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Biological Performance of *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) Upon Exposure to Sublethal Concentration of Imidacloprid

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ABSTRACT

The effects of sublethal exposure to imidacloprid (Kendor® 18.3SL) on the biological performance of the ladybird beetle, *Menochilus sexmaculatus* F., the most common coccinellid beetle found feeding on aphids, were studied under ambient laboratory conditions of 27-32˚C and 50-75% RH. The corn leaf aphid, *Rhopalosiphum maidis*, was offered as prey. The LC_{50} obtained from the contact bioassay at 24 h post-treatment for the regression slope, b=1.08, indicated that imidacloprid was likely to be selective. Sublethal exposure to imidacloprid caused reduction in survival with the females reaching 50% mortality by the 24th day, while that of the control was the 36th day. Meanwhile, demographic parameters including the net reproductive rate (R_o), generation time (T), innate capacity of increase (r_m) and the finite rate of increase (λ) of the treated females were markedly inferior as compared to the untreated females. The R_o value indicated that the control female was capable of producing 17.59 female offspring but treated female could only produce 2.55 female offspring during their generation time of 25.17 and 23.04 days, respectively. The capacity or instantaneous rate of increase (r_c) declined from 0.114 to 0.041, which was parallel with the decrease in the intrinsic rate of increased (r_m) value from 0.125 to 0.041. The values of λ were 1.133 and 1.042 for the control and treated population, respectively. In the meantime, the doubling time (DT) increased sharply to 16.86 days for the treated population, whereas DT for the control was 6.09 days. The sex ratio was biased towards the female and generally the females survived slightly longer (48 days) than the males (46 days), as observed in the control population.

Keywords: Biological performance, demographic parameters, imidacloprid, *Menochilus sexmaculatus*, sublethal effects

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INTRODUCTION

Coccinellid beetles are the most common and intensively studied aphidophagous insects for biological control. In fact, their biology and ecology have been extensively researched (Hodek, 1967; Hagen, 1974). Meanwhile, *Menochilus sexmaculatus* has been reported as the most common of these species feeding on aphids and being the most voracious and has the highest fecundity among the ladybird beetles in Malaysia (Parker & Singh, 1973).

Biological control practitioners usually assume that natural enemies are capable of keeping pest populations below economic thresholds but they have frequently failed to do so. This is because the natural enemies are often harmed by pesticides without regards, especially by non-selective chemicals that can greatly disrupt the stability in an agro-ecosystem (Parker *et al*., 1976). However, it is unthinkable to eliminate the use of insecticides in the near terms since the ladybird beetles alone may not be able to control the aphids below the economic thresholds. Without eliminating the use of pesticides, Integrated Pest Management (IPM) is greatly encouraged, and with the use of predators and parasitoid, the control strategies adopted are ecologically sound and environmentally friendly. To ensure its success, it is essential that natural enemies are not eliminated by insecticides. In this context, imidacloprid has been widely used in Malaysia. At a lower application rate and with fewer applications, imidacloprid has provided a long lasting efficacy; its contact, ingestion and systemic properties and its slightly selective nature, combined with modern application technologies, have in fact made it an important component in the IPM programmes (Iwaya & Kagabu, 1998). In the United States of America (USA), imidacloprid has been used to manage a wide range of insect pests such as aphids, mealybugs, whiteflies and certain scale insects (Ware & Whitacre, 2004), and extensively against the Colorado potato beetle, *Leptinotarsa decemlineata*. However, it was highly toxic to the adults and larvae of the natural enemy of the potato beetle, *Coleomegilla maculate* (Lucas *et at*., 2004). The impacts of neonicotinoid imidacloprid on the natural enemies in greenhouse and interiorscape environments have also been reported by Cloyd *et al*. (2010). This paper examines the sublethal effects of imidacloprid in the laboratory on the demographic performance of *M. sexmaculatus* when fed with corn leaf aphid, *Rhopalosiphum maidis*.

MATERIALS AND METHODS

Chemical Tested

Imidacloprid (Kendor® 18.3SL) was used in this study. Serial dilutions were prepared in distilled water with an initial concentration of 0.2 mg a.i. L^{-1}. For the next concentration of 0.02 mg a.i. L^{-1}, 1 ml was pipetted from the initial concentration and added into 9 ml of distilled water. The procedure was repeated for the subsequent dilutions until the fifth dilution of 0.00002 mg a.i. L^{-1}. The treatments were used immediately after the preparation so as to minimize any decomposition. The control consisted only distilled water.

Insect Culture and Maintenance

The predatory ladybird beetles, *M. sexmaculatus,* were collected from the corn field of Universiti Putra Malaysia (UPM) and mass reared in standard plastic containers (36 x 20 x 30 cm) containing a bouquet preparation of detached brinjal leaves kept fresh in a conical flask as shelters and oviposition substrate. The container was covered with muslin cloth to provide better aeration. Each container contained four pairs of adults. The corn leaf aphids, *R. maidis*, of all stages on detached corn leaves and tassels were provided as prey for the ladybirds. Smaller plastic containers (5 cm diameter x 4 cm) were used for rearing the newly emerged larvae. This culture was used for the experiments as well as for colony maintenance.

Experimental Environment

All the experiments were conducted in a laboratory under an ambient environment of 27-32˚C and 50-75% RH., with 24 h illumination (DURO-Test, 40-W True-Lite, Duro-Test International, Fairfield, New Jersey).

Contact Dose-mortality Bioassay

Filter paper discs, with a standard size of 9 cm in diameter, were used as a testing arena whereby they were dipped in the respective solutions of imidachloprid to complete immersion with light agitation for 5s and left to drip dry. Meanwhile, paper disc treated with distilled water was used as a control. Each of the treated filter paper disc was placed in a plastic container (9 cm diameter x 7.5 cm). Twenty adult female predators were transferred to each of the experimental arenas. Each experiment was replicated six times, and the predators were sustained with unlimited prey.

Mortality was recorded 24 h after the treatments. The beetles, which did not respond when probed with a fine brush, were considered as dead. An estimate of LC_{50} and the regression equation for the dose-mortality line was obtained using a probit programme based on the procedure of Finney (1971) (software: EPA version 1.5, USA). Nonetheless, no mortality was recorded in the control. The LC_{50} value was used for the sub-lethal treatments in the subsequent life-table studies.

The Effects of Sublethal Exposure of Imidacloprid on Survivorship and Pertinent Demographic Parameters

To initiate the study, a cohort of young male and female predators from the stock culture was selected to lay eggs overnight. All demographic data were obtained with these newly deposited eggs beginning with a batch of 15 males and 29 females for the control, and five other subsequent batches producing 121 females for the sublethal treatment, and ended with the death of the last adult. Initially, the eggs were removed the following day and placed in a plastic container (5 cm diameter x 4 cm) which was lined with a filter paper (5 cm diameter) for hatching. Upon emergence, the larvae were individually isolated with a soft brush into a plastic container lined with trimmed filter paper which was moistened daily with a few drops of distilled water. Corn aphids were provided ad libitum every day.

Two days after adult emergence, the control adults were individually transferred into plastic containers (9 cm diameter x 7.5 cm) lined with 9 cm clean filter paper disc, while 121 other females were individually transferred into plastic containers which had been lined with the filter paper disc treated with sublethal concentration of imidacloprid (0.002 mg a.i. L^{-1}) adopted from the bioassay study. In order to maintain continuous residual activity, each beetle was subsequently transferred daily to a freshly treated filter paper disc. Each surviving female was provided with an untreated male from the stock colony. They were fed with corn aphids every day. Daily records for their longevity and fecundity were made at about the same time to minimize any differences in sensitivity that might be associated with diet rhythm. Life table parameters were taken until the death of the last individual.

The demographic parameters were calculated as described by Birch (1948) and Laughlin (1965). A female biased sex ratio of 2:1 obtained from the previous control observation was assumed.

RESULTS AND DISCUSSION

Contact Dose-mortality Bioassay

The mortality of adult females increased with increasing concentration of imidacloprid This was indicated by 0.83% mean mortality with 0.00002 mg a.i. L^{-1} of imidacloprid compared with the highest concentration (0.2 mg a.i. L^{-1}) which resulted in 100% mortality.

No death was observed in the control. Other mortalities were 85.8% at 0.02 mg a.i. L^{-1}, 55.0% at 0.002 mg a.i. L^{-1} and 20.8% at 0.0002 mg a.i. L^{-1}. Cursory observations showed that imidacloprid illicited repellent effect, while disoriented movements away from the treated paper disc were observed. From the probit analysis, a small change in kill for a given variation in the concentration was indicated from the regression equation for dose-mortality line (y = 8.05 + 1.08x), and LC_{50} was 0.002 mg a.i. L^{-1} (Table 1). A study by Xue and Li (2001) showed that imidacloprid was relatively safe to *Coccinella septumpunctata*, a coccinellid species that is closely related to *M. sexmaculatus*, and this is thus comparable with the result of the current study. Nonetheless, field studies need to be conducted since it has been reported by Sur and Stork (2003) that imidacloprid is converted into a number of metablites within certain plants and become more water soluble and can be more toxic to natural enemies.

TABLE 1
Results of probit analysis for imidacloprid on females of *Menochilus sexmaculatus.*

No. treated	a	b ±S.E. (mg a.i. L^{-1})	LC_{50}	95% FL
720[a]	8.05	1.08 ± 0.07	0.002	0.001-0.002

[a] 20 females per replicate, with 6 replicates per concentration, 6 series of concentrations per assay

Survivorship

Fig.1 shows the age-specific survivorship for both the treated and untreated cohorts of *M. sexmaculatus* population. Overall, the survivorship was longer for the control than

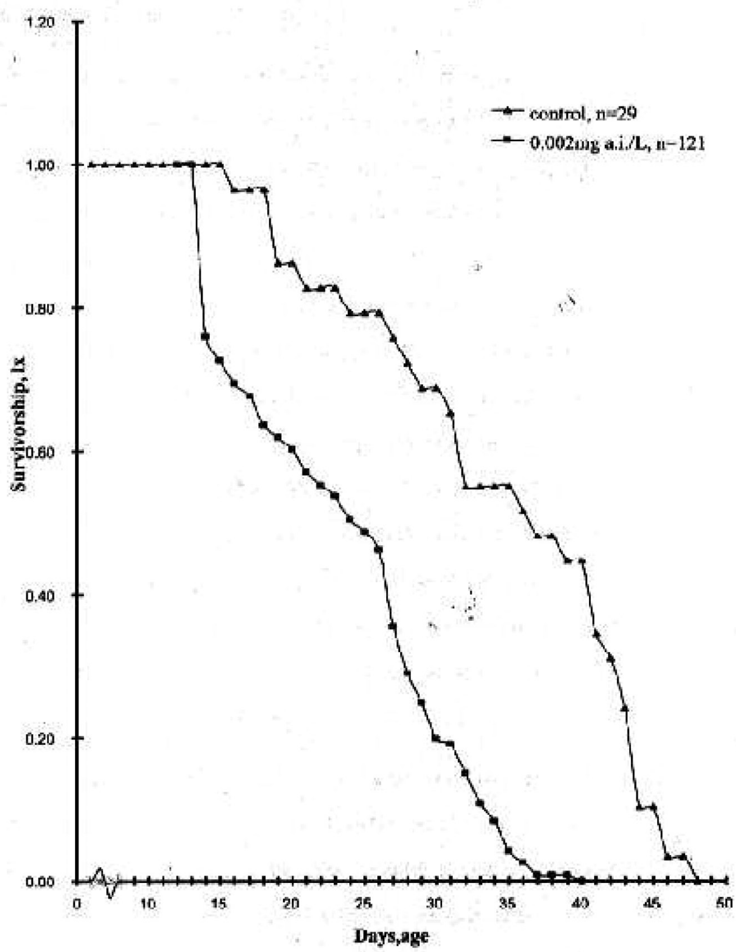

Fig.1: Survivorship of *Menochilus sexmaculatus* upon exposure to sublethal concentration of imidacloprid compared to the control.

with the treated population. Meanwhile, the females in the control group maintained a 100% survivorship for the first fifteen days, declined gradually and continued to survive with the last female to survive up to the 48th day as compared to 40th day for the treated females. On the contrary, the survivorship of the females declined sharply to 76% upon initial exposure to sublethal amount of imidacloprid and steadily declined to reach 50% mortality on the 24th day as compared to a longer survival of up to the 37th day for the control population. This indicates that a continuous exposure to sublethal doses of imidacloprid does not immediately affect the survivorship of *M. sexmaculatus*, and may be acting selectively at sublethal concentration.

Demographic Parameters

It was observed that both the treated and untreated females mated more than once during their reproductive lives. The females began to lay eggs on the 15th to 17th days, which was 3-5 days after they had emerged. From Fig.2, the peak oviposition period lasted for seven days for the control population and this was five days for the treated population. The peak oviposition fell on the 23rd day for the control, with a maximum of 3.21 eggs per female per day, while that of the treated population fell on the 22nd and 24th day with a maximum of 0.93 eggs and 0.86 eggs female^{-1} day^{-1}, respectively. The oviposition activity for the treated population declined sharply after the peak oviposition with the last egg being oviposited on the 36th day. The second peak oviposition for the control appeared on the 21st day with 2.42 eggs female^{-1} day^{-1}, and the third peak on the final 44th day with 1.33 eggs female^{-1} day^{-1}; the females did not oviposit any more eggs on the remaining three days of survival. For the control population, the total eggs deposited by the 29 females were 765, while those deposited by 121 females of the treated population amounted to 487 eggs. Therefore, each female had deposited an average of 26.4 and 4.0 eggs during its lifetime for both the control and treated populations, respectively. These data clearly showed that although *M. sexmaculatus* was capable of surviving through the sublethal exposure of imidacloropid, the reproduction was apparently and greatly reduced.

A study by Tank and Korat (2007) revealed that *M. sexmaculatus* could lay at least 195 eggs during its lifetime when fed with its preferred host *Aphis gossypii*; however, its mean life span was 34.15 days, and this was comparatively shorter than that recorded in the current study, i.e. 48 days, when fed with corn aphid *R. maidis*.

From the control population, a female biased sex ratio of 2:1 was obtained, while a 1.7:1 sex ratio was obtained from the treated population. It could be deduced that imidacloprid had slightly affected the sex ratio of *M. sexmaculatus* by laying fewer that would become females as compared to the control. The survivorship of the treated females relative to the number of female offspring born per female is shown in Fig.3. The maximum female offspring born per female was 0.63, which was achieved by the 24th day, and this was maintained through the 24th day. Barely half of the females survived up to this point. Thereafter, the number of the female offspring born dropped drastically as the female survivorship declined and reached 100% mortality on the 40th day.

The demographic parameters, including adult longevity and fecundity, showed slight differences between the control and treated populations (Table 2). Even though the treated adults survived to reproduce, the mean fecundity per female declined from 25.16 eggs in the control to 6.10 eggs in the treated population. The net reproductive rate, the intrinsic rate of increase and the finite rate of increase were inferior to the control and these were achieved within a shorter mean generation time of 23.0 days as compared to 25.2 days in the control. With these reductions, the doubling time increased sharply to 19.9 days, which was

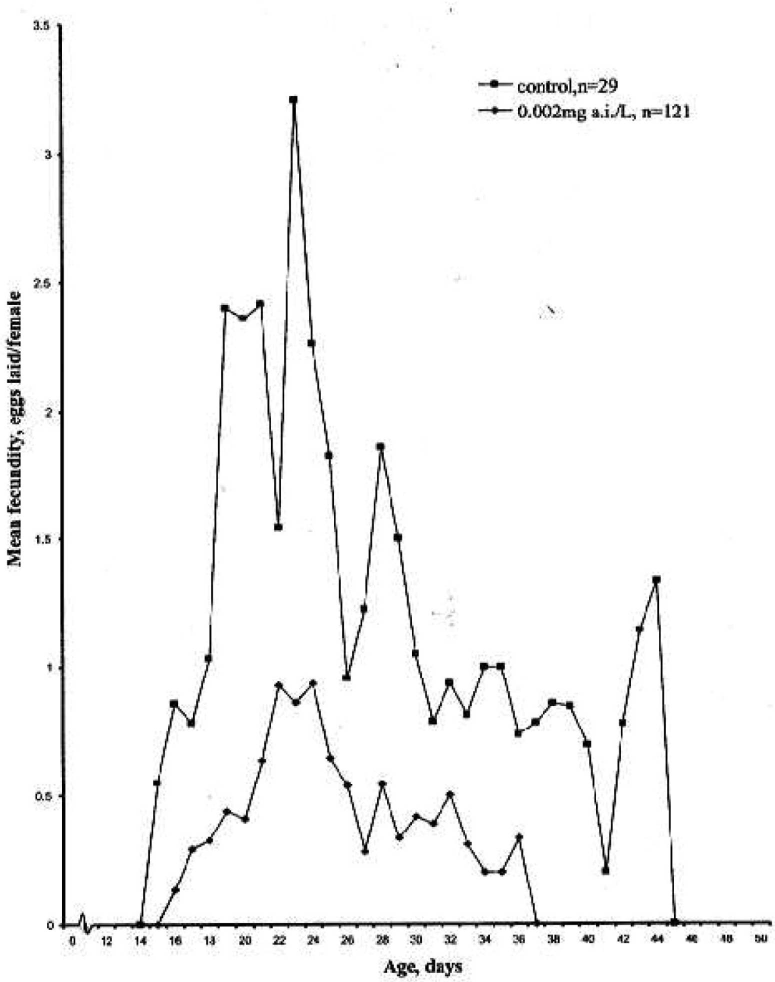

Fig.2: A comparison of the control and imidacloprid treatments on the fecundity of *Menochilus sexmaculatus*

10.8 days longer than that of the control population.

Meanwhile, the conservation of naturally occurring biological control agents is frequently limited by the incompatibility between insecticides and natural enemies. Therefore, knowledge on the sublethal effects of imidacloprid is important in adjusting the predator-prey ratio in an IPM programme. This study showed that although the reproductive performance of *M. sexmaculatus* was inferior to the control, the longevity was not drastically affected and neither was the sex ratio from the eggs laid by the treated females. Maintaining a female biased sex ratio is important since the females actively hunt and serve as reproductive agents. Thus, results from the current population demographic studies should provide sufficient basic

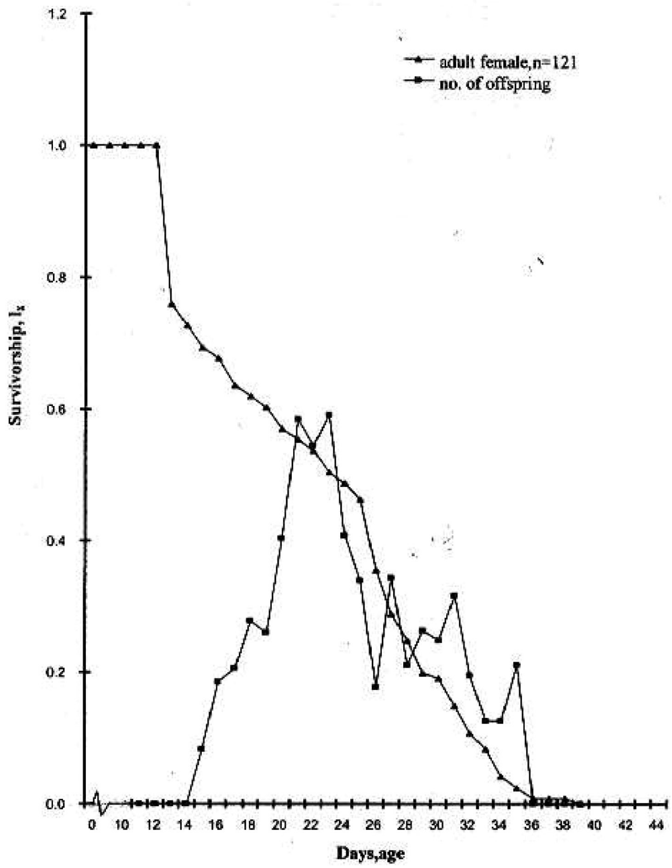

Fig.3: Survivorship of *Menochilus sexmaculatus* relative to the female offspring born per female upon exposure to sublethal concentration of imidacloprid

TABLE 2
A comparison of the demographic parameters of *M. sexmaculatus* upon sublethal exposure to imidacloprid

Parameters	Treatment concentration	
	Control (n=29)	0.002 mg a.i. L^{-1} (n=121)
Life span (days)	48.00	40.00
Fecundity	25.16	6.10
Net reproductive rate, R_0	17.590	2.550
Generation time, T (days)	25.171	23.037
Capacity of increase, r_c	0.114	0.041
Doubling time, DT (days)	6.085	16.855
Intrinsic rate of increase, r_{m*}	0.125	0.041
Finite rate of increase, λ	1.133	1.042

* $r = r_m$ when $\sum e^{-xr} l_x m_x = 1$ was fulfilled.

understanding on whether imidacloprid can be successfully integrated with biological control. As such, IPM studies in the field should follow to determine the full impact of imidacloprid on the bionomics of *M. sexmaculatus* in corn.

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