these isolates were successfully reisolated from the rotting tissues, proving that the isolates were the causal pathogen of vegetable rot. Wounded treatment showed severed symptoms compared to unwounded treatment. Rotting symptoms shown on different types of vegetable were similar and characterized by the development of rotting areas with brown discolouration and water soaked appearance (Fig.2). Rotting symptoms were observed on the 7th day after inoculation and the size of the rotting areas gradually increased.

On Solanaceae crops, four species were pathogenic (namely, *F. solani, F. oxysporum*, *F. proliferatum* and *F. sacchari*). Four isolates of *F. solani*, three isolates of *F. oxysporum* and one isolate of *F. proliferatum* were pathogenic on tomato. On chilli, three isolates of *F. solani*, one isolate of *F. proliferatum* and one isolate of *F. sacchari* were pathogenic (Table 2). Disease severity ranging from 60% - 90% and only one isolate of *F. solani* (LMH T6) caused infection using both wounded and non-wounded treatment. On eggplant, one isolate of *F. solani* (TBP S3) was pathogenic (with 85% disease severity) using wounded treatment, while one isolate of *F. proliferatum* (TPJ T3) was pathogenic using both wounded and non-wounded treatment with 65% and 50% disease severity, respectively. Meanwhile, only one isolate of *F. solani* (TMT T3) was pathogenic on tomato using non-wounded treatment with 15% disease severity. Among the four species, *F. oxysporum* and *F. solani* are commonly reported to be associated with rotting of vegetable crops. *Fusarium oxysporum* has been recorded to cause fruit rot of tomatoes (Lockhart, 1970; Akinmusire, 2011) and peppers (Micosa & Ilag, 1977; Fletcher, 1994) and *F. solani* on eggplants, pepper (Ramdial & Rampersad, 2010) and brinjal (Pandey, 2010). Other *Fusarium* species have also been reported to be associated with the rot of Solanaceae crops such as *F. equiseti* on tomatoes and pepper (Adisa & Lekunze, 1986; Oladiran & Iwu, 1993), *F. chlamydosporum* (Oladiran & Iwu, 1993) and *F. avenaceaum* on tomatoes (Marras *et al*., 1979).

Two isolates of *F. solani* and one isolate of *F. oxysporum* were pathogenic on moringa, with disease severity ranging

TABLE 3. *Fusarium* isolates pathogenic to their host

Host	Isolate	*Fusarium* species	Pathogenicity			
			Wounded	Scale / Disease severity	Non-wounded	Scale / Disease severity
Tomato	TMT G7	F. solani	P	4 / 100%	NP	0
Tomato	TMT M1	*F. solani*	P	3 / 80%	NP	0
Tomato	TMT M5	*F. solani*	P	4 / 90%	NP	0
Tomato	TMT T2	*F. solani*	P	3 / 65%	NP	0
Tomato	TMT T3	*F. solani*	P	4 / 100%	P	1 / 15%
Tomato	TMT G3	*F. oxysporum*	P	3 / 80%	NP	0
Tomato	TMT M3	*F. oxysporum*	P	2 / 50%	NP	0
Tomato	TMT T1	*F. oxysporum*	P	4 / 90%	NP	0
Tomato	TMT C1	*F. proliferatum*	P	3 / 70%	NP	0
Chilli	LMH S1	*F. solani*	P	3 / 70%	NP	0
Chilli	LMH T3	*F. solani*	P	3 / 70%	NP	0
Chilli	LMH T6	*F. solani*	P	4 / 90%	P	2 / 50%
Chilli	LMH T4	*F. sacchari*	P	3 / 70%	NP	0
Chilli	LMH S4	*F. proliferatum*	P	3 / 60%	NP	0
Cucumber	TMN S1b	*F. solani*	P	4 / 85%	NP	0
Moringa	MNG R1	F. solani	P	1 / 7%	P	1 / 2%
Moringa	MNG R3	*F. solani*	P	3 / 65%	NP	0
Moringa	MNG R2	*F. oxysporum*	P	2 / 42%	P	1 / 5%
Eggplant	TBP S3	*F. solani*	P	4 / 85%	NP	0
Eggplant	TPJ T3	*F. proliferatum*	P	3 / 65%	P	2 / 50%
Long bean	KPJ N1	*F. semitectum*	P	4 / 100%	NP	0

* P – Pathogenic, NP – Non- pathogenic

from 2% - 65%. Although *F. solani* (MNG R1) showed infection using both wounded and non-wounded treatments, lower disease severity was observed with 7% and 2% severity, respectively. So far, there has been no report on the occurrence of *Fusarium* species that cause rotting of moringa pod.

Although *F. semitectum*, *F. solani* and *F. oxysporum* were recovered from cucumber and loofah, only *F. solani* (TMN S1B) was pathogenic on cucumber with 85% severity using wounded treatment. *Fusarium solani* has been found to be associated with rot of cucumber by Joffe and Plati (1972). In the present study, *F. oxysporum* was not found to be pathogenic on cucumber but it has been reported to be pathogenic on cucumber in the USA (Jenkins & Wehner, 1983; McMillan, 1986). In the present study, *F. semitectum* was not pathogenic to loofah. However, *F. semitectum* was found to cause decay on *Luffa cylindrica* (Tandon & Jamaluddin Bhargava, 1976) and was the most virulent species causing rotting on fruit tissues of *Luffa cylindrical* (Hilal *et al.,* 2003).

Pod rot of okra caused by *F. solani* has been reported by Esuruoso *et al*. (1975). However, in the present study, *F. solani* isolated from okra was not pathogenic, just like the six other species, namely, *F. oxysporum, F. proliferatum, F. solani, F. pseudocircinatum and F. verticilliodes*. Meanwhile, *Fusarium semitectum* was found to be the most common species isolated from okra but was non-pathogenic. This was not surprising as the species has not been known as an important plant pathogen although it has been reported to be pathogenic on several plants (Leslie & Summerell, 2006). In the present study, only *F. semitectum* recovered from long bean was pathogenic with 100% disease severity. On other types of legume, *F. semitectum* has been reported to have caused pod rot and seed rot of snap bean in India and field disease of common bean in Brazil (Dhingra & Muchovej, 1979; Dhingra *et al.,* 2002).

Most of the *Fusarium* isolates infected the vegetable crops on wounded treatment, indicating that the *Fusarium* species associated with vegetable rot are weak pathogen that causes infection when the crops are weakened or stressed through mechanical injuries and impact damage (Coates & Johnson, 1997). Moreover, some vegetable crops such as tomato, chilli, brinjal and cucumber have thin skin which causes them to become more prone to injuries. Injuries on the surface of vegetables are caused by cuts or abrasion during harvesting, handling operations, storage pressure and impact damage as well as poor sanitary practice and contamination during transportation and marketing (Coates & Johnson, 1997; Eckert, 1978; Barth *et al*., 2009).

The injuries and presence of pathogenic microbes on vegetable crops, combined with suitable environmental factors, provide the conditions for disease expression and development by spoilage fungi including *Fusarium* species. From non-wounded treatment, three *F. solani* isolates, one *F. oxysporum* isolate and one *F. proliferatum* isolate were found to be pathogenic to their host. These *Fusarium* isolates might produce pectin-degrading enzymes to degrade pectin component of the cell wall which assist the pathogen to penetrate the host. In the *Fusarium* species, endopolygalacturonases are among the enzyme produced during infection especially for tissue penetration and colonization (Mariotti *et al*., 2009).

Although only 21 isolates were found to be pathogenic, the isolates showed variation in term of their degree of pathogenicity. Most *F. solani* and *F. oxysporum* isolates were pathogenic on different types of vegetable crops and showed variation in their pathogenicity. The range of variation in pathogenicity could be associated with genetic diversity as both *F. solani* and *F. oxysporum* are regarded as species complex (Baayen *et al*., 2000; O'Donnell, 2000). Species in a species complex exhibit high level of genetic diversity. Moreover, both species occur on a wide host range and have several forma specialis and races which infect specific plant species and cultivars. The same condition can be applied to *F. proliferatum* isolates which were pathogenic

on tomato, chilli and brinjal. *Fusarium proliferatum* is grouped in *Gibberella fujikuroi* species complex and can be found on a wide host range as well as pathogenic on various agricultural crops.

Other *Fusarium* species isolated with low frequency from vegetable rot were *F. sacchari, F. pseudocircinatum, F. verticillioides* and *F. equiseti*. Among the three species, only *F. equiseti* and *F. verticillioides* have been reported to be associated with vegetable crops. *Fusarium equiseti* has been isolated from rotten tomato fruits (Oladiran & Iwu, 1993) and the host range includes several numbers of Leguminosae (Goswani *et al*., 2008). *Fusarium verticillioides* has been recovered from internal fruit rot of pepper (Howard, 2005) and from apical segment of asparagus (Elmer, 2000).

The non-pathogenic *Fusarium* isolates recovered from the rotting tissues of the vegetable crops could be part of epiphytic mycoflora which occur naturally on the surfaces of the vegetables. Epiphytic mycoflora occurs on the plant surfaces of vegetables as vegetables have high water activity (more than 0.99) and the pH ranges from 4.9 – 6.5 which allow the growth of many fungi (Lund, 1992). Most epiphytic fungi including *Fusarium* are benign to the crops and in many ways can provide a barrier to infestation by plant pathogenic microbes (Janisiewicz & Korsten, 2002).

The present study showed that the *Fusarium* species are prevalent on vegetable crops. Many isolates are not pathogenic or not capable of causing diseases, while some species are opportunists. Opportunistic species can colonize plant tissues and this leads to infection by *Fusarium* when the crops are predispose to abiotic and biotic factors.

Fusarium spp. are among toxigenic fungi causing contamination on vegetables and fruits. Although detected at low level, *Fusarium* mycotoxins have been reported in asparagus, herbs, fig, potato, celery, beans, chilli, ginger, coriander and medicinal plant. The occurrence of *Fusarium* species on these crops may contribute to an intake of *Fusarium* mycotoxins (Logrieco *et al*., 2003). The ability of toxigenic species to produce mycotoxins depends on the substrates. Mycotoxin-producing *Fusarium* species are known as field fungi which require very high moisture content for growth on the substrate and for production of mycotoxin (Logrieco *et al*., 2003). These conditions make vegetables a suitable substrate for toxigenic *Fusarium* growth as the crops have ideal water activity and low pH which are conducive for fungal growth.

Thus, the knowledge on the presence of *Fusarium* on vegetable crops can provide a basis for proper harvesting and storage practices as unsuitable harvesting practices and poor storage conditions may cause growth and proliferation of the mycotoxin-producing *Fusarium* species.

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REFERENCES

Adisa, V. A., & Lekunze, J. K. (1986). Fruit rots of *Capsicum annum* and *C. frulescens* in. Nigeria. *Fitopatologia Brasileira, 11,* 817 - 822.

Akinmusire, O. O. (2011). Fungal species associated with the spoilage of some edible fruits in Maiduguri Norther Eastern, Nigeria. *Advances in Environmental Biology, 5,* 157 – 161.

Baayen, R. P., O'Donnell, K., Bonants, P. J. M., Cigelnik, E., Kroon, L, Roebroeck, E. J. A., & Waalwijk, C. (2000). Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. *Phytopathology, 90*, 891-900.

Barth, M., Hankinson, T. R., Zhuang, H., & Breidt F. (2009). In W.H. Sperber and M.P. Doyle (Eds.), *Compendium of the Microbiological Spoilage 135 of Foods and Beverages, Food Microbiology and Food Safety*. Springer Science+Business Media, LLC.

Benyon, F., Summerell, B. A., & Burgess, L. W. (1996). Association of *Fusarium* species with root rot of *Cymbidium* orchids. *Australasian Plant Pathology 25,* 226-228.

Burgess, L. W. (1981). General Ecology of the Fusaria. In P.E. Nelson, T. A. Toussoun & R. J. Cook (Eds.), *Fusarium*: *Diseases, Biology and Taxonomy.* University Park: The Pennyslvania State University.

Coates, L., & Johnson, G. (1997). Post-harvest diseases of fruits and vegetables. In *Plant Pathogens and Plant Diseases* (pp. 533 – 547). Armidale NSW: Rockvale Publications.

Dhingra, O. D., & Muchovej, J. J. (1979). Pod rot, seed rot, and root rot of snap bean and dry bean caused by *Fusarium semitectum*. *Plant Disease Reporter*, *63*, 84–87.

Dhingra, O. D., Maia, C. B., Lustosa, D. C., & Mesquita, J. D. (2002). Seedborne pathogenic fungi that affect seedling quality of Red Angico (*Anadenanthera marcrocarpa*) trees in Brazil. *Journal of Phytopathology*, *150*, 451 – 455.

Eckert, J. W. (1978). Pathological diseases of fresh fruits and vegetables. *Journal of Food Biochemistry, 2,* 243 – 250.

Elmer, W. H. (2000). Incidence of infection of asparagus spears marketed in Connecticut by *Fusarium* species. *Plant Disease*, *84,* 831 – 834.

Esuruoso, O. F., Ogundiran, S. A., Chheda, H. R., & Fatokun, D. O. (1975). Seedborne fungi and some fungal diseases of okra in Nigeria. *Plant Disease Reporter*, *59,* 660 – 663.

Fletcher, J. T. (1994). *Fusarium* stem and fruit rot of sweet peppers in the glasshouse. *Plant Pathology, 43*.

Goswami, R. S., Dong, Y., & Punja, Z. K. (2008). Host range and nycotoxin production by *Fusarium equiseti* isolates originating from ginseng fields. *Canadian Journal of Plant Pathology*, *30*, 155 – 160.

Hilal, A. A., Abo-El-Ela, A. M., .El-Morsy, S. A., & Nadq, M. G. A. (2003). The prevalence and control of flower and fruit rots of loofa (*Luffa aegyptiaca*) in Egypt. *Egypt Journal of Phytopathology*, 31, 167 – 182.

Howard, R. (2005). *Management of major green house vegetable diseases*. Canadian Greenhouse Conference, 5 Oct 2005. pp. 1 – 6.

Jenkins, S. F. Jr., & Wehner, T. C. (1983). Occurrence of *Fusarium oxysporum* f. sp. *cucumerinum* on greenhouse-grown *Cucumis sativus* seed stocks in North Carolina. *Plant Disease*, *67,* 1024-1025.

Joffe, A. Z., & Palti, J. (1972). *Fusarium* species of the Martiella section in Israel. *Phytopathologische Zeitschrift*, *73*, 123 – 148.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of post harvest disease of fruits. *Annual Review of Phytopathology, 40*, 411 - 441.

Leslie, J. F., & Summerell, B. A. (2006). The *Fusarium* Laboratory Manual. Blackwell Publishing, Ames, IA, USA.

Lockhart, C. L. (1970). Suppression by ethylene of *Fusarium oxysporum* growth in culture and rots of tomato in controlled atmosphere storage. *Canadian Journal of Plant Science*, *50*, 347 – 349.

Logrieco, A., Bottalico, A., Mule, G., Moretti, A., & Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. *European Journal of Plant Pathology*, *109*, 645 – 667.

Lund, B. M. (1992). Ecosystems in vegetable foods. *Journal of Applied Bacteriology*, *73*, Supplement *21*, 115S-135S.

Mariotti, L., Cassoli, M., Caprari, C., & Lorenzo, G-De. (2009). A divergent polygalacturoanse of *Fusarium phyllophilum* shows sequence and functional similarity to the enzyme of *Fusarium verticillioides*. *Journal of Plant Pathology*, *9*, 129 – 139.

Marras, F., Corda, P., & Fiori, M. (1979). *Fusarium roseum* var. *avenaceum* (Sacc.) Synd. Et Hans., agente di un marciume molle dei fruit di pomodoro in coltura protetta. *Studi Sassaresi III*, *27,* 233 – 242.

McMillan, R. T. (1986). Cross pathogenicity studies with isolates of *Fusarium oxysporum* from either cucumber or watermelon pathogenic to both crop species. *Annals of Applied Biology*, *109*, 101 – 105.

Micosa, R. S., & Ilag, L. L. (1977). Fruit rot of pepper caused by *Fusarium* spp. in the Philippines. *Philippine Phytopathology*, *13*, 14 – 23.

Naureen, F., Humaira, F., Viqar, S., Jehan, A., & Syed, E. H. (2009). Prevalence of post harvest rot of vegetables and fruits in Karachi, Pakistan. *Pakistan Journal of Botany*, *41,* 3185 – 3190.

O'Donnell, K. (2000). Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. *Mycologia*, *92*, 919–938.

Oladiran, A. O., & Iwu, L. N. (1993). Studies on the fungi associated with tomato fruit rots and effects of environment on storage. *Mycopatologia*, *121*, 157 – 161.

Palada, M. C., & Chang, L. C. (2003). *Suggested cultural practices for moringa. International Cooperators Guide*. Asian Vegetable Research and Development Centre (AVRCD) pub #03-545.

Ramdial, H. A., & Rampersad, S. N. (2010). First report of *Fusarium solani* causing fruit rot of sweet pepper in Trinidad. *Plant Disease*, *1,* 275.

Snowdon, A. L. (1990). A *colour atlas of post-harvest diseases and disorders of fruits and vegetables, Volume 2*: Vegetables. Wolfe Scientific Ltd. BPCC Hazell Books, Aylesbury, England.

Tandon, M. P., & Jamaluddin Bhargava, V. (1976). Chemical control of *Fusarium semitectum* decay of fruits of *Luffa cylindrical* in marketing channels. *Proceedings of the National Academy of Sciences, 46B*(3), 456 – 458.

Tournas, V. H. (2005a) Mould and yeasts in fresh and minimally processed vegetables and sprouts. *International Journal of Food Microbiology*, *99*, 71 – 77.

Tournas, V. H. (2005b). Spoilage of vegetables crops by bacteria and fungi and related health hazards. *Critical Reviews in Microbiology*, *31*, 33 – 44.

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Preference for *Molineria latifolia* var. megacarpa and *Rhodomyrtus tomentosa* as Native Urban Landscape Plants

Sarah, B.[1]*, Thohirah, L. A.[1], Mustafa Kamal, M. S.[2] and Rosenani A. B.[3]

[1]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Landscape Architecture, Faculty of Design and Architecture, Persiaran Universiti 1, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Factors influencing the perception of landscapes have been the subject of research in the last 40 years. Indigenous and native plants are commonly restricted to informal or naturalistic designed landscapes. This research project investigates the use of native plants as a formal landscape element. As the world is becoming more urbanized (United Nations, 2010), gardens are becoming an increasingly important contributor to people's health and well-being (Dunnett & Qasim, 2000). The research has highlighted some elements that tend to affect visual preferences. This paper discusses a study conducted to determine preferences of Malaysian landscape professionals and students in landscape architecture and horticulture on two native ornamental plants, *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting), that are grown in soilless media with the potential for use in urban landscapes. Participants of this study comprised of landscape architects (20 respondents), architects (20), nursery owners (20), Bachelor of Horticulture students (80) (Faculty of Agriculture, UPM), and Bachelor of Landscape Architecture students (80 respondents) (Faculty of Design and Architecture, UPM), with a total of 220 respondents. Data collected were analyzed through descriptive analysis, Chi square and reliability test using SPSS. Results indicated that 88.2% of the respondents agreed that *Molineria latifolia* var. megacarpa (Lemba) could be a potential urban landscape plant, while 92.7% of them agreed that *Rhodomyrtus tomentosa* (Kemunting) could be domesticated, and is therefore a

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E-mail addresses:
sarahbaharudin85@gmail.com (Sarah, B.),
thohirah@upm.edu.my (Thohirah, L. A.),
musms@upm.edu.my (Mustafa Kamal, M. S.),
rosenani@upm.edu.my (Rosenani A. B.)
* Corresponding author

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potential urban landscape plant. Majority of the respondents (49% to 55%) preferred the plants grown individually, while others (40% to 49%) preferred both plants in the form of mass planting. Meanwhile, using the Likert's Scale, about half (50% to 52%) of the amateurs and professionals of the landscape field rated 4 (Like) for both the plants, whereas 10% to 15% of them marked 5 (Extremely Like) to show their acceptance towards the two new native plants. This finding indicates bright future for the two undomesticated wild native plants to be used as urban landscape plants. Thus, it is concluded that *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting) grown in soilless media have a high potential to become urban, native landscape plants.

Keywords: Landscape preferences, Native plants, Urban landscape plants

INTRODUCTION

The Council of Europe (2000) defines a landscape as "an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors" (Article 1); in parts of the world dominated by humans, landscape design can have significant environmental effects. The aggregate effects of private landscapes can influence habitat and water quality, among other environmental attributes. Nassauer (1993) has found that yards incorporating native plants can be as attractive, or even more attractive, to homeowners as conventional yards that do not include native plants. Gardens are the cumulative result of many individual decisions about plant choice over time that combine to determine the social and biophysical benefits provided.

Factors influencing the perception of landscape have been the subject of a great deal of research during the past 40 years. The research has highlighted some elements that tend to affect visual preferences. In general, natural landscapes are preferred over urban ones (Kaplan & Kaplan, 1989) in the urban areas as natural elements improve landscape quality (Matsuoka & Kaplan, 2008). Lyons (1983) analyzed the landscape preferences of subjects of different ages (children, adolescents, and adults) and concluded that culture plays a very important role in the perception of landscape. The perception of landscape tends to differ on the basis of social group, job type, familiarity, age, and other factors (Herzog *et al*., 2000; Kaltenborn & Bjerke, 2002).

Native plants are commonly restricted to informal or naturalistic designed landscapes. However, this study investigated the use of native plants as a formal landscape element. The goal of this study was to determine preferences of Malaysian landscape professionals and students in landscape architecture and horticulture for two native test plants, *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting) grown in soilless media. Currently, the two plant species are lesser known as landscape plants in the urban areas. The use of native plants is highly encouraged to create sustainable

urban landscapes. Positive preferences for these plants will add to the palette of existing native plants that are available to landscape designers in the tropical regions of the world.

MATERIALS AND METHOD

Test Plants

a. Rhodomyrtus tomentosa (Kemunting)

Rhodomyrtus tomentosa is a flowering plant from the family Myrtaceae, which is native to southern and south eastern Asia, east southern of China, Taiwan, the Philippines, as well as south Malaysia and Sulawesi. It grows in coastal areas, natural forests, riparian zones, wetlands, as well as moist and wet forests that are from sea level up to 2400 m elevation (Flora of China Editorial Committee, 2007).

Botanical Description

It is an evergreen shrub growing up to 4 m tall. The leaves are opposite, leathery, 5-7 cm long and 2-3.5 cm broad, three-veined from the base, oval, obtuse to sharp pointed at the tip, glossy green above, densely grey or rarely yellowish, hairy beneath, with wide petiole and entire margin. The flowers are solitary or in clusters of two or three, with about 2.5-3 cm in diameter and have five petals which are tinged white outside with purplish-pink or all pink. Fruit is a globose, few-seeded berry to 1.3 cm (0.5 in) across, dark purple and with sweet, aromatic flesh. Its edible fruit is about 10-15 mm in length, which is round and purple with three or four-celled and capped with persistent soft calyx lobes, with 40-45 seeds in a double row in each cell; seed dispersal is by frugivorous birds and mammals (Long & Lakela, 1971). Meanwhile, seed production and germination rates are high. It is a very showy shrub when in bloom and the prospects for its use as an ornamental plant are better than for its role as a fruit crop (Latiff, 1992).

b. Molineria latifolia var. megacarpa. (Lemba)

Botanical Description

Molineria latifolia var. megacarpa is from the Hypoxidaceae family. It is a herbaceous plant that grows in a relatively large group. It has simple leaves that are green, hard or strong, and oval. The shape of the leave is oblong and with 30 – 100 cm x 5cm in length and width. They have numerous parallel primary veins. The leafstalks are about one-third the length of its leaves, overlapping with one another at their bases to form a thick stem. The leaves are very tough, thin and broad (Keng, 1983). Inflorescence is ovoid to cylindrical, compact, 2-6 cm x 2-6 cm, bracts 1 - 6cm long, has green colour and white yellow or dark pink petals, fruit ovoid, and white to green tiny seeds and sweet (Shaari, 2005).

Cultivation and Management

This plant is cultivated through vegetative propagation. Cutting propagation utilizes a portion of the stem, root, or leaf that is cut from the parent or stock plant and induced to form roots and shoots by

chemical, mechanical, and environmental manipulation (Hudson, *et al.*, 2002).

Case Study Area

The study was conducted through a survey at nurseries located in the Klang Valley and Muar, Johor. Two nurseries chosen were Sungai Buloh, Selangor plant nurseries as well as Parit Jawa and Parit Sulung plant nurseries in Muar, Johor. Sungai Buloh was chosen because it has the most number of nurseries that are centralized in one area, i.e. in the Klang Valley. It is also a centre for plant-shopping to growers, garden-lovers and many home-owners around the Klang Valley, while nurseries in Muar area are selected due to their reputation as plant exporters to Singapore and they are also specializing in mass planting of landscape plants and are suppliers to many municipal councils and landscape companies throughout Malaysia.

The survey was conducted in architect and landscape architect firms in Petaling Jaya, Shah Alam, Subang Jaya and Kuala Lumpur. The two professions are considered as the professionals of their field, and thus, surveys were carried out to get their professional opinions on the two new, native and potential test plants.

The survey was also conducted with students of Bachelor of Horticulture Science at the Faculty of Agriculture and students of Bachelor of Landscape Architecture at the Faculty of Design and Architecture, Universiti Putra Malaysia, Serdang, Selangor as they have relevant background, exposure and knowledge on plant botanical perspectives and plant aesthetical values.

Survey Design

Pictorial Stimuli

A photo-questionnaire with photographs of *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting) was utilized in the survey. Over the last 20 years, there have been numerous developments in visualization tools, design processes and techniques that assess landscape preferences (Wherrett, 1999; Yamashita, 2002). In addition to other methods such as onsite surveys or slide projection, the use of photos in landscape preference studies has become generalized. The use of photos is extremely appealing as they show landscape in a holistic way (Hagerhall, 2001); photos also provide visual stimuli that closely assemble the real-life experience of the landscape. The use of photos is generally favoured because they enable larger samples of observers and judgments made based photo surveys are close (with a correlation of 80% or more) to those from on-site surveys (Natori & Chenoweth, 2008). Therefore, the assumption can be made that photos are capable of providing stimuli that enable the mind to associate sensory information with other knowledge and thus form opinions about what is perceived through intuitive recognition of an aesthetic quality (Bell, 2001).

Questionnaire

The questionnaire consisted of 13 items, in which the respondents were asked about their understanding of native plants and familiarity with the two plants, preferences for the different parts of the plants, as well as functional and economic values of the plants. In addition, the questionnaire also elicited the demographic information of the respondents such as age, gender and profession. Responses were either rated by using the Likert-like scale (1 = Extremely unlike to 5 = Extremely like) or by ticking the most appropriate answers.

Target Groups

The target groups in this survey are landscape architects (20 respondents), architects (20 respondents), nursery owners (20 respondents), horticulture students (80 respondents), landscape architecture students (80 respondents), making it a total of 220 respondents (n=220).

Survey Procedure

This survey method (pair-wise comparison) had previously been used to the study of relationships between landscape preferences and personal factors (Ruiz & Bernaldez, 1983; Abello & Bernaldez, 1986). According to the previous literature cited, this method presents three main advantages: the possibility of using a great number of photographs, simple and fast on-site application, and its methodological approach is based on the exploration of preference differences or contrasts among parts of the studied population. Presented with a collection of 50 mm x 60 mm photos of test plants in an album or power point presentation, the respondents were asked to complete the questionnaire based on the pictorial stimuli.

The survey was conducted in four months, i.e. from August to December 2012. Three methods were applied during the survey. First, emails consisting of the questionnaire and pictorial stimuli in Microsoft Presentation format were sent with the intention to make the survey process easier, faster and paperless. It is also technology savvy and pictorial stimuli materials can be viewed very clearly by the target groups. This method was applied to Architect and landscape architects.

The second method was to interview respondents in the target groups and to get them answer the questions based on the printed pictorial stimuli. The survey was done within one interview session with the nursery owners. As the nurseries are centralized, the process was done much faster and it was easier to achieve the maximum number of target groups. This method was mainly applied to nursery owners, with one session done in Sungai Buloh, Selangor and another session in Muar, Johor. All the nursery owners participated and responded positively to the questions forwarded to them and they were also willing to spare some of their time to partake in the survey.

The last method made use of survey that was done through answering session in a class where the target groups were gathered in a room and they were provided

with the questionnaires with pictorial stimuli projected on white screen. The respondents answered based on the pictures of the two native test plants *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting). This survey was conducted in 4 different sessions of 4 different cohorts.

Session one was done with the first-year students of Bachelor of Horticulture Science, while the second session involved the third-year students of Bachelor of Horticulture Science from the Faculty of Agriculture. The third session was conducted with the first-year students of Bachelor of Landscape Architecture and the final session involved the final year students of Bachelor of Landscape Architecture from the Faculty of Design and Architecture, Universiti Putra Malaysia, Serdang, Selangor. The sessions were carried out on different days. The students involved indicated their willingness to participate in the survey and to answer the questionnaire with some help from their lecturers and tutors (see Fig.1 – Fig.9).

Data Analysis

Variables and Statistical Method

Results from the survey were analyzed using Reliability test, Descriptive analysis and Chi Square using SPSS Version 16 Equinox. Frequency and descriptive statistics were employed to describe the demographic characteristics of the respondents. Analysis of these data indicated that a wide cross section of the respondents responded to the questionnaire.

The responses were subsequently quantified and analyzed using the SPSS software package. Qualitative data coding was conducted in an inductive manner or with no predetermined categories but they were defined on the basis of the survey results, with key response themes identified

Fig.1: Whole plant of *Rhodomyrtus tomentosa* (a)

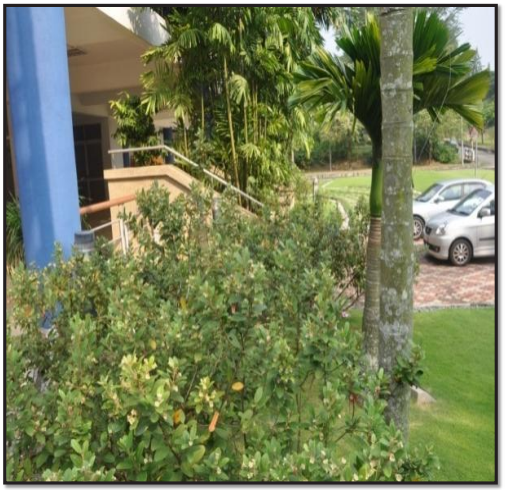

Fig.2: Whole plant of *Rhodomyrtus tomentosa* (b)

Fig.3: *Rhodomyrtus tomentosa* Flowers (a)

Fig.4: *Rhodomyrtus tomentosa* Flowers (b)

Fig.5: *Rhodomyrtus tomentosa* Buds (a)

Fig.6: *Rhodomyrtus tomentosa* Buds (b)

Fig.7: *Rhodomyrtus tomentosa* Fruits (a)

Fig.8: *Rhodomyrtus tomentosa* Fruits (b)

Fig.9: *Rhodomyrtus tomentosa* Fruit (c)

from the open-ended descriptions given by respondents. Descriptive statistics revealed different trends depending on the parameters.

Reliability Test

Reliability test is an indicator of consistency which shows how stable a test score or data is across applications or time. In this study, the Cronbach's Alpha was used to measure how closely related a set of items are as a group. The lower the errors caused, the higher the reliability of the instrument would be (Kumar, 1999). Therefore, any Cronbach's Alpha value that is greater than 0.60 indicates consistency among the theories. The Cronbach's Alpha value for the respondents' opinion towards *Molineria latifolia* var. megacarpa was 0.708, and this means there is consistency among the model fit for this study (Table 1).

Table shows the reliability test for R*hodomyrtus tomentosa* (Kemunting). The Cronbach's Alpha value for the respondents' opinion towards R*hodomyrtus tomentosa* (Kemunting) is 0.714, indicating the consistency among the theories (Table 1).

TABLE 1
Reliability Test for *Molineria latifolia* var. *megacarpa* (Lemba) and *Rhodomyrtus tomentosa* (Kemunting)

Test Plant	Cronbach's Alpha	N of Items
Molineria latifolia var. megacarpa (Lemba)	0.708	6
Rhodomyrtus tomentosa (Kemunting)	0.714	6

RESULTS AND DISCUSSION

Descriptive Analysis

Yu (1995) found living environment (urban or rural) and education level could significantly affect landscape preferences. Landscape preferences have also been found to differ with age (Balling & Falk, 1982; Lyons, 1983; Zube *et al*., 1983). In particular, it has been shown that the preferences of

children can vary significantly to that of adults. The above table shows the socio-demographic profile of the respondents in the target groups. There were 58.2% female respondents as compared to only 41.8% males for *Molineria latifolia* var. megacarpa (Lemba) and 33.6% male and 66.4% female respondents for the R*hodomyrtus tomentosa* (*K*emunting) survey. The ratio of the female respondents is slightly more compared to that of the male respondents. Age-wise, most of the respondents are between the age of 21-25 year old, with 34.5% and 40%, respectively.

This age category consists of Bachelor of Horticulture Science or Landscape Architecture students as the respondents. They were given pictorial stimuli to be evaluated based on their perception and preferences of the respective native test plants. Meanwhile, the other age groups (26 to 40 year old and above 40 years) comprised of students, nursery owners, architects and landscape architects. As mentioned before, the largest respondent group of this survey comprised of Bachelor of Horticulture or Landscape Architecture or Agriculture students of Universiti Putra Malaysia, with 49.1% for *Molineria latifolia* var. megacarpa (Lemba) and 45.4% for R*hodomyrtus tomentosa* (*K*emunting), followed by nursery owners in the Klang Valley area as well as in Muar, Johor, with 26.4% and 27.3% respectively (Table 2).

Ranking of Preferences

According to the ranking made by the respondents, the part of the plants that they found most attractive for *Molineria latifolia* var. megacarpa (Lemba) was the leaves (80.9%), while many others (88.2%) agreed that the flowers of R*hodomyrtus tomentosa* (Kemunting) were the most attractive part of the plant. This is quite obvious because

TABLE 2
Socio-demographic profile of the respondents (n=220)

	***Molineria latifolia* var. megacarpa (%)**	***Rhodomyrtus tomentosa* (%)**
Gender		
Male	41.8	33.6
Female	58.2	66.4
Age		
19-20	28.2	21.8
21-25	34.5	40.0
26-40	16.4	13.6
above 40	20.9	24.5
Category		
Landscape architect	14.5	18.2
Architect	10.0	9.1
Nursery owners	26.4	27.3
Landscape architecture /Horticulture student	49.1	45.4

the flowers of R*hodomyrtus tomentosa* (kemunting) are more attractive as opposed to those of the *Molineria latifolia* var. megacarpa (Lemba) which grew at the basal stem of the plant, making it harder to see them (see Fig.10 - Fig.14).

Most of the respondents (64.5%) agreed that *Molineria latifolia* var. megacarpa (Lemba) is mostly suitable as a potted plant, while the majority of the respondents (58.2%) agreed that R*hodomyrtus tomentosa* (Kemunting) is best planted as border planting plant. Meanwhile, 78%-88% of the respondents also agreed that both the plants have high aesthetic values, and 52%-62% others agreed that both plants have commercial values. These findings depicted that the two test plants are high in value and have the potential as landscape plants.

This research is based on Nassauer's past research (1992, 1993, 2004) on the cultural sustainability of ecological design. According to this theory, ecologically landscape designs that also are valued for their appearance are more likely to exist over the long term in a human-dominated landscape. Using digital simulations depicting residential landscapes with varying degrees of these characteristics, Nassauer (1993) found that "care," "neatness," and "naturalness" were significant predictors for the attractiveness of landscape designs, some of which included native plants in residential and urban yards (see Tables 3-5).

Fig.10 : *Molineria latifolia* var. *megacarpa* Whole plant (a)

Fig.11 : *Molineria latifolia* var. megacarpa Whole plant (b)

Fig.12 : *Molineria latifolia* var. megacarpa Flower (grown on basal stem) (a)

Fig13: *Molineria latifolia* var. megacarpa Flower (grown on basal stem) (b)

Fig.14: *Molineria latifolia* var. megacarpa Fruit

TABLE 3
Ranking of Preference - Which part of the plant you found most attractive?

	Molineria latifolia var. megacarpa (%)	*Rhodomyrtus tomentosa* (%)
Leaves	**80.9**	19.1
Flower	39.1	**88.2**
Stem	0.9	1.8
Fruits	6.4	14.5

TABLE 4
Ranking of Preference - In your opinion, this plant is most suitably planted as:

	Molineria latifolia var. megacarpa (%)	*Rhodomyrtus tomentosa* (%)
Potted plant	**64.5**	54.5
Ground cover plant	25.5	21.8
Indoor plant	55.5	13.6
Border planting plant	26.4	**58.2**
Other: The ground itself	0.9	0

TABLE 5
Ranking of Preference - What do you think are the prominent values of the plants?

	Molineria latifolia var. megacarpa (%)	*Rhodomyrtus tomentosa* (%)
Aesthetic values	**78.2**	**88.2**
Commercial values	61.8	52.7
Medicinal values	18.2	7.3
Edible values	20.0	24.5
Other	0	1.8

Chi Square Results

Hypothesis

Analysis of Chi Square was done using SPSS Equinox version 16.0 and the results depicted the relationship between respondents' demographic profile and a few variables with the following hypotheses:

H_o: There is no relationship between the public's perception on this plant and socio-demographic profiles of consumers such as gender, age and category.

H_1: There is relationship between the public's perception on this plant and socio-demographic profiles of consumers such as gender, age and category.

Landscape aesthetics is a complex issue, the basis of which can be found in human biological make-up and cultural experience (Appleton, 1975; Kaplan, 1987; Bourassa, 1990). The influence of individuals' personalities has been suggested as another important factor in understanding landscape aesthetics. The first analysis was the relationship between demographic profile and respondent's knowledge of *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa (*Kemunting). The only variable that failed to accept H_o was the profession variable of the respondents. This finding showed that the amateurs, i.e. degree students have less knowledge about the plants as compared to the professionals in the field.

Meanwhile, the relationship between demographic profile and respondents' perception towards both the plants also showed that the variable of profession had failed to accept H_o, and this was particularly due to the landscape architects' perception of the test plants. In more specific, the landscape architects were found to be rather sceptical about the ability of the test plants to be used as urban landscape plants. This is due to the growth of the plants or the probability of customers buying this new test plants or the issue of familiarity or knowledge of the plants which remains arguable.

However, the relationship between respondents' demographic profile and suitability of the plants as urban landscape plants provides different feedback for *Molineria latifolia* var. megacarpa (Lemba). two variables, namely gender and profession, failed to accept H_o. The female respondents showed lower percentage for using *Molineria latifolia* var. megacarpa (Lemba) as a landscape plant and this was most likely due to the lack of aesthetic value on the flowers as compared to the leaves as they are big. As for the profession category, nursery owners did not seem to agree that *Molineria latifolia* var. megacarpa (Lemba) had the potential to be used as urban landscape plant in the near future, with more or less the same reason, i.e. lacking commercial values as compared to *Rhodomyrtus tomentosa* (Kemunting). Based on this finding, it could be concluded that *Molineria latifolia* var. megacarpa (Lemba) is not popular amongst landscape

plant growers and nursery owners are also not interested in selling this particular plant. Thus, there is a lack of supply for this native test plant in the landscape plants market.

For *Rhodomyrtus tomentosa* (Kemunting), those who did not accept H_o involved those who are landscape architects. In particular, they did not agree that this plant is suitable for urban landscaping and the reason was most likely due to the probability of customers buying this new test plant or the issue of familiarity or knowledge of the new plants itself. In specific, 3 out of 20 respondents made a remark that the plant was hard to shape and did not look bushy enough.

Different respondents, who were grouped according to their activities, experiences, attitudes and behaviour, would have different preferences for landscaping. The differences were partly explained by varying levels of knowledge regarding the landscapes under examination. Darmstadt *et al*. (2006) also found that different groups of people (e.g., students and locals) would often have very different landscape preferences and argued that the differences underlined the need for care when interpreting indicator values (see Table 6-8).

CONCLUSION

Based on the descriptive analysis and Chi square results presented, it could be concluded that the two native test plants, namely *Molineria latifolia* var. megacarpa (Lemba) and *Rhodomyrtus tomentosa* (Kemunting), have high possibilities to be used as urban landscape plants. Amateurs and professionals in the landscape field both responded well to the test plants. Both the plants have good visual and aesthetical values and they may also have high commercial values. The plants are easy to propagate and have low maintenance, making them good and suitable candidates for urban landscape plants. The two native plants should be domesticated and widely propagated and popularized by nursery and landscape architect companies nationwide.

TABLE 6
Relationship between Demographic Profile and Respondent's Knowledge of (a) & (b)

a. *Molineria latifolia* var. megacarpa (Lemba)

Variables	Chi-square	Significant	Decision
Gender	2.277	0.131	Fail to Reject H_0
Age	4.005	0.261	Fail to Reject H_0
Category (Horticulture student)	9.305	0.097*	Reject H_0

b. *Rhodomyrtus tomentosa* (Kemunting)

Variables	Chi-square	Significant	Decision
Gender	0.125	0.723	Fail to Reject H_0
Age	3.748	0.290	Fail to Reject H_0
Category (Horticulture student)	19.760	0.001***	Reject H_0

***Statistically significant at 0.01 level, **at 0.05 level, and *at 0.10 level

TABLE 7
Relationship between Demographic Profile and Respondents' Perception on Test Plant (a)(b);

a. Molineria latifolia var. megacarpa (Lemba)

Variables	Chi-square	Significant	Decision
Gender	3.824	0.430	Fail to Reject H_0
Age	8.030	0.783	Fail to Reject H_0
Category (Landscape Architecture)	28.630	0.095*	Reject H_0

b. Rhodomyrtus tomentosa (Kemunting)

Variables	Chi-square	Significant	Decision
Gender	4.201	0.380	Fail to Reject H_0
Age	13.823	0.312	Fail to Reject H_0
Category (Landscape architect)	29.809	0.073*	Reject H_0

***Statistically significant at 0.01 level, **at 0.05 level and *at 0.10 level

TABLE 8
Relationship between Demographic Profile and Plant's Suitability as Urban Landscape Plant (a)(b);

a. Molineria latifolia var. megacarpa (Lemba)

Variables	Chi-square	Significant	Decision
Gender (Female)	4.234	0.040**	Reject H_0
Age	5.621	0.132	Fail to Reject H_0
Category (nursery owners)	9.760	0.082*	Reject H_0

b. Rhodomyrtus tomentosa (Kemunting)

Variables	Chi-square	Significant	Decision
Gender	1.727	0.189	Fail to Reject H_0
Age	4.672	0.197	Fail to Reject H_0
Category (Landscape architecture)	29.762	0.000***	Reject H_0

***Statistically significant at 0.01 level, **at 0.05 level and *at 0.10 level

REFERENCES

Abello, R. P., & Bernaldez, F. G. (1986). Landscape preferences and personality. *Landscape Urban Plann.,* 13, 19-28.

Appleton, J. (1975). *The Experience of Landscape*. John Wiley, London.

Balling, J. D., & Falk, J. H. (1982). Development of visual preference for natural environments. *Environment and Behavior, 14*, 5–28.

Bell, S. (2001), Landscape pattern, perception and visualisation in the visual management of forests. *Original Research Article Landscape and Urban Planning, 54*(1–4), 201-211.

Bourassa, S. C. (1990). A paradigm for landscape aesthetics. *Environ. Behav., 22*, 787–812.

Council of Europe (2000). European landscape convention. Retrieved from http://conventions.coe.int/Treaty/en/Treaties/Html/176.htm

Dunnett, N., & Qasim, M. (2000). Perceived benefits to human well-being of urban gardens. *HortTechnology, 10*(1), 40–45.

Flora of China Editorial Committee (2007). *Fl. China, 13*, 1–548. Science Press & Missouri Botanical Garden Press, Beijing & St. Louis. Retrieved from http://www.tropicos.org/Reference/1031194

Hagerhall, C. M. (2001). Consensus in Landscape Preference Judgements. *Original Research Article Journal of Environmental Psychology, 21*(1), 83-92.

Herzog, T. R., Herbert, E. J., Kaplan, R., & Crooks, C. L. (2000). Cultural and developmental comparisons of landscape perceptions and preferences. *Environ. Behav., 32*, 323–346.

Hudson, J. W. (2002). *Response to Climate Variability in the Livestock Sector in the North-West Province, South Africa.* M.A. thesis. Colorado State University, Fort Collins, Colorado.

Kaltenborn, B. P., & Bjerke, T. (2002). Associations between environmental value orientations and landscape preferences. *Landscape and Urban Planning, 59*, 1–11.

Kaplan, S. (1987). Aesthetics, affect and cognition; environmental preference from an evolutionary perspective. *Environ. Behav., 19*, 3-32.

Kaplan, R., & Kaplan, S. (1989). *The Experience of Nature*. Cambridge, UK: Cambridge University Press.

Keng, H. (1983). Annotated List of Seed Plants of Singapore (VIII), *36, Part 1 of Gardens Bulletin, Singapore.*

Kumar, U. D. (1999). Optimization models for recovery block schemes. *European Journal of Operational Research, 115*, 368-379.

Latiff, A. M. (1992). Rhodomyrtus tomentosa (Aiton) Hassk. In R.E. Coronel & E.W.M. Verheij (Eds.), *Plant Resources of South-East Asia*, No. 2 (pp. 276-277). Edible fruits and nuts. Prosea Foundation, Bogor, Indonesia.

Long, R. W., & Lakela, O. K. (1971). Fl. Trop. *Florida i–xvii, 1–962*. University of Miami Press, Coral Cables. Retrieved from http://www.tropicos.org/Reference/1506.

Lyons, E. (1983). Socio-economic correlates of landscape preference. *Environment and Behavior, 15*, 487–511.

Matsuoka, R. H., & Kaplan, R. (2008). People needs in the urban landscape: Analysis of Landscape and Urban Planning contributions. *Review Article Landscape and Urban Planning*, *84*(1), 7-19.

Nassauer, J. I., & Pitt, D. G. (1992). Virtual reality systems and research on the perception, simulation and presentation of environmental change. *Landscape L'rbanPlann., 2*(1), 269-271.

Nassauer, J. I. (1993). Ecological Function and the Perception of Suburban Residential Landscapes. In P.H. Gobster (Ed.), *Managing Urban and High Use Recreation Settings*. General Technical Report, USDA Forest Service North Central Forest Exp. Sta., St. Paul, MN.

Nassauer, J. I. (1997). Cultural sustainability: aligning aesthetics and ecology. In Nassauer, J.I. (Ed.), *Placing Nature: Culture and Landscape Ecology* (pp. 65–83). Washington, D.C.: Island Press.

Nassauer, J. I. (2004). Using normative scenarios in landscape ecology. *Landscape Ecology, 19*, 343-356.

Natori, Y., & Chenoweth, R. (2008). Differences in rural landscape perceptions and preferences between farmers and naturalists; Original Research Article. *Journal of Environmental Psychology, 28*(3), 250-267.

Ruiz, J. P., & BernBldez, F. G. (1983). Landscape perception by its traditional users. The ideal Landscape of Madrid livestock raisers. *Landscape Plann., 9,* 279-297.

Shaari, N. (2005). Lemba (*Curculigo latifolia)* Leaf as a New Materials for Textiles. Environmentally Conscious Design and Inverse Manufacturing. Eco Design 2005. *Fourth International Symposium*.

United Nations (2010). *World urbanization prospects: The 2009 Revision.* New York: United Nations Department of Economic and Social Affairs, Population Division.

Wherrett, J. R. (1999). Issues in using the Internet as a medium for landscape preference research. *Landscape and Urban Planning, 45*, 209-217.

Yamashita, S. (2002). Perception and evaluation of water in landscape: use of Photo-Projective Method to compare child and adult residents' perceptions of a Japanese river environment Original Research Article. *Landscape and Urban Planning*, *62*(1), 3-17.

Yu, K. (1995). Cultural variations in landscape preference: comparisons among Chinese sub-groups and Western design experts. *Landscape and Urban Planning, 32*, 107–126.

Zube, E. H., Pitt, D. G., & Evans, G. W. (1983). A lifespan developmental study of landscape assessment. *Journal of Environmental Psychology, 3*, 115–128.

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Timber use Practices in Malaysia's Construction Industry: Single-family Residential Building Sector

Mohamed, S.* and Abdullah, R.

Faculty of Forestry, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

The importance of domestic timber market in Malaysia is recognised with the launch of the National Timber Industry Plan in 2009, which calls for promoting and encouraging the use of timber products by the local construction industry. A study was conducted to provide information on the current use of timber products in the single-family residential building sector as it is one of the major development projects undertaken by the construction industry. In specific, the study aimed to identify the types and to estimate the amount of timber products used in the construction and those installed in the completed single-family residential building units. Data for the study were collected using a self-administered, open-ended questionnaire sent to constructions firms in Selangor and the Federal territory of Kula Lumpur advertising the sale of their residential building units in the local newspapers and the internet during the survey period. The respondents of the study were project managers or quantity surveyors who were involved directly in the supervision and monitoring of the residential projects constructed by the construction firms. The amount of sawn timber and plywood used for the construction of the residential units ranges from 0.05 to 0.07 cu. m/sq. m. and 0.01 to 0.07 cu. m./sq. m., respectively. The most common timber products installed in the completed residential units are wooden/timber doors. Other traditional timber-based products are still being used but they are continually replaced by other building materials such as aluminium, steel and glass. Efforts to promote timber products to the single-family residential building sector should target on their uses in the completed residential building units.

Keywords: Wood, timber products, residential units, substitution

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E-mail addresses:
shukri@upm.edu.my (Mohamed, S.),
rahimhpr@gmail.com (Abdullah, R.)
* Corresponding author

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INTRODUCTION

The timber industry in Malaysia caters timber products for not only the domestic but also the international markets. Despite being an important market outlet for timber products, most domestic markets including Malaysia's, generally receive less attention as they are almost always overshadowed by exports (Bourke, 1991). In 2008, for example, domestic consumption of primary timber products and furniture in Malaysia worth about RM7.6 billion, while export of timber and timber products was about RM22.5 billion (MPIC, 2009). However, the scenario in Malaysia was expected to change with the launch of the National Timber Industry Plan (NATIP) in 2009 which recognized the importance of the domestic timber market. NATIP calls for promoting and encouraging the use of timber products in the domestic market to sustain the growth of the country's timber industry (MPIC, 2009). The plan targets the construction industry as it has been the largest consumer of sawn timber, plywood and other wood-based panels in the country.

Even though Malaysia is a timber-rich country, the use of timber products in the construction industry is almost negligible (Wong, 2008). Jumaat *et al*. (2006b) lamented that the construction industry in Malaysia is not very keen in using the material. Various reasons such as poor and inconsistent quality, association with low social status and fire performance, as well as high and fluctuating cost of the material have been cited for the construction industry's disinterest in using timber products (Tan *et al*., 2005; Ismail *et al*., 2008). Similarly, the lack of consumer awareness on the availability of Malaysian timber species and products in the local market has also been cited for the continued use of imported timber or alternative materials such as plastic in the building and housing sectors (MPIC, 2009). The diminishing number of timber craftsmen was also another factor for the use concrete and masonry materials for residential buildings in rural communities in Malaysia (Ismail *et al*., 2008). The industry is also increasingly using other alternative materials such as bricks and concrete (Nor Haniza *et al*., 2007; Fujita *et al*., 2009; Abu Hassan *et al*., 2011). In 2008, for instance, timber products constitute only 8% of the total materials used by the Malaysian construction industry compared to 23% each for iron and steel, and cement and concrete (SEAISI, 2008).

There is, however, a general lack of detailed studies on the use of timber products not only in the Malaysian construction industry but also in other major timber product consuming sectors. A study was, thus, conducted to provide information on the use of timber products by the Malaysian construction industry, especially in the residential building sector as it is one of the major development projects undertaken by the construction industry. In more specific, the study aimed to identify the types and to estimate the amount of timber products used in the construction and those installed in the completed single-family residential building units. In 2011, about 28% of the total 5,555 projects awarded to the construction

industry were for residential building construction (CIDB, undated). This study focused on the use of timber products in the construction of single-family residential units by building construction firms; hence, it did not include units constructed by individual house owners. Single-family residential units include detached, semi-detached and terraced houses, in which each unit is separated by a ground-to-roof wall and where no other units are constructed above or below it. During the third quarter of 2011, about 63% of the 4.49 million residential units constructed in Malaysia were single-family residential units (NAPIC, 2011).

METHOD

Initial visits to four construction sites were done to identify the type of timber products used in the construction of single-family residential units. Other applications of timber product in the completed residential units were also identified. The information gathered from the visits was used to construct a questionnaire that was later employed in collecting the required data for the study. During the initial visits, the most appropriate persons to whom the questionnaire was to be sent were also ascertained.

The self-administered, open-ended questionnaire used in the study consists of two sections, which firstly collect the types, number, as well as the build-up area of each type of residential units the company is currently constructing. The second section of the questionnaire requires the respondents to provide information on the types and amount of timber products used in the construction, as well as those used in the completed units of each type of house constructed. The questionnaire had been pre-tested with the four residential building construction firms visited earlier for its clarity and ease of obtaining the data on the types and amount of timber and timber products used in the residential building construction projects. The main concern raised by the respondents during the pre-test was the data on the amount of timber products used in the construction of each type of residential unit, as well as those used in the completed units. As it was deemed difficult to request data for a single unit of house constructed, the questionnaire was modified to obtain data on the types and total amount of timber and timber products estimated for each type of housing units currently or would be constructed by the construction firms.

The respondents for the study were the project managers or the quantity surveyors who were directly involved in supervising and monitoring the residential projects by the construction firms during the survey period. The survey was conducted in Selangor and the Federal Territory of Kuala Lumpur as high residential building construction had been recorded at the two states, as well as because of other logistic reasons. Out of 1,567 residential projects awarded in 2011, about 39% were awarded in these two states (CIDB, undated). Those construction firms advertising their residential building units in the local newspapers and the internet were contacted for the study. The questionnaire

was distributed to 58 project managers/ quantity surveyors who had initially agreed to participate in the study.

RESULTS AND DISCUSSION

Types of Single-family Residential Units Constructed

Data from 17 fully-completed questionnaires were used in the analysis. The most common type of single-family residential unit constructed by the respondents' firms is the double-storey terraced houses, with an average floor area of about 186.27 sq. m., followed by semi-detached and bungalows, and single-storey terraced houses (Table 1). In the fourth quarter of 2011, 2 to 3-storey terraced houses formed about 51% of the single-family residential units constructed in the Federal Territories of Kuala Lumpur and Putrajaya and in Selangor (NAPIC, 2011). In fact, 2- to 3-storey terraced houses are the most common types of single-family residential unit being constructed nationwide (Erdayu *et al*., 2010).

Timber Products in Single Family Residential Units

Timber products are used both during the construction and for completed residential units. A majority of the respondents' firms used timber formworks, fabricated of sawn timber and plywood, for the construction of concrete structures of the units. This conventional construction method is a common practice in Malaysia where reinforced concrete frame and brick, beam, column, wall, and roof are cast *in situ* using these timber formworks (Lou & Kamar, 2012). Sawn timber is also used as props and scaffoldings. The usage rates of sawn timber and plywood for the construction of the different types of residential units are shown in Table 2. The value was obtained by dividing the total volume of timber products that was being or would be used in the construction of the residential units (in cu. m.) with the total floor area of the units that were or would be built (in sq. m.).

The amount of sawn timber and plywood used for the construction of the residential units ranged from 0.05 to 0.07 cu. m/sq. m. and 0.01 to 0.07 cu. m./sq. m., respectively. The study by Monerasinghe (1985) reported about 0.05 cu. m. of timber materials was required for the construction of a square meter floor area for all types of houses. Meanwhile, Fujita *et al*. (2009) reported a much higher timber usage rate of 0.29 cu. m./sq. m. for low-cost and single-storey terrace houses,

TABLE 1
Type and average floor area of single-family residential units constructed by the respondent firms

Type of residential unit	Number of units constructed	Number of projects	Average floor area (sq. m.)
Single-storey terraced	247	3	120.07
Double-storey terraced	2357	13	186.27
Semi-detached & bungalows	772	8	290.68

and 0.13 cu. m./sq. m. for 2- to 3-storey terraced, semi-detached and detached houses. These researchers, however, did not indicate whether both sawn timber and plywood were included in their calculations. Furthermore, the researchers did not provide further information pertaining to the method used in obtaining the amount of timber and timber products in the construction of the building units.

The common sizes of sawn timber used by the respondents' firms ranged from 25.4 mm x 50.8 mm to 50.8 mm x 101.6 mm without any general indication of species or grade preference, while the 12 mm thick plywood were commonly used during the construction of the residential units. The timber formworks, props and scaffoldings are used several times before they are disposed, which is due to damages during dismantling, as construction waste. Similarly, Lee *et al*. (2013) reported that timber formworks would be used at least three times before they were disposed. Wood materials are reported to be one common waste component generated by the construction industry in the country (Begum *et al*., 2006; Lee *et al*., 2013). However, the use of sawn timber and plywood in the construction of residential buildings is expected to continue in the future as the construction industry prefers this conventional construction method (Thanoon *et al*., 2003; Abdul Kadir *et al*., 2006).

In addition to being used during construction, timber products are also used in the completed residential units. Traditionally, these timber products included roof trusses and related members, doors and door frames, windows and window frames, decorative panels, skirting and flooring. Table 3 shows the various timber products used in the residential building projects surveyed in the study. The most common timber product found in all types of completed residential units surveyed in the study is doors. Solid timber doors are normally used for the main entrance, and sometimes for the master bedroom, while plywood flush doors are mainly used for other rooms. Nonetheless, there seems to be no standard number or dimensions for these doors as they vary from one residential building project to another.

The next common timber products are roof trusses and related members. Other timber products which are less commonly found in these completed residential units are door frames, fascia boards, stair parts

TABLE 2
Type and amount of timber products used in the construction of single-family residential units

Type of residential unit	Average floor area (sq. m.)	Usage rate (cu. m./sq. m.)	
		Sawn timber	Plywood
Single-storey terrace	120.07	0.07	0.01
Double-storey terrace	186.27	0.05	0.02
Semi-detached & bungalows	290.68	0.07	0.07

(especially handrails and post) and flooring. However, it is not possible to quantify the average amounts of the various timber products found in these residential units as there are variations in the units of measurements reported by the respondents even though they were requested to report using a common unit of measurement. Some reported that data were not available as these timber products were procured from their suppliers in assembled forms, and thus, they were not able to provide any details on the amount used in each completed residential unit. Roof trusses, for example, are normally prefabricated in the factories and transported to the construction sites (Jumaat *et al*., 2006a). In general, most of the respondents interviewed reported that timber products constituted about 5% to 10% of the total volume of the materials used in a completed single-family residential unit.

Substitution of Traditionally Used Timber-based Products in Residential Building Units

It is evident, as shown in Table 3, that timber products which were traditionally found in the completed residential units are being substituted with other materials. Wooden roof trusses are still being used in residential buildings, with a high percentage of the construction firms building semi-detached and bungalows now opted for steel roof trusses. Termite infestation, among others, has been cited as the reason for the construction industry's tendency to use steel roof trusses (Mahmood *et al*., 2005; Ngian *et al*., 2012).

In addition to the roof/ceiling, other common locations of termite infestation in residential buildings are door and window frames, as well as flooring and baseboard/ skirting (Lee, 2002). This has led the industry to substitute wooden door frames with those made of steel. It is more common now for houses to be fitted with casement windows with aluminium frames. While ceramic and porcelain tiles are replacing parquet flooring and skirting.

Cement boards and metal sheets are now being used by a number of residential building construction firms in replace of timber fascia boards. The most common reasons given by the respondents are the difficulty of obtaining straight, long pieces of timber and the tendency for timber fascia

TABLE 3
Timber products in the completed residential units

Type of timber product	Incidence - (% of number of projects)			
	Single-storey terrace	Double-storey terrace	Semi-detached & bungalows	Average
Doors	100	100	100	100
Roof trusses and members	100	62	25	62.3
Door frames	67	31	38	45.3
Fascia boards	33	46	25	34.7
Stair parts	-	31	38	23.0
Flooring	-	23	38	20.3

boards to rot as they are continuously exposed to the weather elements. Some timber products such as stair parts are replaced with stainless steel and glass as these new and modern materials have become easily available and well accepted by both residential building developers and buyers.

CONCLUSION

The most common type of single-family residential units constructed by the respondents' firms is the double-storey terraced houses, followed by semi-detached and bungalows, and single-storey terraced houses. Sawn timbers and plywood timbers are used in the construction of the single-family residential units but the usage rate is rather low. The most common timber products installed in the completed residential units are wooden/timber doors. Although other traditional timber-based products are still used, they are continually being replaced with other building materials such as aluminium, steel and glass.

REFERENCES

Abdul Kadir, M. R., Lee, W. P., Jaafar, M. S., Sapuan, S.M., & Ali, A. A. A. (2006). Construction performance comparison between conventional and industrialised building system in Malaysia. *Structural Survey, 24*, 412-424.

Abu Hassan, A. B., Mahyuddin, R., Mazlina, J., & Aulina, A. (2011). Awareness assessment framework for implementing the sustainable housing in Malaysia. *Asian Journal of Management Research, 1*, 703-713.

Begum, R. A., Siwar, C., Pereira, J. J., & Jaafar, A. H. (2006). A benefit–cost analysis on the economic feasibility of construction waste minimisation: The case of Malaysia. *Resources, Conservation and Recycling, 48*, 86-98.

Bourke, I. J. (1991). Domestic timber markets: important outlets for the developing countries. Retrieved from http://www.fao.org/DOCREP/U4200E/u4200e05.htm

CIDB. (undated). Number and value of projects awarded by category as of December 2011. Retrieved from http://www.cidb.gov.my/cidbweb/images/pdf/buletin/2011/BahagianKeduaQ42011.pdf

Erdayu, O. O., Esmawee, E., & Masran, S. (2010). Adapting by altering: Spatial modifications of terraced houses in the Klang Valley area. *Asian Journal of Environment-Behaviour Studies, 1*, 1-10.

Fujita, Y., Matsumoto, H., & Ho, C. S. (2009). Assessment of CO_2 emissions and resource sustainability for housing construction in Malaysia. *International Journal of Low-Carbon Technologies, 4*, 16-26.

Ismail, S., Abdul Malek, D., & Syed Ahmad Iskandar, S. A. (2008). A study of constructing timber architecture: Merging the skills of architect, carpenter and masonry workers. *Jurnal Alam Bina, 12*, 97-108.

Jumaat, M. Z., Rahim, A. H. A., Othman, J., & Midon, M. S. (2006a). Strength evaluation of oil palm stem trussed rafters. *Construction and Building Materials, 20*, 812-818.

Jumaat, M. Z., Rahim, A. H. A., Othman, J., & Razali, F. M. (2006b). Timber engineering research and education in Malaysia. In *9th World Conference on Timber Engineering* (pp. 2494-2497). Portland, OR., USA.

Lee, C. F., Ismail, A, R., Ade, A., Sasitharan, N., & Khairul. I. K. (2013). Classification and quantification of construction waste at housing project site. *International Journal of Zero Waste Generation, 1*, 1-4.

Lee, C. Y. (2002). Subterranean termite pests and their control in the urban environments in Malaysia. *Sociobiology, 40*, 3-9.

Lou, E. C. W., & Kamar, K. A. M. (2012). Industrialized Building Systems: Strategic outlook for manufactured construction in Malaysia. *Journal of Architectural Engineering, 18*, 69-74.

Mahmood, M. T., Thang, C. M., & Tan, C. S. (2005). Performance of locally produced cold-formed steel sections for roof truss system. *Jurnal Teknologi, 42*(B), 11-28.

Monerasinghe, M. N. (1985). Research needs and priorities in housing and construction in Malaysia. *Habitat International, 9*, 37-57.

MPIC. (2009). National Timber Industry Plan, 2009-2020. Ministry of Plantation Industries and Commodities, Malaysia.

NAPIC. (2011). Property Stock Report - Residential Property Stock Report Table Q4 2011. Retrieved from http://napic.jpph.gov.my/portal/content/Publication_PDF/q411residential.pdf

Ngian, S. P., Tahir, M.M., Siang, T. C., Hong, A. K. B., & Mohammad, S. (2012). Experimental investigation on locally produced cold-formed steel sections for roof truss system. *Advanced Science Letters, 13*, 620-623.

Nor Haniza, I., Zuraini, M.A., Yacob, O., & Helena, A. H. (2007). Case studies on timber defects of selected traditional houses in Malacca. *Journal of Design and the Built Environment, 3*, 81-90.

SEAISI. (2008). Construction sector in Malaysia Retrieved from www.seaisi.org/news/news_view.asp?news_id=688

Tan, Y. E., Ong, C. B., Khairul, A., & How. S. K. (2005). Use of laminated timber for trusses. *Master Builders Journal,* 3rd Quarter, 62.

Thanoon, W. A., Lee, W. P., Abdul Kadir, M. R., Jaafar, M. S., & Salit, M. S. (2003). The experiences of Malaysia and other countries in industrialised building system. In *International Conference on Industrialised Building Systems* (pp. 255-267). Kuala Lumpur, Malaysia.

Wong, T. M. (2008). Ensuring quality assurance in timber applications. *Master Builder Journal,* 1st Quarter, 84-87.

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Molecular Characterization of Fowl Adenoviruses Isolated from Inclusion Body Hepatitis Outbreaks in Commercial Broiler Chickens in Malaysia

Juliana, M. A.[1], Nurulfiza, I.[1]*, Hair-Bejo, M.[2,4], Omar, A. R.[2,4] and Aini, I.[3,4]

[1]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Laboratory of Vaccines and Immunotheraputics, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Fowl adenoviruses (FAdVs), belonging to the *Aviadenovirus* genus of the family *Adenoviridae*, have been classified into five species (A to E) and further divided into 12 serotypes. The objective of this study was to identify the serotype classification of five Malaysian FAdV isolates obtained from field outbreaks of IBH in commercial broiler chickens. Hexon-based polymerase chain reactions (PCR), combined with restriction enzyme analysis (REA), were applied. Viral DNA reacted positively with H1/H2 and H3/H4 primer pairs which hybridised to highly conserved regions of the hexon genes. The restriction enzyme profiles of the H1/H2 fragment digested with *HaeII* and the H3/H4 fragment digested with *Hpa*II revealed that all five isolates shared identical patterns and are characterised as being FAdV-8b, species E. Meanwhile, sequence analysis of the L1 loop region of the hexon gene revealed 98.1% identity with FAdV-8b strain 764. High bootstrap values in phylogenetic analysis supported the clustering of the Malaysian FAdV isolates into FAdV species E. The present study has provided a very useful reference for further studies of FAdVs in Malaysia. Vaccination strategies should be developed against FAdVs infection in commercial broiler chickens to prevent IBH outbreaks in the country.

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E-mail addresses:
akiera07@yahoo.com.my (Juliana, M. A.),
nurulfiza@upm.edu.my (Nurulfiza, I.),
mdhair@upm.edu.my (Hair-Bejo, M.),
aro@upm.edu.my (Omar, A. R.),
aiini@upm.edu.my (Aini, I.)
* Corresponding author

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INTRODUCTION

Fowl adenoviruses (FAdVs) are common infectious agents in poultry farms and are distributed worldwide. The *Aviadenovirus's* members are classified into five species (A to E) based upon their restriction enzyme fragment patterns, phylogenetic relationships, pathogenicity, cross-neutralization and recombinant potential (Zsák & Kisary, 1984; Virus Taxonomy, 2011). In particular, FAdVs consist of 12 serotypes with varying pathogenicities and can be isolated from both healthy and sick birds (McFerran *et al*., 1972). This is due to the presence of maternal antibodies and low virulence of some strains. Economically, these icosahedral viruses are mainly responsible for naturally acquired outbreaks of inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome, respiratory tract disease and gizzard erosions that have high economic impacts (Alvarado *et al*., 200; Grime *et al*., 1977; Nakamura *et al*., 1999; Mase *et al*., 2009; Ono *et al*., 2004).

There are several methods that have been routinely employed for the diagnosis of FAdVs including isolation in cell culture, immunodiffusion test, immunofluorescence techniques, enzyme-linked immunosorbent assay (ELISA) and electron microscopy (Balamurugan & Kataria, 2004; Cowen *et al*., 1978). However, despite their time-consuming and expensive nature, these techniques are only of minor relevance due to the widespread occurrence of antibodies to avian adenoviruses. In recent years, several methods based on the polymerase chain reaction (PCR), combined with restriction enzyme analysis (REA), have been developed and proven to be not only rapid but more sensitive and specific for the detection of group 1 avian adenovirus (Ganesh *et al*., 2002; Hess, 2000; Jiang *et al*., 1999; Raue & Hess,1998; Singh *et al*., 2002).

Furthermore, variable regions that exist in the loop regions of the hexon protein, specifically the L1 and L2 loops (Crawford-Miksza & Schnurr, 1996) had been manipulated to generate a more precise evolutionary profile compared to the restriction profiles of the whole genome. These regions of the hexon gene consisting of seven hypervariable regions in mastadenovirus have been shown through neutralization test to have type-specific domains (Toogood *et al*., 1992). Previous studies used L1 loop sequences for the generation of a phylogenetic tree of FAdVs owing to this loops greater variability when compared to the L2 loop in FAdV (Meulemans *et al*., 2004). The L1 loop was also found to be the longest and most complex loop in mastadenovirus.

In an endeavour to identify and characterise FAdV serotypes associated with IBH outbreaks in Malaysia, hexon-based PCR and REA, as described by Raue and Hess (1998), was employed to test its usefulness for the detection of our isolates,

followed by the analysis of the nucleotide sequences and the phylogenetic clustering of the isolates based on the L1 loop region.

MATERIALS AND METHODS

Viruses

The FAdVs isolates (designated as UPM04217, UPM08158, UPM08136, UPM11142 and UPM11134) used in this study were obtained from the collection maintained by the Faculty of Veterinary Medicine, Universiti Putra Malaysia. They were isolated previously from IBH field outbreaks in commercial broiler chickens from different states in Malaysia in 2004, 2008 and 2011. Samples in the form of CAM (UPM04217, UPM08158) and liver (UPM08136, UPM11142 and UPM11134) tissues were frozen and thawed three times before being macerated with a sterile mortar and pestled to prepare a 1 in 2 (w/v) suspension in sterile phosphate buffer saline (PBS; pH 7.4, 0.1 M). The suspension was centrifuged at 3000 rpm for 30 minutes for clarification. The collected supernatant was filtered through a 0.45-µm filter and treated with a commercial antibiotic and antimycotic preparation (GIBCO Laboratories, New York, USA) at a 1 in 10 (v/v) dilution and incubated at 4°C for 1 hour prior to inoculation. All the isolates were then inoculated into 10-day-old SPF embryonated chicken eggs via the chorioallantoic membrane (CAM) route following a standard procedure (Dagmar & Becht, 1975). The CAM and embryonic liver from dead embryos were harvested under sterile conditions.

Viral DNA Extraction

The salting-out DNA extraction method was used with some modifications (Mirmomeni *et al*., 2010). DNA was extracted from 500 µl homogenates of CAM (UPM04217 and UPM08158) and liver (UPM08136, UPM11142 and UPM11134). Aliquots of 50-70 µl of 10% sodium dodecyl sulfate (SDS) and 1 µl of proteinase K were added into each tube, which were then vortexed. The tubes were placed into a warm water bath (65°C) for 30 minutes and shaken every minute. After keeping the cells in a freezer (-20°C) for 5 minutes, 5M ammonium acetate was added into each tube, and the mixture was then vortexed. The cell debris was pelleted and 850 µl of supernatant was transferred into a new Eppendorf tube. Ice-cold isopropanol (700 µl) was added into each tube and it was inverted 30-40 times. The DNA was pelleted and purified by two ethanol washes. The pellets were dried and resuspended in ddH_20 and the resultant DNA extract was qualitatively checked and quantified at 260/280 nm using a spectrophotometer (Beckman, USA).

PCR and Restriction Enzyme Analysis

DNA amplification was carried out using H1/H2 and H3/H4 primer sets (Raue & Hess, 1998). The PCR products were then separated by using electrophoresis in 1% agarose gel (Promega, USA) at 75 V for 50 minutes, stained with ethidium bromide and visualised through UV transillumination. PCR fragments amplified by the H1/H2 and H3/H4 primer sets were cleaved by *Hae*II and *Hpa*II restriction enzyme

respectively according to the manufacturer's recommendations (Fermentas, Life Sciences). The *Hae*II cleavage products were separated by electrophoresis in 1% agarose, 80 V for 45 minutes while the *Hpa*II cleavage products were separated in 3% agarose gel at 88 V for 75 minutes followed by ethidium bromide staining and visualisation by UV transillumination. The profiles created by restriction enzyme digestion were compared with those of the FAdV 1-12 reference strains (Raue & Hess, 1998).

Nucleotide Sequencing and Phylogenetic Analysis of the Hexon Genes

The PCR products for H1/H2 of each isolate containing the loop 1 and loop 2 regions were cloned into the pCR®2.1-TOPO® vector using the TOPO TA Cloning kit (Invitrogen, USA). Then, plasmid from the positive colonies were extracted and purified using GeneAll® Exprep™ Plasmid Quick (Generabiosystems, Australia) and subjected to DNA sequencing at least three times for each isolate on an automatic sequencer (ABI PRISM 377 DNA) using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer) to get a precise consensus sequence. The sequences were assembled, edited, and analyzed using the BioEdit version 7.0.9 package and translated using ExPASy Proteomic server available online.

Twenty-nine available FAdVs hexon sequences representing each serotype were retrieved online from GenBank including 11 sequences published by Meulemans (2004) (Table 1). The nucleotide and deduced amino acid sequences were aligned and compared using BioEdit™ version 7.0.9, GeneDoc version 2.7.000 and Clustal-X™ version 2.0.12. For the generation of a phylogenetic tree, a segment of 198 amino acids was selected to correspond to residues 101 to 298 of the reference strain HG (Steer *et al.*, 2011). MEGA software version 5 was used to compute the distance matrix using the Jones-Taylor-Thorton (JTT) model and subsequently used to generate a phylogenetic tree using the neighbour-joining (NJ) method with 1000 bootstrap replicates.

GenBank Accession Numbers

The sequences were submitted to GenBank and the accession numbers assigned were: UPM04217 [GenBank: JF917237], UPM08158 [GenBank: JF917238] and UPM08316 [GenBank: JF917239].

RESULTS AND DISCUSSION

The main structural component of the capsid of fowl adenovirus is the hexon, which plays important roles in establishing immune responses (Russel, 2009). The hexon determines the type, group and subgroup antigenic determinants of fowl adenovirus (Norrby, 1969) and has been proven to be very useful in serotype identification. Therefore, several classification methods have been developed recently based on the hexon region for FAdV typing (Marek *et al*., 2010; Meulemans *et al*., 2001; Steer *et al*., 2011).

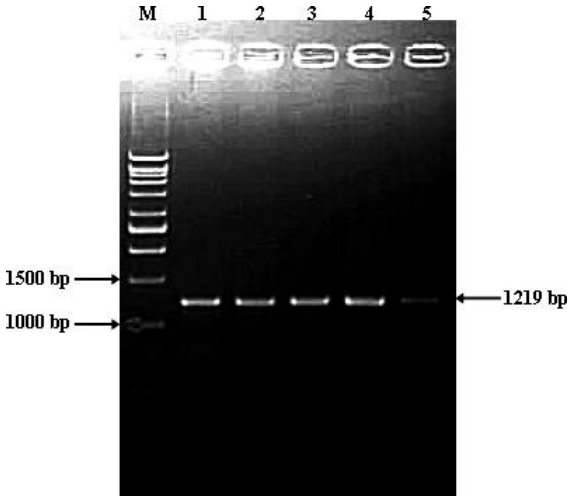
TABLE 1
FAdV hexon genes used in the sequence and phylogenetic analysis

No	Strain	Accession number	Reference
1	CELO	AF339914	Meulemans *et al*., 2001
2	340	AF508952	Meulemans *et al*., 2004
3	IBH-2A	AF339916	Meulemans *et al*., 2001
4	TR22	AF508953	Meulemans *et al*., 2004
5	506	AF508950	Meulemans *et al*., 2004
6	J-2A	AF339917	Meulemans *et al*., 2001
7	KR5	AF508951	Meulemans *et al*., 2004
8	C2B	AF339923	Meulemans *et al*., 2001
9	685	AF508947	Meulemans *et al*., 2004
10	SR48	AF508946	Meulemans *et al*., 2004
11	75	AF508949	Meulemans *et al*., 2004
12	75-1A-1	AF339921	Meulemans *et al*., 2001
13	SR49	AF508948	Meulemans *et al*., 2004
14	A2-A	AF339918	Meulemans *et al*., 2001
15	380	AF339925	Meulemans *et al*., 2001
16	CR119	AF508954	Meulemans *et al*., 2004
17	X11	AF339920	Meulemans *et al*., 2001
18	X11A	AF339924	Meulemans *et al*., 2001
19	YR36	AF508955	Meulemans *et al*., 2004
20	58	AF508957	Meulemans *et al*., 2004
21	TR59	AF508956	Meulemans *et al*., 2004
22	T8-A	AF339919	Meulemans *et al*., 2001
23	764	AF508958	Meulemans *et al*., 2004
24	B-3A	AF339922	Meulemans *et al*., 2001
25	430-06	GU120266	Steer *et al*., 2011
26	607-06	GU120267	Steer *et al*., 2011
27	Australian FAdV Vaccine	GU120268	Steer *et al*., 2011
28	Stanford	DQ323986	Alvorado *et al*., 2007
29	HG	GU734104	Grgić *et al*., 2011

The present study describes the molecular characterisation of five Malaysian isolates of FAdVs that were associated with field outbreaks of IBH. Among the five isolates, only UPM04217 was studied in detail. Meanwhile, UPM04217 had been previously isolated (Hair-Bejo, 2005) from a commercial broiler farm in Perak and identified as FAdV by electron microscopy (Alemnesh *et al*., 2012) and other molecular techniques (Jason *et al*., 2008). This isolate caused 100% mortality in SPF embryonated chicken eggs but showed low pathogenicity to 9-day-old SPF chicks since no clinical signs, mortality and gross lesions were found. This finding led to the present study

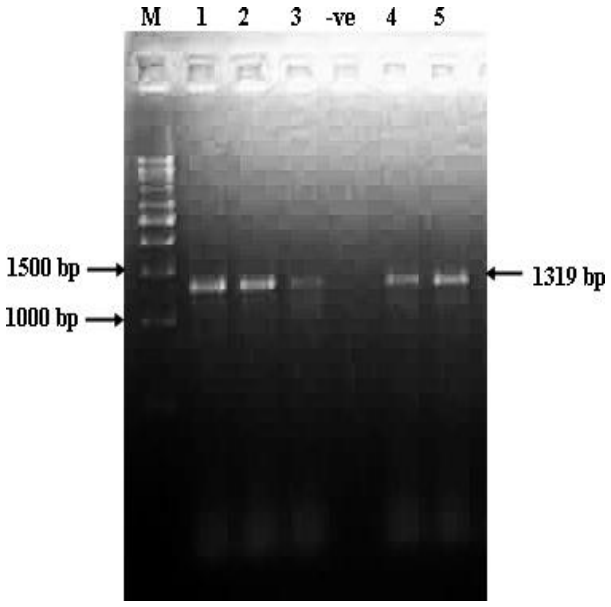
for the characterisation of the Malaysian isolates as no such report on them has been published to date.

The presence of FAdVs was detected by virus isolation. All infected embryos inoculated with UPM04217, UPM08158, UPM08136, UPM11134 and UPM11142 isolates showed obvious gross lesions of swelling, paleness, haemorrhages and multi-focal necrosis on the liver (data not shown). The presence of adenovirus was demonstrated by PCR using the H1/H2 and H3/H4 primer sets to amplify conserved regions of the hexon genes (Fig.1 and Fig.2), where fragments of expected sizes with the estimated lengths of 1219 bp and 1319 bp, respectively, were obtained. When subjected to RE analysis, all the isolates produced identical cleavage patterns. The *Hae*II restriction profiles of the H1/H2 PCR products of UPM04217, UPM08158, UPM08136, UPM11142 and UPM11134 isolates yielded three segments between 200 and 1200 bp (Fig.3A and Fig.3B), whilst the cleavage of the H3/H4-amplified segments generated five fragments between 100 to 400 bp (Fig.4). Interestingly, the digestion patterns of *Hae*II are similar to the RE profiles for FAdV strain 764 (Raue and Hess, 1998) that had been used as a reference strain of FAdV-9, on the basis of molecular weight in agarose gels. However, in accordance with the current ICTV nomenclature system (Benko *et al*., 2000), FAdV strain 764 which had formerly been classified as European serotype 9 was renamed as FAdV-8b (Pizzuto *et al*., 2010; Steer *et al*., 2009). The serotype identification was further strengthened by the generation of five fragments when the *Hpa*II restriction enzyme digested the H3/H4 PCR products of these isolates. These



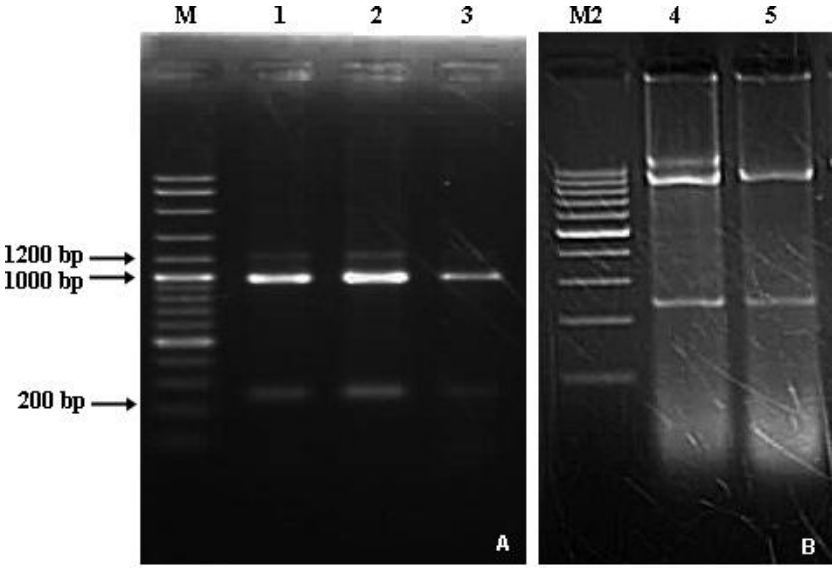
Lane 1, UPMPH04217; 2, UPM08158; 3, UPM08136; 4, UPM11142; 5, UPM11134 and M, molecular weight DNA marker (1kb DNA ladder, Promega).

Fig.1: Demonstration of the presence of FAdV using the hexon-based primer pair H1/H2



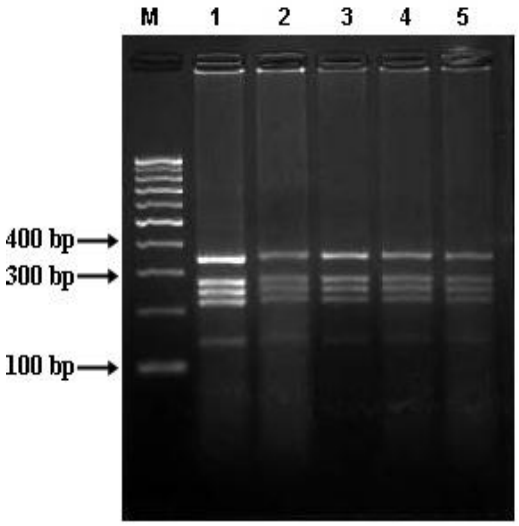
Lane 1, UPMPH04217; 2, UPM08158; 3, UPM08136; 4, UPM11142; 5, UPM11134; M, molecular weight DNA marker (1kb DNA ladder, Promega) and –ve, negative control.

Fig.2: Amplification of the hexon gene using the H3/H4 primer set



A) Lane 1, UPM04217; 2, UPM08158; 3 UPM08136; M, DNA size marker (VC 100bp Plus DNA ladder, Vivantis). B) Lane 1, Positive control of UPM04217; Lane 2, UPM11142; Lane 3, UPM11134; M, DNA size marker (VC 100 bp DNA ladder, Vivantis).

Fig.3: Ethidium bromide-stained agarose gel showing *Hae*II restriction enzyme patterns of the FAdV local isolates



Lane 1, UPM04217; 2, UPM08158; 3, UPM08136; 4, UPM11142 and 5, UPM11134 and M, DNA size marker (VC 100 bp DNA ladder, Vivantis).

Fig.4: Ethidium bromide-stained agarose gel showing *Hpa*II restriction enzyme patterns of the FAdV local isolates

profiles were identical to those of DNA group E fowl adenoviruses (Raue & Hess, 1998; Singh *et al*., 2002) which comprised FAdV-6, -7, -8a and -8b (Pizzuto *et al*., 2010; Steer *et al*., 2009).

Strong evidence for this classification was demonstrated when comparing the amino acid sequences of the L1 loop of the hexon gene of the Malaysian isolates with several reference strains representing each of the 12 serotypes. The sequence analysis of the deduced amino acids of 388 residues of the hexon protein between Malaysian isolates revealed 100% identity among them. The pairwise comparisons and the phylogenetic analysis of the 198 amino acids corresponding to residues 101 to 298 of the reference strain HG (Benko *et al*., 2000) confirmed the classification of UPM04217, UPM08158, UPM08136, UPM11134 and UPM11142 as being FAdV species E. The number of nucleotide differences between Malaysian and selected FAdV strains is shown in Table 2. Low nucleotide differences between Malaysian and several FAdV-8 species E (strains X11-A, T8-A, 764, Stanford, HG, 430-06, 607-06 and Australian FAdV vaccine) ranging from 13 to 23 nucleotides were also observed. In contrast, FAdV-A to -D showed up to 257 nucleotide differences. The highest identity of 98.1% was revealed between Malaysian strains and FAdV strain 764 followed by strains HG, 430-06, 607-06, Australian FAdV Vaccine and T8-A which showed 97.6% identity. An overall identity percentage of only 68% sequence identity was shown between the Malaysian isolates and FAdV species D, followed by FAdV species B and A with the identity percentage

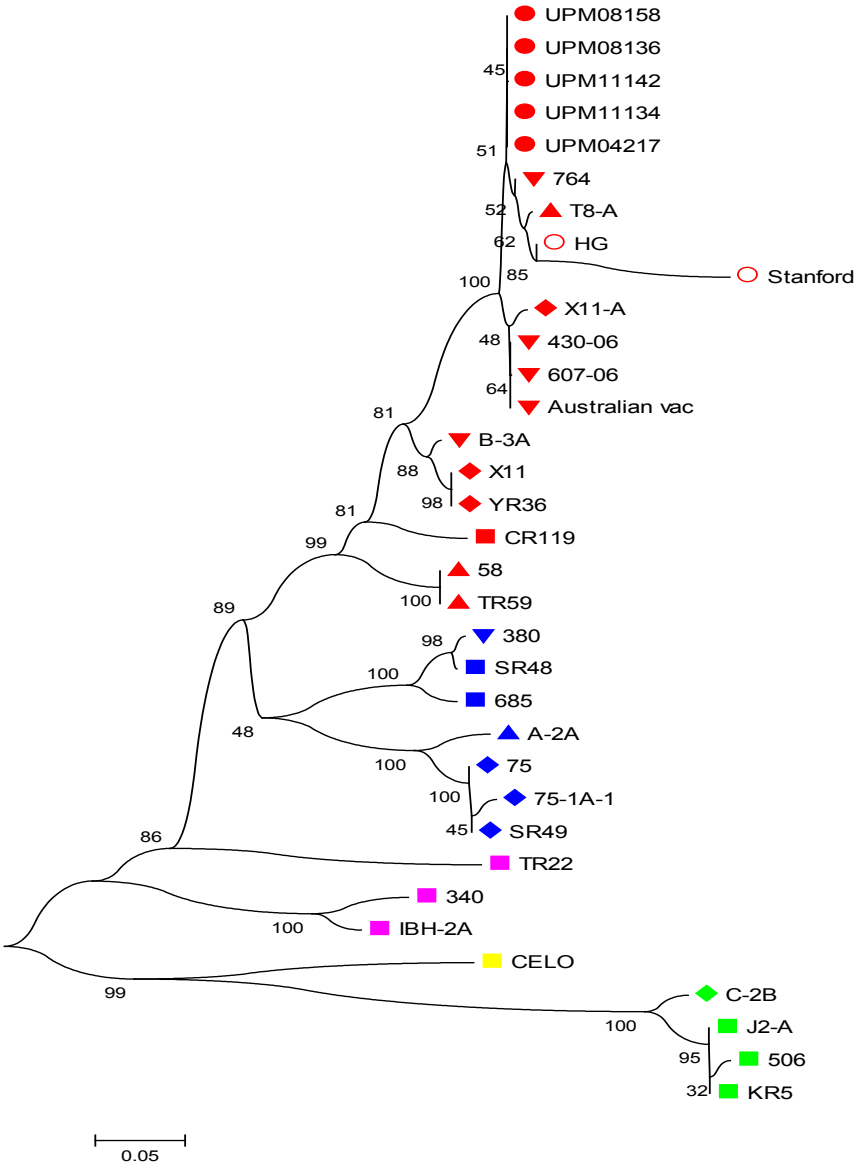
TABLE 2
Comparisons between the L1 sequences of Malaysian isolates with other published sequences

Strain	Species	Serotype	N. diff	Sequence identities (%)
CELO	A	1	237	60.4
340	B	5	213	64.6
IBH-2A	B	5	210	65.1
TR22	B	5	206	65.4
506	C	4	257	57.0
J2-A	C	4	254	57.5
KR5	C	4	254	57.5
C-2B	C	10	255	57.4
685	D	2	187	68.6
SR48	D	2	189	68.2
75	D	3	192	67.7
75-1A-1	D	3	194	67.4
SR49	D	3	191	67.9
A-2A	D	9	193	67.6
380	D	11	191	67.9
CR119	E	6	117	80.3
X11	E	7	77	87.0
X11-A	E	7	23	96.1
YR36	E	7	75	87.4
58	E	8a	106	82.2
TR59	E	8a	106	82.2
T8-A	E	8a	13	97.8
764	E	8b	11	98.1
B-3A	E	8b	75	87.4
Stanford	E	8[a]	14	97.6
HG	E	8[a]	13	97.8
430-06	E	8b	13	97.8
607-06	E	8b	13	97.8
Australian FAdV Vaccine	E	8b	13	97.8

[a] Unspecific group of FAdV-8 published data.

of 65% and 60%, respectively. FAdV species C showed the lowest evolutionary relationship with the Malaysian isolates, with only 57% identity.

The results of the distance-based method analysis on the 34 aligned amino acid sequences of the L1 loop hexon sequences are summarised in Fig.5. Five major groups are clearly shown in the phylogenetic tree. Each group represents species of fowl adenovirus from A to E of the current ICTV classification. The Malaysian isolates were most likely to have evolved together with several FAdV-8 such as strains 764, HG,



The datasets were bootstrapped 1000 times before being analysed using the Neighbour-Joining method. Species of FAdV are represented by colours; FAdV-A (yellow), FAdV-B (pink), FAdV-C (green), FAdV-D (blue) and FAdV-E (red). FAdV serotypes are represented by symbols as follows; FAdV-1 (yellow, square), FAdV-2 (blue, square), FAdV-3 (blue, diamond), FAdV-4 (green, square), FAdV-5 (pink, square), FAdV-6 (red, square), FAdV-7 (red, diamond), FAdV-8a (red, triangle), FAdV-8b (red, inverted triangle), FAdV-8 of unknown group (red, empty circle), Malaysian isolates (red, circle), FAdV-9 (blue, triangle), FAdV-10 (green, diamond) and FAdV-11 (blue, inverted triangle).

Fig.5: Phylogenetic analysis of amino acid sequences of 34 FAdV strains

Stanford and T8-A and seemed to be derived from a common ancestor. This was validated by the good matches to these viruses when analysed using pairwise alignments. A close relationship was also found between Malaysian and Australian field isolates (430-06 and 607-06) and an Australian FAdV vaccine strain that were identified as FAdV-8b (Steer *et al*., 2011).

In addition, a positive correlation was also found between FAdV-6 and -7 and our isolates but they were subgrouped into different subclusters by using the distance clustering method. The distance between these serotypes was increased equivalent to every mismatch occurring in the pairwise calculation. This topology was supported by the new ICTV classification of fowl adenovirus with five independent clusters representing FAdV-A, -B, -C, -D and –E. Contrary to expectation, strain TR22 was diverged from the phylogenetic clustering with FAdV-5. This evidence for the existence of six clusters has also been reported in some previous studies based on amino acid sequences of the loop regions (see Marek *et al*., 2010; Meulemans *et al*., 2001) and thought to result from cross-reactions among the serotypes.

In other Asian countries like India, Pakistan, Japan and Korea, most FAdVs infections were reported to be associated with hepatitis-hydropericardium syndrome and the main causative agent was FAdV-4 species C (Asthana *et al*., 2011; Kim *et al*., 2008; Mase *et al*., 2009; Mansoor *et al*., 2009; Park *et al*., 2011). Recently, IBH cases reported in Japan are associated with FAdV-2 (Nakamura *et al*., 2011). An increased number of IBH outbreaks associated with FAdVs infection were also reported in Korea (Lim *et al*., 2011). The researchers have identified four serotypes of FAdVs (FAdV-4, -5, -8b and -11) responsible for the IBH outbreaks and these are considered as dominant serotypes in Korea with FAdV-4 isolated in most cases. In Malaysia, outbreaks of IBH have been reported from several states, yet the serotype of the FAdV responsible is not known. Interestingly, the IBH cases in Malaysia caused solely by FAdV-8b, species E and are similar to those strains originating from Northern Ireland (strain 764), U.S.A (strain Stanford), Canada (strain HG) and Australia (strains 430-06, 607-06 and Australian FAdV Vaccine) (Alvarado *et al*., 2007; Calnek & Cowen, 1975; Grgić *et al*., 2011; McFerran *et al*., 1972) that have minimal number of nucleotides different. In previous studies, FAdV species E was also reported to be isolated in majority of the outbreaks in Ireland, England, Australia and New Zealand with FAdV-8 exhibiting the highest virulence and being the dominant serotype in Ontario, Canada, although the presence of other serotypes was also encountered (El-Attrache & Villegas, 2001; Erny *et al*., 1991; Grgić *et al*., 2011; McCracken *et al*., 1976; Steer *et al*., 2011; Toro *et al*., 1999; Ojkic *et al*., 2007). However, the relationship between geographical areas and genotype of FAdV remains poorly understood and we are unable to conclusively establish the origin.

An accurate identification of the serotypes involved in IBH outbreaks is very useful for epidemiological tracing and controlling the disease. Thus, vaccination

strategies against FAdV-8b should be developed for the prevention of IBH outbreaks in Malaysia. However, additional numbers of isolates are preferable for the establishment of the role of this virus in relation to IBH outbreaks.

CONCLUSION

This study describes the molecular characterisation of fowl adenoviruses isolated from several states of Malaysia through a combination of hexon-based PCR, REA and phylogenetic analysis. The outbreaks of IBH investigated in this study were solely caused by FAdV-8b and seemed to be the dominant serotype in Malaysia. Therefore, there is a need for the development of vaccines against FAdV-8b to control FAdV infections in the poultry industry.

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REFERENCES

Alemnesh, W., Hair-Bejo, M., Aini, I., & Omar, A. R. (2012). Pathogenicity of fowl adenovirus in specific pathogen free chicken embryos. *Journal of Comparative Pathology*, *146*, 223-229.

Alvarado, I. R., Villegas, P., El-Attrache, J., Jensen, E., Rosales, G., Perozo, F., & Purvis, L. B. (2007). Genetic characterization, pathogenicity and protection studies with an avian adenovirus isolate associated with inclusion body hepatitis. *Avian Diseases*, *51*, 27-32.

Asthana, M., Singh, V. K., Kumar, R., & Chandra, R. (2011). Isolation, cloning and *In Silico* of hexon gene of fowl adenovirus 4 (FAdV4) isolates associated with hydro pericardium syndrome in domestic fowl. *Journal of Proteomics & Bioinformatics*, *4*, 190-195.

Balamurugan, V., & Kataria, J. M. (2004). The hydropericardium syndrome in poultry- A current scenario. *Veterinary Research Communications*, *28*, 127-148.

Benkö, M., Harrach, B., & Russell, W. C. (2000). Family Adenoviridae. In M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, & R. B. Wickner (Eds.), *Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego, CA, 227–238.

Calnek, B. W., & Cowen, B. S. (1975). Adenoviruses of chickens: serologic groups. *Avian Diseases, 19,* 91-103.

Cowen, B., Mitchell, G. B., & Calnek, B. W. (1978). An adenovirus survey of poultry flocks during the growing and laying periods. *Avian Diseases, 22,* 115-121.

Crawford-Miksza, L. K., & Schnurr, D. P. (1996). Adenovirus serotype evolution is driven by illegitimate recombination in the hypervariable regions of the hexon. *Virology*, *224*, 357-367.

Dagmar, C., & Becht, H. (1975). *In vitro* cultivation of cells from the chorioallantoic membrane of chick embryos. *Medical Microbiology and Immunology*, *161*, 3-10.

El-Attrache, J., & Villegas, P. (2001). Genomic identification and characterization of avian adenoviruses associated with inclusion body hepatitis. *Avian Diseases*, *45*, 780-787.

Erny, K. M., Barr, D. A., & Fahey, K. J. (1991). Molecular characterisation of highly virulent fowl adenoviruses associated with the outbreaks of inclusion body hepatitis. *Avian Pathology*, *20,* 597-606.

Ganesh, K., Suryanarayana, V. V. S., & Raghavan, R. (2002). Detection of fowl adenovirus associated with hydropericardium hepatitis syndrome by a polymerase chain reaction. *Veterinary Research Communication*, *26*, 73-80.

Gomis, S., Goodhope, R., Ojkíc, D., & Willson, P. (2006). Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. *Avian Diseases*, *50*, 550-555.

Grgić, H., Yang, D., & Nagy, E. (2011). Pathogenicity and complete genome sequence of a fowl adenovirus serotype 8 isolate. *Virus Research*, *156*, 91-97.

Grimes, T. M., King, D. J., Klven, S. H., & Fletcher, O. J. (1977). Involvement of a type-8 avian adenovirus in the etiology of Inclusion Body Hepatitis. *Avian Diseases, 21*, 26-38.

Hair-Bejo, M. (2005). Inclusion body hepatitis in a flock of commercial broiler chickens. *Journal of Veterinary Malaysia*, *17*, 23-26.

Hess, M. (2000). Detection and differentiation of avian adenoviruses: a review. *Avian Pathology*, *29*, 195-206.

Jason, P. S., Hair-Bejo, M., Omar, A. R., & Aini, I. (2008). Molecular detection of fowl adenovirus in commercial broiler chickens in Malaysia. In *Proceedings 20th Veterinary Association Malaysia Congress: 15-17 August 2008; Bangi, Malaysia*: 72.

Jiang, P., Ojkić, D., Tuboly, T., Huber, P., & Nagy, È. (1999). Application of the polymerase chain reaction to detect fowl adenoviruses. *Canadian Journal of Veterinary Research, 63*, 124-128.

Kim, J. N., Byun, S. H., Kim, M. J., Kim, J. J., Sung, H. W., & Mo, I. P. (2008). Outbreaks of hydropericardium syndrome and molecular characterization of Korean fowl adenoviral isolates. *Avian Diseases 52,* 526-530.

Lim, T., Lee, H., Lee, D., Lee, Y., Park, J., Youn, H., Kim, M., Youn, H., Lee, J., Park, S., Choi, I., & Song, C. (2011). Identification and virulence characterization of fowl adenoviruses in Korea. *Avian Diseases, 55,* 554-560.

Mansoor, M. K., Hussain, I., Arshad, M., Muhammad, G., Hussaini, M. H., & Mehmood, S. (2009). Molecular characterization of fowl adenovirus serotype 4 (FAdV-4) isolate associated with fowl hydropericardium-hepatitis syndrome in Pakistan. *Pakistan Journal of Zoology*, *41*, 269-276.

Marek, A., Günes, A., Schulz, E., & Hess, M. (2010). Classification of fowl adenoviruses by use of phylogenetic analysis and high-resolution melting-curve analysis of the hexon L1 gene region. *Journal of Virological Methods*, *170,* 147-154.

Mase, M., Chuujou, M., Inoue, T., Nakamura, K., Yamaguchi, S., & Imada, T. (2009). Genetic characterization of fowl adenoviruses isolated from chicken with hydropericardium syndrome in Japan. *Journal of Veterinary Medicines Sciences, 71*, 1455-1458.

McCracken, R. M., McFerran, J. B., Evans, R. T., Connor, T. J. (1976). Experimental studies on the aetiology of inclusion body hepatitis. *Avian Pathology*, *5,* 325-339.

McFerran, J. B., Clarke, J. K., & Connor, T. J. (1972). Serological classification of avian adenoviruses. *Archive für die Gesamte Virusforschung, 39*, 132-139.

McFerran, J. B., & Smyth, J. A. (2000). Avian adenoviruses. *Review of Science and Technology Off. International Epiz*, *19,* 589-601.

Meulemans, G., Boschmans, M., van den Berg, T. P., & Decaesstecker, M. (2001). Polymerase chain reaction combined with the restriction enzyme analysis for detection and differentiation of fowl adenovirus. *Avian Pathology*, *30*, 655-660.

Meulemans, G., Couvreur, B., Decaesstecker, M., Boschmans, M., van den Berg, T. P. (2004). Phylogenetic analysis of fowl adenovirus. *Avian Pathology*, *33*, 164-170.

Mirmomeni, M. H., Majd, S. S., Sisakhtnezhad, S., & Doranegard, S. (2010). Comparison of the three methods for DNA extraction from paraffin-embedded tissue. *Journal of Biological Sciences, 10*, 261-266.

Nakamura, K., Mase, M., Yamamoto, Y., Takizawa, K., Kabeya, M., Wakuda, T., Matsuda, M., Chikuba, T., Yamamoto, Y., Ohyama, T., Takahashi, K., Sato, N., Akiyama, N., Honma, H., Imai, K. (2011). Inclusion body hepatitis caused by fowl adenovirus in broiler chickens in Japan, 2009-2010. *Avian Diseases, 55*, 719-723.

Nakamura, K., Mase, M., Yamaguchi, S., Shibahara, T., & Yuasa, N. (1999). Pathologic study of specific-pathogen-free chicks and hens inoculated with adenovirus isolated from hydropericardium syndrome. *Avian Diseases*, *43*, 414-423.

Norrby, E. (1969). The relationship between the soluble antigens and the virion of Adenovirus type 3: IV. Immunological complexity of soluble components. *Virology, 37*, 565-576.

Ojkic, D., Krell, P. J., Tuboly, T., & Nagy, È. (2007). Characteristic of fowl adenoviruses isolated in Ontario and Quebec. *The Canadian Journal of Veterinary Research*, *72*, 236-241.

Ono, M., Okuda, Y., Yazawa, S., Shibata, I., Sato, S., & Okada, K. (2004). Pathogenicty by parental injection of fowl adenovirus isolated from gizzard erosion and resistance to reinfection in adenoviral gizzard erosion in chickens. *Veterinary Pathology*, *41*, 483-489.

Park, H., Lim, I., Kim, S., Kim, T., & Yeo, S. (2011). Isolation and characterization of fowl adenovirus serotype 4 from chickens with hydropericardium syndrome in Korea. *Korean Journal of Veterinary Research, 51*, 209-216.

Pizzuto, M. S., Battisti, C. D., Marciano, S., Capua, I., Cattoli, G. (2010). Pyrosequencing analysis for a rapid classification of fowl adenovirus species. *Avian Pathology*, *39*, 391-398.

Raue, R., & Hess, M. (1998). Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. *Journal of Virological Methods, 73*, 211-217.

Russell, W. C. (2009). Adenoviruses: update on structure and function. *Journal of General Virology*, *90*, 1-20.

Saifuddin, M., Wilks, C. R., & Murray, A. (1992). Characterization of avian adenoviruses associated with inclusion body hepatitis. *New Zealand Veterinary Journal, 40*, 52-55.

Singh, A., Oberoi, M. S., Grewal, G. S., Hafez, H. M., & Hess, M. (2002). The use of PCR combined with restriction enzyme analysis to characterize fowl adenovirus field isolates from Northern India. *Veterinary Research Communication, 26*, 577-585.

Steer, P. A., Kirkpatrick, N. C., O'Rourke, D., & Noormohammadi, A. H. (2009). Classification of fowl adenovirus serotypes by use of high-resolution melting curve analysis of the hexon gene region. *Journal of Clinical Microbiology*, *47*, 311-321.

Steer, P. A., O'Rourke, D., Ghorashi, S. A., & Noormohammadi, A. H. (2011). Application of high-resolution melting curve analysis for typing

of fowl adenoviruses in field cases of inclusion body hepatitis. *Australian Veterinary Journal*, *89*, 184-192.

Toogood, C. I. A., Crompton, J., & Hay, R. T. (1992). Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. *Journal of General Virology*, *73*, 1429-1435.

Toro, H., Prusas, C., Raue, R., Cerda, L., Geisse, C., & Hess, M. (1999). Characterization of fowl adenoviruses from outbreaks of Inclusion Body Hepatitis/Hydropericardium Syndrome in Chile. *Avian Diseases*, *43*, 262-270.

Virus Taxonomy. (2011). Release (current) [http://ictvonline.org/virusTaxonomy.asp?version=2011]

Yates, V. J., Rhee, Y. O., Fry, D. E., El-Mishad, A. M., & McCormick, K. J. (1976). The presence of avian adenoviruses and adeno-associated viruses in healthy chickens. *Avian Diseases, 20,* 146-152.

Zsák L., & Kisary J. (1984). Grouping of fowl adenoviruses based upon the restriction patterns of DNA generated by *BamH*I and *Hind*III. *Intervirology, 22*, 110-114.

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Comparative Study on Callus Induction, Proliferation and Plantlets Regeneration In Two Cultivars of *Stevia rebaudiana* Bertoni – The Only Non Caloric Natural Sweetener

Sharuti Rathore[1], Kuldeep Yadav[1], Narender Singh[1]* and S. K. Singh[2]

[1]Plant Tissue Culture Laboratory, Department of Botany, Kurukshetra University, Haryana 136119, India
[2]Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110 012, India

ABSTRACT

The present report describes a comparative study for plant regeneration through callus morphogenesis in two different cultivars 'CIM madhu' and 'CIM mithi' of *S. rebaudiana*. The leaf explants were cultured on MS medium supplemented with IBA (1.0-4.0 mg/l) in combination with BAP (0.2 mg/l) for callus induction. Among the various tested combinations for shoot regeneration, maximum multiplication was recorded with MS + Kn (2.0 mg/l) + NAA (0.2 mg/l) + ADS (40 mg/l). Half-strength MS medium with IBA (0.2 mg/l) + AC (100 mg/l) was the best medium for the *in vitro* rooting of regenerated shoots. A comparison of different hardening media was also studied between two cultivars. The micro-plantlets hardened in plastic pots filled with sand: soil: vermiculite (1:2:1), covered with transparent polythene bags took minimum time to glass house transfer with maximum survival rate. CIM-madhu showed good callus induction, proliferation and regeneration ability in comparison to CIM-mithi. In comparison, higher rooting percentage was obtained in CIM-madhu with 97 % survival rate. The ability of 'CIM-madhu' to induce callus and regenerate successful plantlets under these conditions suggests that this cultivar is moderately suitable for micropropagation purposes.

Keywords: Stevia rebaudiana, growth regulators, leaf segment, callus induction, plant regeneration

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E-mail addresses:
shrutihp@gmail.com (Sharuti Rathore),
kuldeep0608@gmail.com (Kuldeep Yadav),
nsheorankukbot11@gmail.com (Narender Singh),
sanjayhor@rediffmail.com (S. K. Singh)
* Corresponding author

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INTRODUCTION

Stevia rebaudiana Bertoni (family Asteraceae) commonly known as "Sweet Weed", "Sweet Leaf", "Sweet Herbs" and "Honey Leaf", is the most valuable tropical natural sweetener perennial herb indigenous

to the Paraguay and Southern Brazil (Ali *et al.,* 2010; Singh *et al.,* 2011). Currently, stevia is commercially grown in Central America, Japan, South East Asia, Israel, China and Canada (Patel & Shah, 2009). Its leaves contain secondary metabolites such as stevioside, rebaudioside A., rebaudioside C. and dulcoside A. which produce a sweet taste having no caloric value (Kinghorn, 1987; Din *et al.,* 2006). So, it produces non-toxic, non-calorie, non-plaque, non-fermentative, flavour enhancing, non-carcinogenic and non-addictive sweetness absolutely safe for diabetics, phenyl ketonuria patients and diet conscious persons (Gregersen *et al.,* 2004). Its leaves also contain protein, fibres, carbohydrates, phosphorus, iron, calcium, potassium, sodium, magnesium, rutin, iron, zinc, vitamin A and vitamin C. It is used for different therapeutic effects in diabetes, obesity, hyperactivity, hypertension, carbohydrate cravings, tobacco and alcohol cravings, hypoglycaemia, indigestion, yeast infections, skin toning and healing (Yasukawa *et al.,* 2002; Lailerd *et al.,* 2004; Singh *et al.,* 2011; Verma *et al.,* 2011). It is also used in sweet sauces, pickles, bakery, beverages and confectionery sectors in Japan and Korea (Preethi *et al.,* 2011).

The seeds of *S. rebaudiana* show a very low germination percentage and do not produce uniform emergence, resulting in great variability in plant growth and maturity (Sivaram & Mukundan, 2003; Verma *et al.,* 2011). Vegetative propagation is too slow having the possibilities of pathogen attack on the tissues (Debnath, 2008; Mishra *et al.,* 2010). Therefore, *in vitro* plant culture techniques may be an effective alternative for propagation and conservation of plants of such an economic importance in which conventional methods show limitations (Yadav *et al.,* 2013a, 2013b).

Although attempts have been made by several workers for *in vitro* studies on *S. rebaudiana* using various physical and biological factors (Kornilova & Kalashnikova, 1997; Din *et al.,* 2006; Ahmed *et al.,* 2007; Ibrahim *et al.,* 2008; Patel & Shah, 2009; Satpathy & Das, 2010; Ali *et al.,* 2010; Singh *et al.,* 2011; Verma *et al.,* 2011), considerable efforts are still required to make it more economical and practical.

The genotype used and its interaction with the various physical and biological factors also influence the different stages of *in vitro* multiplication (George, 1993; Yadav & Singh, 2012; Yadav *et al.,* 2012). In the light of the above-referred importance and demand, the present investigation was performed in order to gain information on the comparative effects for *in vitro* suitability of two varieties (CIM- madhu and CIM- mithi) of *S. rebaudiana.*

MATERIAL AND METHODS

Collection of Plant Material

The experiment was carried out in the Central Tissue Culture Laboratory of Lal Bahadur Shastri Building IARI, New Delhi. Two varieties of *S. rebaudiana* (CIM-madhu and CIM- mithi) were collected from the nursery of Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow (India) and maintained under glasshouse conditions. Leaf segments (1cm

x 1cm) excised from these two different varieties served as a source of explants for micropropagation.

Surface Sterilization

The explants were first washed with running tap water to remove dust particles, and then surface sterilized with Tween-20 (2 drops per 100 ml water) for 5 min, followed by washing under tap water to remove all the detergent traces. The sterilized explants were then treated with 0.1% (w/v) mercuric chloride for 3-5 min under aseptic conditions. These explants were thoroughly washed 4-5 times with autoclaved double distilled water to remove the traces of mercuric chloride. Excess water adhering on explants surface was removed using autoclaved blotting paper under a laminar air flow chamber.

Culture Condition

The explants were trimmed with sterilized blade and finally inoculated on MS (Murashige and Skoog, 1962) medium with different concentrations and combinations of growth regulators (BAP and IBA) containing 30 g/l sucrose and 8 g/l agar (Himedia, India) to initiate callus culture. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl and finally autoclaved at 1.05 kg/cm^{-2} at 121°C for 20 min. The cultures were maintained at a temperature of 25±2ºC with a 16/8 h light/ dark photoperiod under an illumination of 20 µmol m^{-2} s^{-1} photosynthetic photon flux intensity provided by cool- white fluorescent light.

Callus Induction

The calli formed from the leaf explants were periodically sub-cultured every four week for multiplication and maintenance on callus proliferation medium, MS nutrients supplemented with IBA (4.0 mg/l) + BAP (0.2 mg/l).

Indirect Shoot Regeneration

For shoot regeneration, the calli were further sub-cultured on shoot induction medium, MS salts fortified with different concentrations and combinations of Kn (0.5- 2.0 mg/l), BAP (0.5-2.0 mg/l), ADS (40-60 mg/l) and NAA (0.1- 0.2 mg/l). The best resulting medium formulation was identified in terms of response, number and length of shoots.

In vitro Rooting and Acclimatization

For *in vitro* root induction, individual shoots (2 cm long) were excised from the shoot clump and transferred to half-strength MS medium (3% sucrose and 0.8 % agar) with different concentrations of IBA (0.2-0.5 mg/l) alone and in combination with activated charcoal (AC). The cultures were maintained under the same conditions as for shoot induction. When adequate rooted shoots were obtained, the plantlets were carefully pulled out from the medium and kept under running tap water, using a fine brush to remove the medium sticking to the root system. These plantlets were then transferred to pots containing different substrates, viz. vermicompost: peat moss: sand (1:1:1) and sand: soil: vermicompost (1:2:1). The pots were covered with

polyethylene membranes to ensure about 80% relative humidity. The potted plants were irrigated with MS (half strength) salt solution devoid of sucrose and myo-inositol every 3 days. After about 4 weeks, these plants were transferred to larger pots and maintained under glasshouse conditions. Survival rate was assessed after 3 months.

Statistical Analysis

Complete Randomized Design (CRD) was used for the *in vitro* culture experiments. Each single explant was considered as an experimental unit. Each treatment consisted of 3 replicates.

RESULTS

The initiation of callus started from the cut ends of the explants in the beginning and gradually extended to all over the explant. The responses of the leaf explants varied with the growth regulator combinations. However, the nature of the response of different cultivars to a particular growth regulator combination was more or less similar. The control MS medium without any hormone was also capable of inducing callus but only in trace amount (Table 1).

The effects of different concentrations of IBA and BAP on callus induction of *S. Rebaudiana* are presented in Table 1. The increase of callus formation efficiency was observed with the increase of the IBA concentration. The best callus growth (in terms of biomass) was obtained when 4.0 mg/l IBA + 0.2 mg/l BAP was used in the medium. Friable, greenish yellow callus was observed irrespective of the genotypes (Fig.1a-1b).

The above mentioned calli derived from leaf explants were subcultured on MS medium supplemented in different concentrations and combinations of Kn (0.5 - 2.0 mg/l), BAP (0.5 - 2.0 mg/l), ADS (40-160 mg/l) and NAA (0.1 - 0.2 mg/l) for shoot regeneration (Table 2). After, 1-2 weeks of transfer to regeneration medium, the sub-cultured calli enlarged rapidly and started emerging out green shoot buds from the surface.

Among the various combinations, the presence of Kn was found to be more

TABLE 1
Effects of IBA and BAP on callus induction from leaf explants in two *stevia* genotypes (CIM-Madhu and CIM-Mithi). Data were recorded after 45 days of culture.

	Callusing %		Days of callus induction	
Treatments (mgL^{-1})	CIM-madhu	CIM-mithi	CIM-madhu	CIM-mithi
MS devoid of Hormones (control)	10.55	.44)	42.56	43.76
MS+IBA (1.0) + BAP (0.2)	66.26	56.67	30.92	32.16
MS+IBA (2.0) + BAP (0.2)	79.48	78.17	22.20	24.13
MS+IBA (3.0) + BAP (0.2)	93.62	92.32	18.08	18.44
MS+IBA (4.0) + BAP (0.2)	97.79	96.50	13.10	13.58

Results represent CD at 5% of three replicated experiments.
Whereas: Treatments (A) = 0.23; 0.23, Cultivars (B) = 0.36, 0.36, A x B = 0.51, 0.51

effective than BAP. The combination of Kn with NAA/ADS induced the highest number of shoot buds in the least number of days. Among the cultivars, CIM-madhu produced the highest number of shoot buds in less number of days in comparison to CIM-mithi (Table 2; Fig.1c-1d). Plantlets of *S. rebaudiana* longer than 2 cm were transferred into MS medium (half-strength) supplemented with IBA (0.2- 05 mg/l) and active charcoals (AC) to initiate roots. MS basal medium without any growth regulator showed a delayed and weak response (Table 3). The results showed 96 and 95% rooting rates in CIM madhu and CIM mithi in medium ½ MS + 0.2 mg/l IBA + 100 mg/l AC respectively (Fig.1e-1f, Fig.2).

The micro-plantlets hardened in plastic pots filled with vermicompost: soil: sand (1:2:1), covered with transparent polythene bags took minimum time (15 days) to glass house transfer and showed maximum survival rate (Fig.1g-1h; Table 4). It was observed that gradual acclimatization of *in vitro* grown plants to the external environment is most essential for s*tevia*.

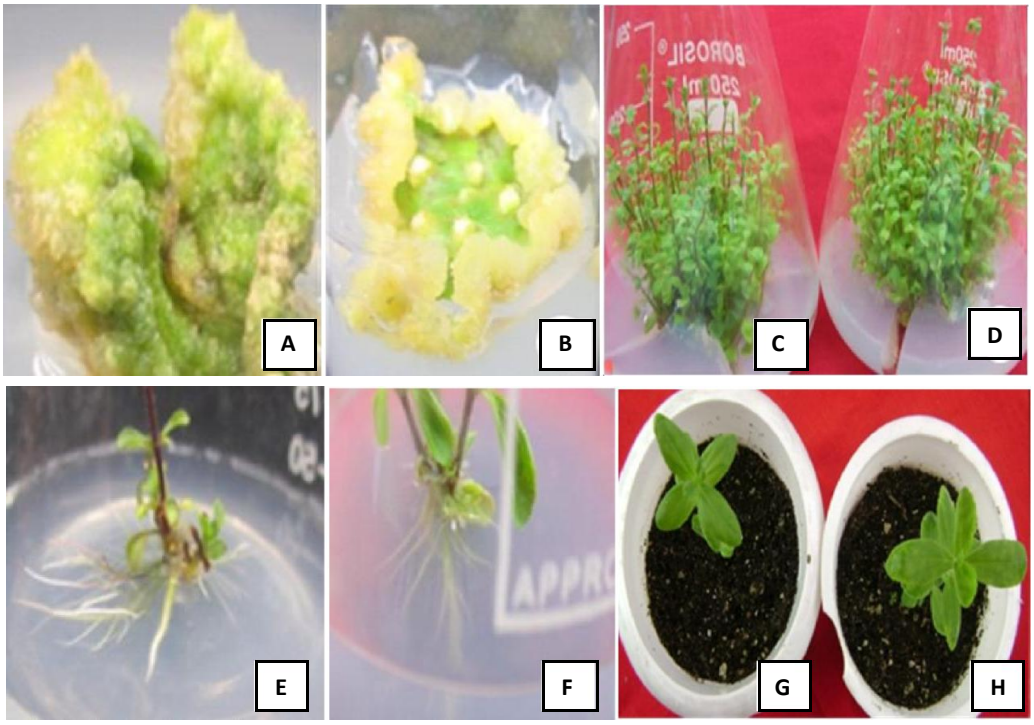

Fig.1: Callus morphogenesis and plant regeneration in *S. rebaudiana*. (A-B): Callusing on MS + IBA(4.0 mgL^{-1}) + BAP (0.2 mgL^{-1}); (C-D): organogensis on MS + Kn (2.0mgL^{-1}) + AS (40mgL^{-1}) + NAA (0.2mgL^{-1}); (E-F): Rooting on MS + IBA (0.2 mgL^{-1})+ AC (100mgL^{-1}); (G-H) hardening of plantlets in vermiculite: soil: sand (1:2:1).

TABLE 2
Effects of growth regulators on shoot regeneration on callus derived from leaf explants in two *stevia* genotypes. Data were recorded after 45 days of culture.

	CIM-madhu			CIM-mithi		
Treatments (mgL^{-1})	Regeneration percent	Days for shoot initiation	Number of microshoot /calli	Regeneration percent	Days for shoot initiation	Number of microshoot /calli
Control	8.00	41.68	1.22	6.02	43.11	1.00
MS+2.0BAP+0.2NAA	33.43	26.45	2.31	33.13	26.93	2.72
MS+1.0BAP+0.2NAA	39.76	28.13	2.05	38.55	30.22	2.16
MS+2.0 Kn+ 0.2NAA	69.04	16.78	7.16	67.33	20.13	8.51
MS+2.0Kn+0.2NAA+40AS	78.91	12.21	11.98	77.13	12.84	12.44
MS+1.0Kn+0.2 NAA+40AS	73.96	15.15	12.51	73.06	18.24	12.10
MS+0.5BAP+0.5Kn+0.1NAA	41.89	26.24	3.23	40.11	29.25	4.86
MS+1.0BAP+0.2NAA+40AS	66.78	21.22	6.98	64.37	24.87	7.87
MS+2.0BAP+0.2NAA+40AS	57.92	20.44	4.53	57.25	22.53	6.88

Note: Results represent CD at 5% of three replicated
Cultivars (A) = 0.18; 0.16; 0.09, Treatments (B) = 0.39; 0.35; 0.21; AXB = 0.56; 0.50; 0.29

TABLE 3
Effects of IBA and AC on number of roots per micro cuttings and mean length in two *stevia* genotypes CIM madhu and CIM mithi. Data were recorded after 30 days of culture.

	No. of roots/microcutting		Root length (cm)	
Treatments (mgL^{-1})	CIM-madhu	CIM-mithi	CIM-madhu	CIM-mithi
½ MS devoid of auxins	3.23	3.06	1.10	1.02
½ MS + 0.2 IBA	9.13	8.76	3.30	3.05
½ MS + 0.5 IBA	7.23	7.06	2.43	2.13
½ MS + 0.2 IBA + 100 mgL-1 AC	13.13	12.73	4.23	4.00
½ MS + 0.5 IBA + 100 mgL-1 AC	9.43	9.16	2.93	2.50

Results represent CD at 5% of three replicated experiments.
Treatment (A) = 0.07, 0.09; Cultivar (B) = 0.12, 0.14; A X B= N.S., N.S.

TABLE 4
Effects of hardening strategies on plantlet survival and time taken to glass house transfer.

Treatments (mgL^{-1})	CIM-madhu		CIM-mithi	
	Survival	Time taken to glass house transfer	Survival	Time taken to glass house transfer
Vermicompost: peat: moss: sand (1:1:1)	50.23	31.40	49.63	31.76
Vermicompost: soil: sand (1:2:1)	97.53	15.20	97.30	15.43

Results represent CD at 5% of three replicated experiments.
Treatments (A) = 0.17, N.S.; Cultivars (B) = 0.17, 0.44; A X B = 0.24, N.S.

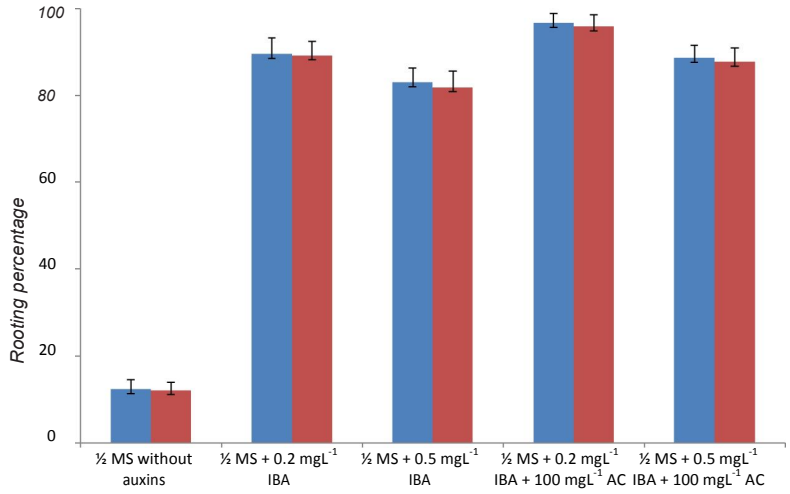

Fig.2: Effects of IBA and AC on rooting in two *stevia* genotypes CIM madhu and CIM mithi

DISCUSSION

Type of growth regulator and genotypes are considered to be important factors for callus induction. Several researchers reported the induction of callus from the leaf explants in *Anthurium andreanum* and *Stevia* (Atak & Celik, 2009; Patel & Shah, 2009).

Growth and developmental processes are generally regulated by growth regulators, which are present in various concentrations in different parts of plant and nutritive media. Phytohormones are essential to disturb the established polarity in the organ for the initiation of cell division. A requirement for exogenous auxin in callus initiation has also been established by Lahiri *et al.* (2012). The combination of a cytokinin with an auxin has been reported to strongly enhance callus induction in many plant species (George, 2008; Irvani *et al.,* 2010; Lahiri *et al.,* 2012). The combination of IBA and BAP may act synergetically with each other and induce the cells to dedifferentiate to form callus. In contrast, no callus induction from leaf

explants was seen with any concentrations of IBA in *Saccharum officinarum* (Gopitha *et al.,* 2010). Meanwhile, the frequency of callus induction may vary from one species to another due to the endogenous level of hormone, their uptake, type of auxins and cytokinins used and also on the mode of action (Gupta *et al.,* 2010).

Similar observation of emerging out green shoot buds from the surface was also observed by Irvani *et al.* (2010) in *Dorem ammoniacum*. Similar reports on efficacy of Kn over BAP are also available for other plant species (Kumar, 1992; Rahman *et al.,* 2004; Zibbu & Batra, 2010). The quantitative interactions between the appropriate plant growth regulators at optimum culture conditions play an important role towards the success of callus cultures (Benmoussa, 1996; Mukhopadhyay *et al.,* 2008; Atak & Celik, 2009). The *in vitro* response of plant tissues towards callus induction, growth and regeneration often seem to be under an over-riding genetic control with other factors exerting only a minor effect (George, 2008; Atak & Celik, 2009).

The roots emerged from the base of the regenerated shoots. The difference among plant parts to the IBA treatment may relate to the differences in their internal growth regulators. The efficiency of IBA in root induction from shoots *in vitro* has been reported for several plant species (Yadav & Singh, 2010; Singh *et al.,* 2010; Yadav & Singh, 2011). AC attributes to the reduction of light at the base of the shoots and provides the environment for auxins accumulation. Druart *et al*. (1982) found the positive response of AC on rooting.

The high survival rate of the regenerated plantlets under field conditions indicated their superiority with both physical and chemical environments.

CONCLUSION

In vitro methods provide an effective alternate means for rapid continuous multiplication of species to meet the demand for commercial exploitation. The genotype used and its interaction with the various physical and biological factors also influence the different stages of *in vitro* multiplication.

REFERENCES

Ahmed, M. B., Salahin, M., Karim, R., Razvy, M. A., & Hannan, M. M. (2007). An efficient method for *in vitro* clonal propagation of a newly introduced sweetener plant (*Stevia rebaudiana* Bertoni.) in Bangladesh. *American Eurasian Journal of Science Research*, *2*, 121-25.

Ali, A., Gull, I., Naz, S., & Afgan, S. (2010). Biochemical investigation during different stages of *in vitro* propagation of *Stevia rebaudiana. Pakistan Journal of Botany, 42*, 2827-2837.

Atak, C., & Celik, O. (2009). Micropropagation of *Anthurium andreanum* from leaf explants. *Pakistan Journal of Botany, 41*, 1155-1161.

Benmoussa, M., Mukhopadhyay, S., & Desjardins, Y. (1996). Optimization of callus culture and shoot multiplication of *Asparagus densiflorus*. *Plant Cell Tissue & Organ Culture, 47*, 91-94.

Debnath, M. (2008). Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. *Journal of Medicinal Plant Research, 2*, 45-51.

Din, M. S. U., Chowdhury, M. A., Khan, M. M. H., Din, M. B. U., Ahmed, R., & Baten, M. A. (2006). *In vitro* propagation of *Stevia rebaudiana* Bert. in

Bangladesh. *African Journal of Biotechnology, 5*, 1238-240.

Druart, P., Kevers, C., Boxus, P., & Gaspar, T. (1982). *In vitro* promotion of root formation by apple shoots through darkness effect on endogenous phenols and peroxidases. *Z flanzenphysiology, 108*, 429-436.

George, E. F. (1993). *Plant propagation by Tissue Culture.* Eastern Press, Eversley.

George, E. F. (2008). Plant Tissue Culture Procedure-Background. In E.F. George, M. A. Hall, & G. J. Deklerk (Eds.), *Plant Propagation by Tissue Culture, 3rd edition, Vol. I, The Background* (pp. 1-28). Springer, Dordrecht, The Netherlands.

Gopitha, K., Bhavani, L., & Senthilmanickam, J. (2010). Effect of the different auxins and cytokinins in callus induction, shoot, root regeneration in sugarcane. *International Journal of Pharmaceutical and Bio-Sciences, 1*, 1-7.

Gregersen, S., Jeppesen, P. B., Holst, J. J., & Hermansen, K. (2004). Antihyperglycemic effects of stevioside in type 2 diabetic subjects. *Metabolism, 53*, 73.

Gupta, P., Sharma, S., & Saxena, S. (2010). Callusing in *Stevia rebaudiana* (natural sweetener) for steviol glycoside production. *World Academy of Science, Engineering and Technology, 72*, 572-576.

Ibrahim, A. I., Nasar, M. I., Mohammed, B. R., & Zefzafi, M. E. (2008). Plant growth regulators affecting *in vitro* cultivation of Stevia rebaudiana. *Sugar Technology, 10,* 254–259.

Irvani, N., Solouki, M., Omidi, M., Zare, A. R., & Shahnazi, S. (2010). Callus induction and plant regeneration in *Dorem ammoniacum* D. an endangered medicinal plant. *Plant Cell Tissue and Organ Culture, 100*, 293-299.

Kinghorn, A. D. (1987). Biologically active compounds from plants with reputed medical and sweetening properties. *Journal of Natural Products, 50*, 1009-1024.

Kornilova, O. V., & Kalashnikova, E. A. (1997). Clonal micropropagation of *Stevia* (*Stevia rebaudiana*). *Cercetari Agrton Moldova, 30*, 80-85.

Kumar A. (1992). Somatic embryogenesis and high frequency plantlet regeneration in callus cultures of *Thevetia peruviana*. *Plant Cell Tissue and Organ Culture, 31*, 47-50.

Lahiri, K., Mukhopadhyay, M. J., Desjardins, Y., & Mukhopadhyay, S. (2012). Rapid and stable *in vitro* regeneration of plants through callus morphogenesis in two varieties of *Mucuna pruriens* L. – an anti. Parkinson's drug yielding plant. *Nucleus, 55*, 37- 43.

Lailerd, N., Saengsirisuwan, V., Sloniger, J. A., Toskulkao, C., & Henriksen, E.J. (2004). Effects of stevioside on glucose transport activity in insulin-sensitive and insulin-resistant rat skeletal muscle. *Metabolism, 53*, 101-107.

Mishra, P., Singh, R., Kumar, U., & Prakash, V. (2010). *Stevia rebaudiana* – A magical sweetener. *Global Journal of Biotechnology and Biochemistry*, *5*, 62–74.

Mukhopadhyay, M. J., Lahiri, K., & Mukhopadhyay, S. (2008). *In vitro* microtuberization and enhanced colchicine accumulation in two species of *Gloriosa*. *Cytologia, 73*, 357–63.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plant, 15*, 473-497.

Patel, R. M., & Shah, R. R. (2009). Regeneration of *Stevia* plant through callus culture. *Indian Journal of Pharmaceutical Sciences, 71*, 46-50.

Preethi, D., Sridhar, T. M., & Naidu, C. V. (2011). Carbohydrate concentration influences on *in vitro* plant regeneration in *Stevia rebaudiana*. *Journal of Phytology*, *3*, 61-64.

Rahman, M. M., Amin, M. N., Ahamed, T., Ali, M. R., & Habib, A. (2004). Efficient plant regeneration through somatic embryogenesis from leaf base-derived callus of *Kaempferia galanga* L. *Asian Journal of Plant Sciences, 3*, 675-678.

Satpathy, S., & Das, M. (2010). *In vitro* shoot multiplication in *Stevia rebaudiana* Bert., a medicinally important plant. *General and Applied Plant physiology, 36*, 167-175.

Singh, N., Garg, A., Yadav, K., & Kumari, S. (2010). Influence of growth regulators on the explants of *Commiphora mukul* (Hook. ex Stocks) Engl. under *in vitro* conditions. *Researcher, 2*, 41-48.

Singh, N., Yadav, K., Kumari, S., & Renu. (2011). Metabolic changes during differentiation in callus cultures of *Stevia rebaudiana* (Bertoni). *Journal of Phytology, 3*, 63-67.

Sivaram, L., & Mukundan, U. (2003). *In vitro* culture studies on *Stevia rebaudiana. In vitro cellular and Developmental Biology, 39*, 520-523.

Verma, S., Yadav, K., & Singh, N. (2011). Optimization of the protocols for surface sterilization, regeneration and acclimatization of *Stevia rebaudiana* Bertoni. *American- Eurasian Journal of Agricultural and Environmental Sciences, 11*, 221-227.

Yadav, K., Aggarwal, A., & Singh, N. (2013a). Evaluation of genetic fidelity among micropropagated plants of Gloriosa superba L. using DNA based markers- a potential medicinal plant. *Fitoterapia, 83*, 265-270.

Yadav, K., Aggarwal, A., & Singh, N. (2013b). Arbuscular mycorrhizal fungi (AMF) induced acclimatization, growth enhancement and colchicine content of micropropagated *Gloriosa superba* L. plantlets. *Industrial Crops and Products, 45*, 88- 93.

Yadav, K., Aggarwal, A., & Singh, N. (2012). Actions for *ex situ* conservation of *Gloriosa superba* L. - an endangered ornamental cum medicinal plant. *Journal of Crop Science and Biotechnology, 15*, 297-303.

Yadav, K., & Singh, N. (2010). Micropropagation of *Spilanthes acmella* Murr. - An important medicinal plant. *Nature and Science, 8*, 5-1.

Yadav, K., & Singh, N. (2011). *In vitro* flowering of shoots regenerated from cultured nodal explants of *Spilanthes acmella* Murr. - An ornamental cum medicinal herb. Analele Universităţii din Oradea – *Fascicula Biologie, 18*, 60-64.

Yadav, K., & Singh, N. (2012). Factors influencing *in vitro* plant regeneration of Liquorice (*Glycyrrhiza glabra* L.). *Iranian Journal of Biotechnology, 10,* 161-167.

Yasukawa, K., Kitanaka, S., & Seo, S. (2002). Inhibitory effect of stevioside on tumor promotion by 12-0-TCA in two stage carcinogenesis in mouse skin. *Biological and Pharmaceutical Bulletin, 25*, 1488-499.

Zibbu, G., & Batra, A. (2010). Effect of adeninesulphate on organogenesis via leaf culture in an ornamental plant: *Thevetia peruviana* (pers.) schum. *International Journal of Pharma and Bio-Sciences, 1*, 1-9.

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A New Latent Lovastatin Producer viz. *Fusarium pseudocircinatum* IBRL B3-4, Produced in Laboratory Tray System

Syarifah, A. R.[1]*, Darah, I.[1] and I Nyoman, P. A.[2]

[1]Industrial Biotechnology Research Laboratory (IBRL), School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[2]School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesa No. 10 Bandung, Indonesia 40132

ABSTRACT

A proximate analysis study of local rice bran and brown rice disclosed a distinguish level of chemical compositions. Lipid, carbohydrate and ash occupy in rice bran, while fibre is a predominant component in brown rice. Both perceptible anti-cholesterol substrates were grown with locally isolated *Fusarium pseudocircinatum* IBRL B3-4 to obtain the best lovastatin activity via dichloromethane extraction. Evaluation of lovastatin production at different substrate thickness ranges of 0.25 to 1.5 cm in a static tray system (20x20x6 cm^3) exposed the highest production at 0.5 cm level (1135.0±6.7 µg/g dry solid of lovastatin). Meanwhile, effects of physical parameters investigation interpreted that the original substrate size, 60% (v/w) moisture content and ambient local temperature of 30±2°C as the most suitable conditions to generate lovastatin at the utmost level. Meanwhile, the maximum production was synthesized at day 12th (twelfth) under solid substrate fermentation system. A significant activity increment was revealed after 60 % (v/v) moisture content had been applied into tray system. In more specific, it boosted up to 2271.7±14.4 µg/g dry solid of lovastatin.

Keywords: Solid substrate fermentation, tray system, *Fusarium pseudocircinatum*, rice bran, brown rice, proximate analysis

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E-mail addresses:
sar_1603@yahoo.com (Syarifah, A. R.),
darah@usm.my (Darah, I.),
yoman@sith.itb.ac.id (I Nyoman, P. A.)
* Corresponding author

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INTRODUCTION

World Health Organization (WHO) has reported a threat caused by cardiovascular diseases (CVDs) against human race. Annually, 7.5 million deaths have been

recorded with 51% fatality cases were caused by stroke and 45% due to coronary heart disease. If this pattern continues, it is estimated that by 2030, almost 25 million human beings are predicted to perish due to these silent killers. A waxy steroid substance known as cholesterol is the main element that causes CVDs. It is synthesized by the liver from a small precursor known as acetoacetyl-CoA, a vital isoprene unit (from acetoacetyl-CoA) and then constructed via a lengthy process which leads to cholesterol production. Cholesterol must be wrapped to be transported in the blood circulation by lipoproteins as carriers. There are two types of cholesterol produced, namely, low density lipoprotein (LDL) and high density lipoprotein (HDL). Dietary cholesterol and genetic factors are the controllers of CVDs. These include tobacco use, low intakes of fibrous food, extreme alcohol drinking, physical inactivity and also psychosocial factors. The extension of hypertension, abnormal lipid level and diabetes mellitus can also lead to cardiovascular problem.

Statins are reversible inhibitors of hydroxymethyl glutaryl-CoA (HMG-CoA) reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate limiting step in the biosynthesis of cholesterol via the mevalonate pathway. It is categorized under secondary metabolite cluster of fungi and divided into natural produced statin, semi synthetic and also synthetic. Lovastatin was the first administered as anti cholesterol lactone prodrug, announced by Food and Drug Administration. It appears in two major forms, namely, lactone and acid. Lactone is hydrolyzed *in vivo* to the corresponding β-hydroxyacid which occurs in mammal's liver. The active acid form (also known as mevinolinic acid) acts on the HMG-CoA reductase. Basically, it consists of two polyketide chains viz. nonaketide (go through cyclization to a hexahydronaphthalene ring system) and diketide, 2-methylbutyrate (Hendrickson *et al*., 1999). Research by Endo *et al.* (1985) on lovastatin biosynthesis in *Aspergillus terreus* has proven that polyketide pathway is involved in statin formation. On the other hand, Casas-Lopez *et al.* (2004) reported that lovastatin retarded its own synthesis in *A. terreus* under submerged fermentation (SmF) which indirectly coronated solid substrate fermentation (SSF) as a better medium for lovastatin production. In submerged culture, lovastatin stays in the mycelium that may suppress further synthesis, while in SSF, the provided space in solid substrate allowed absorption for lovastatin. Throughout the time, the definition of SSF keeps on changing because it is distinguished based on substrate utilization during the experiment that is divided into two; natural and inert substrate support. Recently, it is defined as a growing microorganism process on or within natural or inert substrate's particle, in absence or near absence of free flowing water (Pandey, 1992). The hallmarks of SSF are simple, inexpensive and high yield of secondary metabolites.

A special feature of filamentous fungi is their capability to generate assorted of

structurally natural products or secondary metabolites (Cole *et al*., 2003). The first outbreak of lovastatin producer (*Monascus rubber*) was reported by Endo in 1979, followed by Alberts and co-workers (1980) using *Aspergillus terreus*. This 'epidemic' consecutively spread around lovastatin academia and resulted in a variety of other potential producers such as *Aspergillus* sp., *Penicillium* sp., *Monascus* sp., *Paecilomyces* sp., *Trichoderma* sp., *Scopolariopsis* sp., *Doratomyces* sp., *Phoma* sp., *Phythium* sp., *Gymnoascus* sp., *Hypomyces* sp. and *Pleurotus* sp. (Gunde-Cimerman *et al*., 1973; Shindia, 1997). A recent report by Raghunath *et al.* (2012) stated that *Fusarium* sp. isolate has the capacity to synthesize lovastatin too. A lot of secondary metabolites fungi are enriched with polyketide synthase (PKS) genes and *Fusarium* sp. gained this gene (Xiangcheng *et al*., 2007; Brown *et al*., 2012) enabling them to produce lovastatin. Generally, *Fusarium* sp. is known in producing mycotoxin such as beauvericin, fumonisin, zearalenone, moniliformin and trichothecene. Although *Fusarium pseudocircinatum*, it does not produce detectable levels of beauvericin, it can produce moniliformin, low levels of fusaproliferin and trace levels of fumonisins (Fotso *et al*., 2002).

Up until now, various reports of analytical methods, parameters optimization and purification of lovastatin have been well published. Our recent report concentrated on the potentiality of chemical composition in the used substrates (rice bran and brown rice), effects of solvents during extraction programme and effects of cultural conditions (physical parameters) on lovastatin production in a tray system.

MATERIALS AND METHODS

Substrates, F. pseudocircinatum and Chemicals Sources

Substrates viz. rice bran and brown rice were bought at a local rice mill factory in Penang, Malaysia. The *F. pseudocircinatum* culture obtained from Industrial Biotechnology Research Laboratory's stock which had previously been isolated from oil palm farm soil located in the northern region of Malaysia. The culture was revived on potato dextrose agar (PDA) every fortnightly. Only fresh culture was used in performing solid substrate fermentation (SSF) for lovastatin production. All chemicals and solvents used in this experiment were purchased from different companies. Yeast extract (Scharlau Microbiology, Spain), sucrose (Bendosen, Norway) and calcium chloride (Fluka Chemika, Switzerland) were the optimized conditions for the tray system. Organic solvents for the extraction process [namely, acetonitrile (Merck, Germany), dichloromethane (Qrec, New Zealand), buthyl acetate (Merck, Germany), ethanol 99.7 % (Qrec, New Zealand), ethyl acetate (Bendosen, Norway), methanol (Qrec, New Zealand) and toluene (J.T. Baker, USA)] were obtained in AR grade, except for the acetonitrile used during the HPLC analysis. Lovastatin standard was procured from Calbiochem (Merck, Germany) with 99.7 % HPLC purity. Standard powder was dissolved in acetonitrile (HPLC

grade, Merck, Germany) and different concentrations were set up (10 to 100 µg/ml).

Proximate Analysis of Local Brown Rice and Rice Bran

Substrates used in SSF were evaluated for crude protein, ash, carbohydrate, moisture, fibre and lipid content based on the method of AOAC (1997). Total carbohydrate content of the substrates was measured by deducting the sum of the weight of moisture content, crude protein, lipid, ash and fibre from the total dry solid.

Influence of Assorted Solvents in Extraction

Various solvents with different polarity were screened to determine the best extraction agent for lovastatin recovery. Then, 10 mL of polar and nonpolar solvents (namely, methanol, acetonitrile, butyl acetate, ethanol, dichloromethane, ethyl acetate and toluene) were added in 1 g of dried fermented substrate which had been exposed to 80°C for 24 hour. The samples were sonicated for 5 min, followed by shaking at 30 °C, 200 rpm for 2 hours. The aliquot was centrifuged at 3000 g for 8 min to separate the solvent and substrate. Then, 1 ml of supernatant was collected and mixed up with 1 % (v/v) of trifluoroacetic acid (TFA) for lactonization purpose. The mixture was concentrated at 80°C without applying vacuum. They were then subjected into high performance liquid chromatography (HPLC) by diluting the concentrated mixture with 5 ml acetonitrile. Samples were filtered with nylon syringe filter size of 0.45 µm prior to HPLC injection (Pansuriya *et al*., 2010; Panda *et al*., 2010).

Time Course of Lovastatin Production in Different Substrate Thickness

Rice bran and brown rice are broadly known as cholesterol reducing agent. Both substrates consisted lovastatin but not in a very high value. In order to induce lovastatin production, 1.5% (w/w) of sucrose, 1% (w/w) of yeast extract and 0.5 % (w/w) of calcium chloride were supplied into four substrate thicknesses (tray size of 20x20x6 cm^3) under physical environmental of the original particle size, 70% (v/w) of moisture content, ambient incubation temperature (30±2 °C), inoculum size of 1 x 10^5 spore/ml and without mixing effect condition. Substrate thickness ranges of 0.25 cm up to 1.5 cm were autoclaved separately from any solution at 121 °C for 15 min and pH was set as 6.5, prior to autoclave. Afterwards, all the solutions were sterilely pippetted onto the substrate mixture (1:1) and thoroughly stirred for nutrients distribution in a tray. The fermented samples were harvested for every two days interval until day sixteenth.

Modification of Substrate Size, Moisture Content and Temperature

The combination of rice bran (which has an undefined particle size) and brown rice allowed an inter particle space to occur in SSF. Different particle sizes of the brown rice, ranging from 0.1 mm to original size, were investigated. The effect of moisture content was evaluated by varying the moisture percentages from 50% to

90% (v/w). The temperature influence towards lovastatin production was also determined by incubating the trays within the temperature range of 25 to 40°C.

Analytical Methods

Lovastatin Analysis

Retention times of lovastatin's peak produced by the samples were compared with the standard peak via HPLC (Waters, USA) in isocratic motion. The instrument was supplied with aqueous-organic mixture (namely phosphoric acid which was adjusted to pH 3.0) and acetonitrile as a mobile phase (23:77, v/v). A stainless steel column, Symmetry C_{18} (250 x 4.6 mm) was responsible to flow out solution at 1.0 mL/min under 238 nm uv absorbance (Huang *et al*., 2010).

Determination of Fungal Growth

The complexity in valuing the mycelia biomass or the extent to which the mycelia have penetrated into the solid substrate was one of the main problems came across in the SSF study. The combination methods of Tsuji *et al.* (1969) and Swift (1973) have overcome this problem by hydrolyzing the chemical compound presented in the cell wall of the fungi known as poly-N-acetylglucosamine or chitin into glucosamine. Tsuji *et al.* (1969) developed the conversion part of chitin. Later on, Swift (1973) found the glucosamine's assay method using Erhlick reagent. Glucosamine was detected spectrophotometrically at the absorbance of 530 nm.

RESULTS AND DISCUSSION

Proximate Analysis

The choice of substrate in SSF must meet the criteria of non-soluble in water, readily available and cheaper than synthetic substrate, possesses own physical and nutrient support and the most vital characteristic is that whether the substrate can obtain the specific product or not (Pandey, 2003). A study of crude nutrient contents in rice bran and brown rice was executed via proximate analysis. Basically, six categories of chemical properties were investigated and these are moisture content, crude ash, crude protein, fats or lipid, crude fibre and carbohydrate. Table 1 denotes the mean percentage of different compositions of rice bran and brown rice. Our local rice bran consist higher amount of lipid, carbohydrate and ash, while brown rice is enriched with fibre. A previous research by Wanyo *et al.* (2009) indicated that the existence of compositions in rice bran was almost in the same values as those in this study. These essential nutrients play a prominent role in human growth but lipid is always clustered under wrong concept. Both these substrates, rice bran and brown rice, are great carriers of unsaturated fatty acids (with no trans fatty acids) which is a good sign for cholesterol deduction. In addition, a report by the United States Department of Agriculture (USDA) declares that there are also Omega 3-fatty acids (alpha linoleic acid) and omega-6 fatty acid (linoleic acid) in rice bran and brown rice. These elements give an extra boosting factor for lovastatin production by *F. pseudocircinatum* in SSF.

TABLE 1
A study of chemical composition in local rice bran and brown rice (%)

Components	Rice bran	Brown rice
Moisture	10.5±0.3	9.3±0.4
Protein	13.4±0.2	12.1±0.3
Lipid	17.7±0.1	5.1±0.1
Fibre	7.1±1.9	48.5±0.6
Ash	10.04±0.3	2.5±0.3
Carbohydrate	41.3±2.1	22.5±0.5

Values are expressed as means ± standard deviation

Solvents Effect on Lovastatin

In fermentation, fungal mycelia prefers secreting lovastatin more in acid form (beta hydroxy acid) compared to the lactone form. However, acid, which is an active form of lovastatin, is less stable and by considering this matter, it needs to be converted into native form (lactone) via lactonization process. Lovastatin is a dimly non polar compound (Pansuriya & Singhal, 2009) and different polarity of solvents will hand in very much help to drag out lovastatin compound. Commonly, there are three group of solvents encountered namely polar protic, polar aprotic and non polar. As illustrated in Fig.1, dichloromethane, a 'borderline' polar aprotic solvent, showed a tremendous activity of lovastatin (281.7±44.4 µg/g dry solid) compared to acetonitrile (216.7±5.6 µg/g dry solid), butyl acetate (61.7±2.2 µg/g dry solid), toluene (60.0±3.3 µg/g dry solid), ethyl acetate (58.3±4.4 µg/g dry solid), methanol (38.3±2.2 µg/g dry solid) and ethanol which gained the lowest activity (33.8±2.6 µg/g dry solid). A previous report by Pansuriya and Singhal (2009) affirmed that the production of the acid form of lovastatin shed with a decrease in the solvent polarity. For this experiment, the addition of trifluoroacetic acid (TFA), a lactonization agent, drew a polar aprotic solvent as the best solvent to synthesize lactone lovastatin.

Meanwhile, the chromatogram pattern for lovastatin appearance using dichloromethane as a chosen extraction solvent was shown in Fig.2. Fermented sample pointed out a peak at retention time (R_t) of 7.9 min (Fig.2A) which is paralleled with the standard lovastatin (Fig.2B). Verification was made by overlaying those chromatograms and consequently, the peaks were equivalent (Fig.2C). Reports by Alvarez-Lueje *et al.* (2005), Szakacs *et al*. (1998) and Huang *et al*. (2010) recommended a flexible R_t for lactone lovastatin; 8.27 min, 9.20 min and 8.89 min, respectively. The cases of varying R_t depend on a few factors which include size column and types of solution used for mobile phase.

Initial Profiles of Substrate Thickness in Tray System

F. pseudocircinatum is clustered under African clan group which gains a rapid growth on potato dextrose agar. According to Nirenberg and O'Donnell (1998), it exposes short chains of microconidia and sterile coiled hyphae as distinguishable structures with other *Fusarium* sp. (Fig.3). A laboratory scale of simple tray system was done by growing *F. pseudocircinatum* in square tray in five thicknesses ranging from 0.25 cm (50 g) to 1.5 cm (200 g), as shown in Fig.4. Fig.4A indicates that

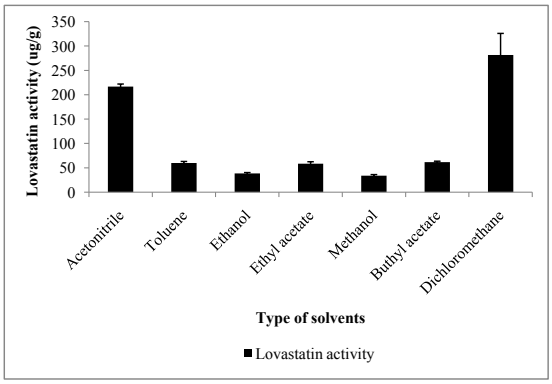

Fig.1: Solvents with different polarity did effect the lovastatin production. The polar aprotic dichloromethane illustrated the best extraction solvent for lovastatin

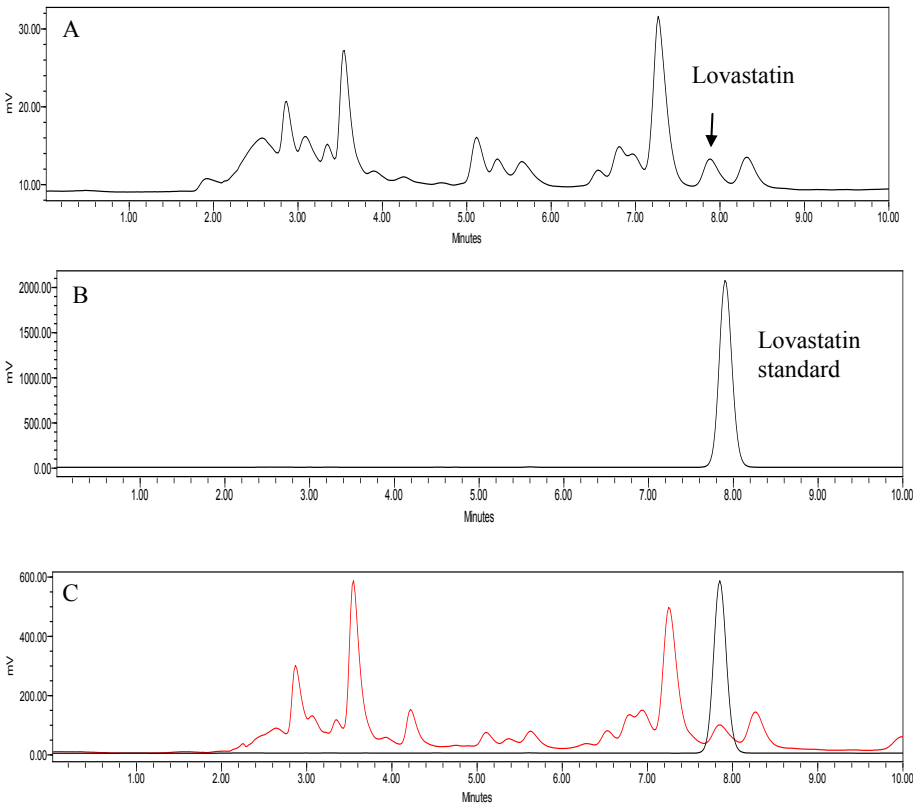

Fig.2: Fermented sample's peak (A) appeared at the same R_t with the standard lovastatin (B). The overlapping peak between sample and standard verified the lovastatin production by *F. pseudocircinatum* (C).

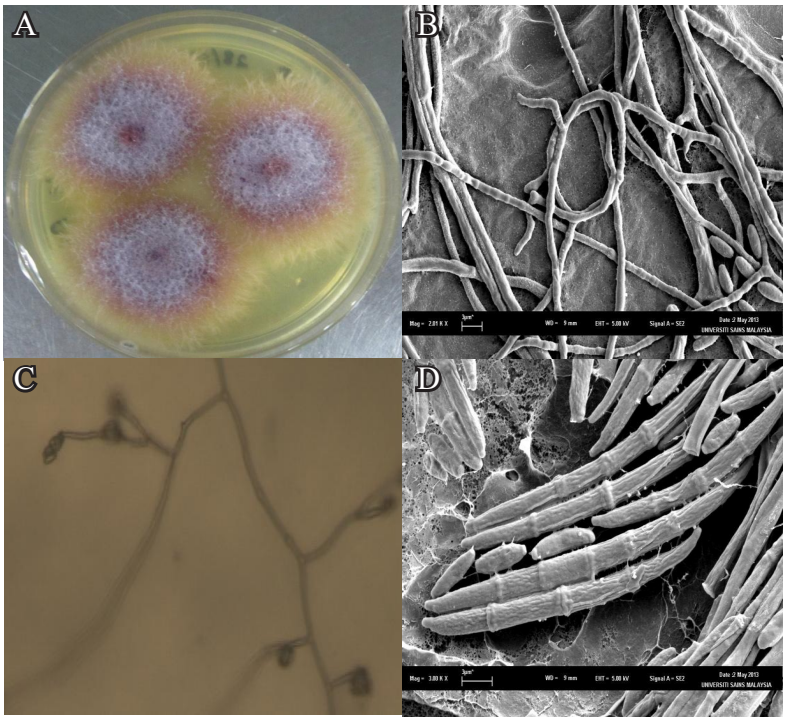

Fig.3: *F. pseudocircinatum* IBRL B3-4 image grew on potato dextrose agar (PDA) for seven day (A) and the special features of it namely coiled hyphae (B). The short chain structure was pointed out in (C) and (D) represented a close up of micro and macroconidia of this fungus

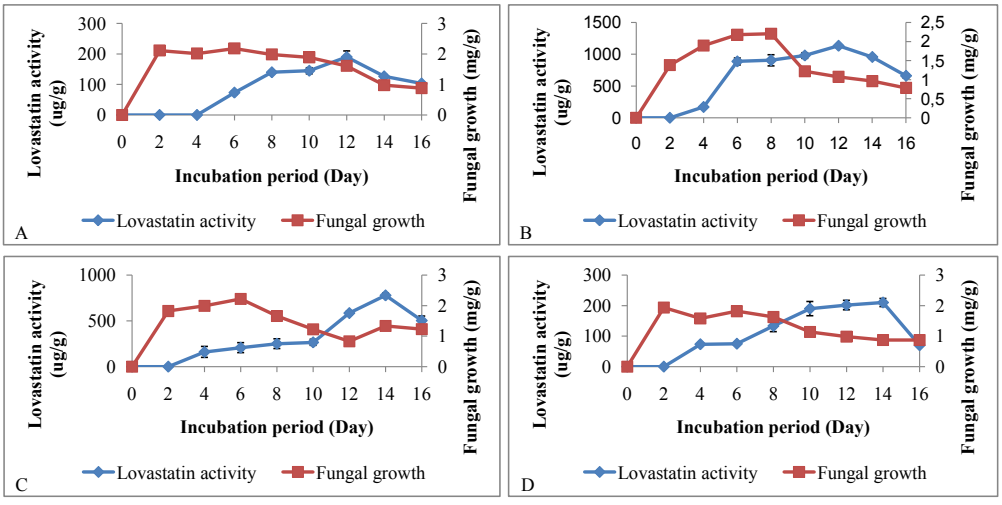

Fig.4: Initial profiles for lovastatin production at different substrate thickness. (A) was 0.25 cm, (B) was 0.5 cm, (C) was 1.0 and (D) was 1.5 cm. The highest activity obtained in (B) which equal to 100 g of substrate

the best production for 0.25 cm thickness is at the same day with 0.5 cm substrate thickness i.e. at day 12th (190.0±20.0 µg/g dry solid and fungal growth of 1.6±0.01 mg glucosamine/g substrate). By comparing all the thicknesses, 0.5 cm thickness (Fig.4B), which is equavalent to 100 g substrate mixture, showed the highest lovastatin production with 1135.0±6.7 µg/g dry solid and fungal growth of 1.1±0.03 mg glucosamine/g substrate. As the thickness got deeper, the production slowly became smaller and the optimum day started to extend. This phenomenon was found for the thickness of 1 cm and above. The activity hit the best production at day14. The best production for 1.0 cm thickness was 778.3±24.4 µg/g dry solid and fungal growth of 1.3±0.02 mg glucosamine/g substrate (Fig.4C). Meanwhile, the activity for 1.5 cm thickness was 210.0±13.3 µg/g dry solid with fungal growth of 0.87±0.09 mg glucosamine/g substrate. Nonetheless, no relationship tangled between lovastatin production and fungal growth.

Previous reports by Pei-Lian *et al.* (2006), Panda *et al.* (2010) and Pansuriya *et al.* (2010) stated that the optimum day for lovastatin produced by fungi was eleventh, fourteenth and tenth, respectively. *F. pseudocircinatum* IBRL B3-4 possesses an efficient mycelia system which permits it to board deep into the bottom part of the tray system and also breaks down the substrate. However, the problem occurred as the oxygen supply was limited beneath the substrate bed. Filamentous fungi preferred spreading between the solid substrate fragments and on the surface of substrate in order to form a mat, a conquering symbol for fungi in SSF. In the static fermentation conditions, the phenomenon related to mass and energy occurs along within the substrate bed. Metabolic heat production, conduction, diffusion gases, convective heat transfer, evaporation and convective mass transfer, are directly influenced the final product in SSF (Mitchell *et al.,* 2006).

The Effect of Substrate Size, Moisture Content and Temperature

Critical parameters in SSF (namely substrate size, moisture content and temperature) were studied. During substrate size investigation, various sizes of brown rice were inspected as the rice bran has an undefined size. Substrate particle size and shape affect the ability to access nutrients by microorganism. *F. pseudocircinatum* IBRL B3-4 demanded the original size of brown rice to achieve the highest production of lovastatin (see Fig.5). In order to beat other sizes (0.1 mm, 1 mm, 3 mm and 6 mm), the original size of brown rice has to set some suitable physical conditions including surface area, porosity, penetration space (Nandakumar *et al*., 1996) and a permission to allow a better ventilation. A study by Panda *et al.* (2010) using the original size of rice mold also showed a positive result of lovastatin production after growing with *Monascus* sp. In this experiment, a combination of brown rice with undefined size of rice bran permitted a large attacking area without causing substrate agglomeration, a common problem that occurred during small particle

size application (Couto & Sanroman, 2006). The indicated result showed that the highest production obtained by *F. pseudocircinatum* IBRL B3-4 was 1171.7±55.5 µg/g dry matter and fungal growth of 1.57±0.05 mg glucosamine/g substrate. Meanwhile, the lowest activity was omitted by the size of 1.0 mm and it produced 53.33±4.44 µg/g dry matter with 1.27±0.05 mg glucosamine/g substrate fungal growth. Couto and Sanroman (2006) informed that smaller sizes only approved the accumulation of substrate that unswervingly resulted in poor growth of fungi. In large scale fermentation, Mitchell *et al.* (2006) stated the particle size could affect the packing within the substrate bed and hence the airing of the bed. By comparing two different bed particle sizes with similar porosity, more trouble of forcing air through a bed of smaller particles (the phenomenon of pressure drop) was found. This condition causes air to choose an extra-large route (channeling phenomenon).

Moisture content is closely related to the definition of the SSF system; a process that involves solids in the absence or near absence of free water and the substrate itself must contained enough humidity to accommodate the growth and metabolism of microorganism. The key to the biological processes in SSF for elongation of hyphae, spores and metabolites production were detained by moisture content and water activity (Lenz *et al*., 2004). It is important to note that the optimal content of water is very crucial to identify the productivity of the SSF process as it comes in restricted amounts. As demonstrated in Fig.6, a moisture content of 60% (v/w) boosted up the lovastatin production up to 2271.7±14.4 µg/g dry matter with fungal growth of 2.3±0.03 mg glucosamine/g substrate. The lowest production was obtained by 50% (v/w) moisture content, with 468.3±64.4 µg/g dry matter and fungal growth of 1.3±0.03 mg/g. Pei-Lian *et al.*

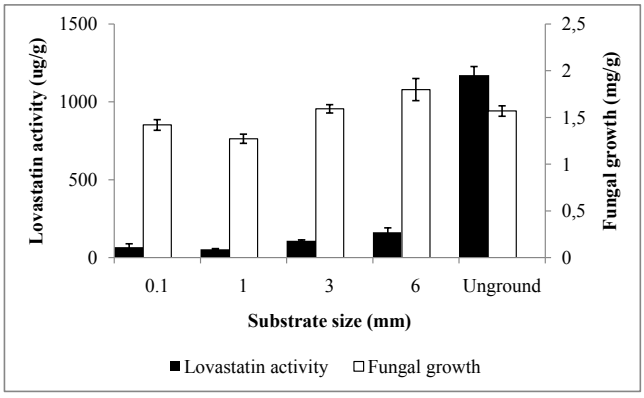

Fig.5: Effects of substrate size towards lovastatin production in a static tray system. After twelve-day incubation time, the natural size of brown rice maintained as the suitable size for lovastatin production by *F. pseudocircinatum* IBRL B3-4

(2006) in a paper work entitles 'Lovastatin production by *Aspergillus terreus* in solid state fermentation', generated 2.9 mg/g lovastatin under moisture level of 50-60%. Water presents in the SSF system in either a complex condition within the solid matrix (substrate) or as a thin layer which can absorb into substrate surface or slightly bonded to the substrate capillary (Raimbault, 1998). Lower moisture content encourages microorganism sporulation and at the same time deducts nutrient absorption, problem during mixing or agitation and results in high pressure of water. A high water percentage persuades decrement of substrate porosity, and this contributes to the viscosity of the medium and enhances chances of contamination. This matter was further amplified by Perez-Guerra *et al.* (2003) who reported that porosity reduction could limit the transfer of oxygen and attract bacteria contamination risk.

A mesophilic temperature is commonly chosen by filamentous fungi as it is similar to the original terrestrial. Many researchers have agreed that the filamentous fungi growth rapidly in 20 to 40°C (Manpreet *et al*., 2005). Fig.7 designates an ambient temperature, 30±2°C, as the best surrounding in the tray system for lovastatin production. The activity increased to 2298.3±8.9 µg/g dry matter with 2.7±0.09 mg glucosamine/g substrate fungal growth. Meanwhile, a temperature of 40°C has been indicated as an unfavourable condition for the production of this secondary metabolite compound. The obtained activity was 75.0±13.3 µg/g dry matter with 1.64±0.04 mg glucosamine/g substrate fungal growth. According to Jahromi *et al.* (2012) and Pei-Lian *et al.* (2007), the temperatures of 25°C and 28°C are the optimum surroundings for lovastatin production by filamentous fungi, *Aspergillus terreus*. The application of high temperature

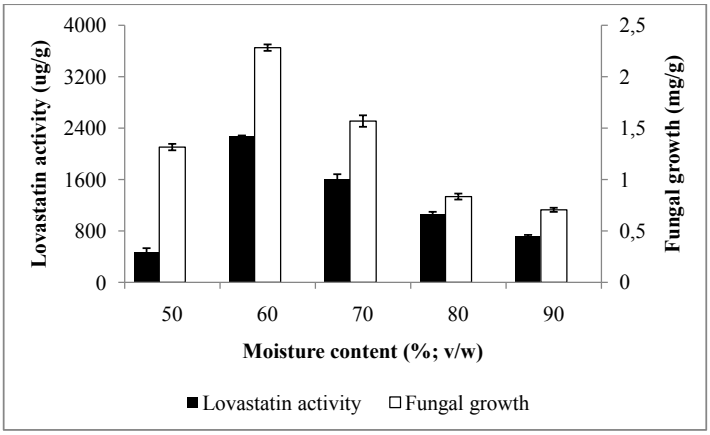

Fig.6: The presence of 60% (v/w) moisture content successfully increased lovastatin activity at the highest rate compared with other percentages

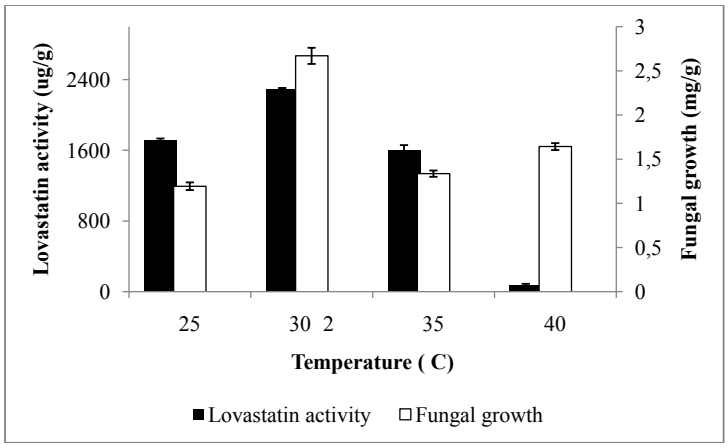

Fig.7: Ambient temperature (30±2°C) sustained as the most suitable parameter for production and fungal growth. This was closely followed by 25°C and 35°C.

will have significant impacts towards fungal growth and final product production. In SSF, a large amount of heat is generated to be used in microorganism metabolic activity which means the heat condition inside the tray is higher than the outside. In addition, there is a heat capacity spawned within substrate bed but this depends on the existing elements such as dry solid, liquid water, dry air and water vapour. As a result, plenty of upscaling bioreactors are fabricated from metal with aeration package to control the overheat problem. Frequently, convection or heat transfer is done in a few alternatives including via bioreactor wall, removal from solids to air and also removal due to air flow through the bed (Mitchell *et al*., 2006).

CONCLUSION

This is the first report of *F. pseudocircinatum* as an anticholesterol agent, lovastatin. Extraction by dichloromethane gained the best solvent mediator in SSF. Under 0.5 cm of substrate thickness, lovastatin activity was found to achieve the highest production with some physical modifications such as unaltered substrate size, 60% (v/w) of moisture content and ambient temperature of 30±2°C.

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REFERENCES

Alberts, A. W., Chen, J., Kuron, G. K., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J., & Springer, J. (1980). Mevinolin, a highly potent competitive inhibitor of hydroxymethyl-glutaryl

coenzyme A reductase and chlosterol lowering agent. *Proceedings Natural Academy of Science USA*, *77*, 3957–3961.

Alvarez-Lueje, A., Patine, J., Squella, J. A., & Nunez-Vergara, L. J. (2005). Assessment of the hydrolytic degradation of lovastatin by HPLC. *Journal of the Chilean Chemical Society, 4*, 639-646.

AOAC. (1997). Official methods of analysis of AOAC International.(16th Ed). In P.A. Cunniff (Ed., *Virginia AOAC International* (pp. 11-116).

Brown, D. W., Butchko, R. A. E., Baker, S. E., & Proctor, R. H. (2012). Phylogenomic and functional domain analysis of polyketide synthases in Fusarium. *Fungal Biology, 116*, 318-331.

Casas-Lopez, J. L., Sanchez-Perez, J. A., Fernandez Sevilla, J. M., Acien Fernandez, F. G., Molina, G. E., & Chisti, Y. (2004). Fermentation optimization for the production of lovastatin by Aspergillus terreus: use of the response surface methodology. *Journal of Chemical Technology and Biotechnology, 79*, 1119–1126.

Couto, S. R. & Sanromán, M. A. (2006). Application of solid-state fermentation to food industry-A review. *Journal of Food Engineering, 76*(3), 291-302.

Endo, A., Negishi, Y., Iwashita, T., Mizukawa, K., & Hirama, M. (1985). Biosynthesis of ML-236B (compactin) and monacolin K. *Journal of Antibiotic, 38,* 444-448.

Endo, A. (1979). Monacolin K, a new hypocholesterolemic agent produced by a Monascus species. *Journal of Antibiotic, 32*, 852–854.

Fotso, J., Leslie, J. F., & Smith, J. S. (2002). Production of beauvericin, moniliformin, fusaproliferin and fumonisins B_1, B_2 and B_3 by ex-type strains of fifteen Fusarium species. *Applied and Environmental Microbiology, 68*, 5195-5197.

Gunde-Cimerman, N., Friedrich, J., Cimerman, A., & Benicki, N. (1973). Screening of fungi for the production of an inhibitor of HMG-CoA reductase: Production of mevinolin by the genus Pleurotus. *FEMS Microbiology Letters, 111*, 203-206.

Hendrickson, L., Davis, C. R., Roach, C., Nguyen, D. K., Aldrich, T., McAda, P. C., & Reeves, C. D. (1999). Lovastatin biosynthesis in Aspergillus terreus: characterization of blocked mutants, enzyme activities and a multifunctional polyketide synthase gene. *Chemistry and Biology, 6*(7), 429-439.

Huang, Z., Xu, Y., Li, Y., & Wang, Y. (2010). Conversion investigation for lovastatin and its derivatives by HPLC. *Journal of Chromatographic Sciences, 48*, 631-636.

Lenz, J., Hofer, M., Krasenbrink, J. B., & Holker, U. (2004). A survey of computational and physical methods applied to solid state fermentation. *Applied Microbiology and Biotechnology, 65*, 9-17.

Mitchell, D. A., Krieger, N., & Berovič, M. (2006). *Solid state fermentation bioreactors: Fundamentals of design and operation*. Springer-Verlag Berlin Heidelberg.

Mohammad Faseleh, J., Juan, B. L., Yin, W. H., Rosfarizan, M., Yong, M. G., & Parisa, S. (2012). Lovastatin production by *Aspergillus terreus* using agro-biomass as substrate in solid state fermentation. *Journal of Biomedicine and Biotechnology*.

Nandakumar, M. P., Thakur, M. S., Raghavarao, K. S. M. S., & Ghildyal, N. P. (1996). Substrate particle size reduction by Bacillus coagulans in solid state fermentation. *Enzyme and Microbial Technology, 18*, 121-125.

Nirenberg, H. I., & O'Donnell, K. (1998). New Fusarium species and combinations within the Gibberella fujikuroi species complex. *Mycologia, 90*, 434-458.

Panda, B. P., Javed, S., & Ali, M. (2010). Optimization of fermentation parameters for higher lovastatin production in red mold rice through co- culture of Monascus purpureus and Monascus ruber. *Food and Bioprocess Technology, 3,* 373-378.

Pandey, A. (1992). Recent process developments in solid-state fermentation. *Process Biochemistry*, *27*(2), 109–117.

Pandey, A. (2003). Solid state fermentation. *Biochemical Engineering Journal, 13*, 81–84.

Pansuriya, R. C., & Singhal, R. S. (2009). Supercritical fluid extraction of lovastatin from the wheat bran obtained after solid state fermentation. *Food Technology and Biotechnology, 47*, 159-165.

Pansuriya, R. C., & Singhal, R. S. (2010). Response surface methodology for optimization of production of lovastatin by solid state fermentation. *Brazilian Journal of Microbiology, 41*(1), 164-172.

Pie-Lian, W., Zhi-nan, X., & Pei-Lin, C. (2007). Lovastatin production by Aspergillus terreus in Solid State fermentation. *Journal of Zheijan University, 8*(9), 1521-1526.

Raghunath, R., Radhakrishna, A., Angayarkanni,, J., & Palaniswamy, M. (2012). Production and cytotoxicity studies of lovastatin from Aspergillus niger PN2 an endophytic fungi isolated from Taxus baccata. *International Journal of Applied Biology and Pharmaceutical Technology, 3*(3), 342-351.

Raimbault, M. (1998). General and microbiological aspects of solid substrate fermentation. *Electronic Biotechnology, 1*(3), 1-15.

Shindia, A. A. (2001). Some nutritional factors influencing mevinolin production by Aspergillus terreus strain. *Folia Microbiology, 46*(5), 413-416.

Swift, M. J. (1973). The estimation of mycelial biomass by determination of the hexosamine content of wood decayed by fungi. *Soil Biology and Biochemistry, 5,* 321-332.

Szakacs, G., Morovjan, G., & Tengerdy, R. P. (1998). Production of Lovastatin by a wild strain of Aspergillus terreus. *Biotechnology Letters, 20*(4), 411-415.

Tsuji, A., Kinoshta, T., & Hosino, M. (1969). Analytical chemical studies of amino sugars: Determination of hexosamines using 3-methyl-2-benzothiazolone-hydrazone hydrochloride. *Chemical and Pharmaceutical Bulletin, 17,* 1505-1510.

Wanyo, P., Chomnawang, C., & Siriamornpun, S. (2009). Substitution of wheat flour with rice flour and rice bran in flake products: Effects on chemical, physical and antioxidant properties. *World Applied Sciences Journal, 7*(1), 49-56.

Xiangcheng, Z., Fengan, Y., Xing-Cong, L., & Liangcheng, D. (2007). Production of dihydroisocoumarins in Fusarium verticillioides by swapping ketosynthase domain of the fungal iterative polyketide synthase Fum1p with that of lovastatin diketide synthase. *JACS Communications, 129*, 36-37.

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   Monographs: Turner, H. N., & Yong, S. S. Y. (2006). *Quantitative Genetics in Sheep Breeding*. Ithaca: Cornell University Press.

   Chapter in Book: Kanwal, N .D. S. (1992). Role of plantation crops in Papua New Guinea economy. In Angela R. McLean (Ed.), *Introduction of livestock in the Enga province PNG* (p. 221-250). United Kingdom: Oxford Press.

8. ***Proceedings***: Kanwal, N. D. S. (2001). Assessing the visual impact of degraded land management with landscape design software. In Kanwal, N. D. S., & Lecoustre, P. (Eds.), *International forum for Urban Landscape Technologies* (p. 117-127). Lullier, Geneva, Switzerland: CIRAD Press.

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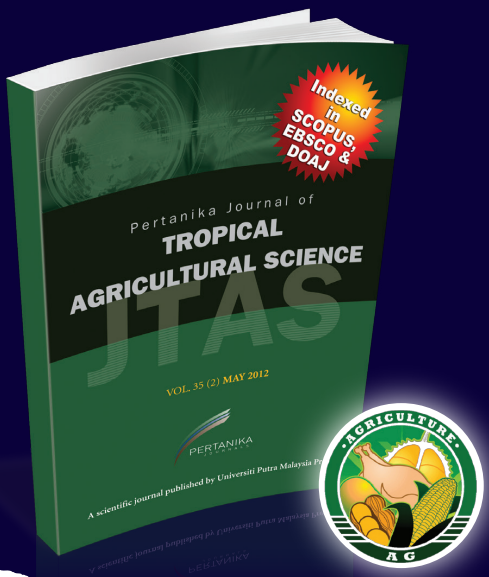

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Pertanika Editorial Office, Journal Division
Office of the Deputy Vice Chancellor (R&I)
1st Floor, IDEA Tower II
UPM-MTDC Technology Centre
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