Efficacy and Sublethal Effects of Imidacloprid on The Survival and Fecundity of *Aulacophora foveicollis* (Lucas) (Coleoptera: Chrysomelidae)

Efikasi dan Kesan Submant Imidacloprid Terhadap Kemandarian dan Fekunditi Aulacophora foveicollis (Rom) (Coleoptera: Chrysomelidae)

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Novelty Statement Imidacloprid was ovicidal against *A. foveicollis* and might not induce physiological resurgence or resistance in the red pumpkin beetle

Abstract

The lethal and sublethal effects of imidacloprid were determined against the red pumpkin beetle, *Aulacophora foveicollis* (Lucas) (Coleopterea: Chrysomelidae). In a bioassay experiment, six varying concentrations of imidacloprid and a control were applied to the adults. The median lethal concentration (LC_{50}) was determined to be 0.005 mg a.i. L^{-1}. Assuming at this level of concentration to be sublethal, i.e. LC_{50} value, imidacloprid was found to significantly exert harmful sublethal effects on the beetle's survival and fecundity. Compared to the control, imidacloprid reduced the longevity of the treated beetle by 29 days and the fecundity to zero. This study showed that imidacloprid was ovicidal against *A. foveicollis* and might not induce physiological resurgence or resistance in the red pumpkin beetle.

Keywords *Aulacophora foveicollis*, bioassay, imidacloprid, sublethal effects, survival

Abstrak

Kesan maut dan submaut imidacloprid telah ditentukan terhadap kumbang labu merah, *Aulacophora foveicollis* (Lucas) (Coleopterea: Chrysomelidae). Dalam satu ujian bioasai, enam kepekatan imidacloprid yang berbeza serta satu kawalan telah dijalankan ke atas kumbang dewasa. Median kepekatan maut (LC_{50}) telah ditentukan sebanyak 0.005 mg a.i. L^{-1}. Apabila diandaikan aras kepekatan ini sebagai submaut, iaitu nilai LC_{50}, imidacloprid didapati secara signifikan mengakibatkan kesan kecederaan submaut ke atas kemandirian dan fekunditi kumbang itu. Berbanding dengan kawalan, imidacloprid merencatkan longeviti kumbang yang dirawat sebanyak 29 hari dan fekunditi kepada sifar. Kajian ini menunjukkan imidacloprid adalah bersifat ovisid terhadap *A. foveicollis* dan mungkin tidak mendorong kepada kemunculan semula secara fisiologi atau keresistanan kumbang labu merah.

Kata kunci *Aulacophora foveicollis*, bioasai, imidacloprid, kesan submaut, kemandirian

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Introduction

The red pumpkin beetle, *Aulacophora foveicollis* (Lucas), is almost exclusively phytophagous and the most important pest on pumpkin, *Cucurbita moschata* (Dutch) in Malaysia. It is known to attack many types of cultivated cucurbits throughout Asia, Africa and Europe (Berti, 1990). Both the larval and adult stages cause damage to the crop. The larva feeds on the roots, fruits and seedlings, while the adult feeds on the foliage, petals and fruits (Bogawat and Pandey, 1967). The larvae that feed on fruits lying on damp soil would allow ingress of microorganisms and cause the fruits to rot (Hoffman and Zitter, 1994). The adults can cause serious damage when aggregating while feeding and result in individual leaf being completely skeletonised, delayed maturation and eventual decimation of the crop. Its unique behaviour by pretending to be dead when disturbed or likely to fall off and then fly away shown the unwillingness to leave the habitat around the host plants; cucurbitaxins are the feeding stimulants regarded as the essential factors for this leaf beetle to recognize the cucurbitaceous host plants (Eben *et al*., 1997; Metcalf and Metcalf, 1992; Nishida *et al*., 1986; Ferguson *et al*., 1983).

This pest is mainly controlled by chemical insecticides. Since chemical has been reported to have adverse effects on humans (Matsumura, 1980) and the environment (Matsumura *et al*., 1972), ecology-based pest management (EBPM) in the form of integrated pest management (IPM) can be strategically environment- and eco-friendly to control this pest. This is especially urgent as it has been reported to have developed resistance to insecticides (Khan, 1992; Roy & Pande, 1991; Al-Ali *et al*., 1990).

Due to continuous insecticide application in the fields, the red pumpkin beetle would be exposed to sublethal concentrations of an insecticide for a long time and consequently may cause a range of sublethal effects. Continuous exposure to sublethal concentrations may result in both physiological and behavioural effects on individuals that survived as well as their offspring (Desneux *et al*., 2007). For example, sublethal effects of endosulfan on the mirid *Apolygus lucorum* significantly reduced the longevity and fecundity of females but slightly affected the longevity of males, and at the same time reduced the hatching rate in F_1 generation (Yangqing *et al*., 2008). Exposure to sublethal concentration of cypermethrin affected the chemical communication, courtship and oviposition of the cabbage looper, *Trichoplusia ni* (Clark & Haynes, 1992). On the other hand, hormoligosis may occur hence increase the reproductive rate of an insect pest (Luckey, 1968). A study by Wang *et al*. (2008) revealed that in both laboratory and green house tests, the number of alate offspring was significantly increased in the green peach aphid, *Myzus persicae*, while the offspring development time was extended in the laboratory.

Sublethal concentrations of insecticides have also adversely affected insect population dynamics. Chronic toxicity data are usually generated after continuous exposure to sublethal insecticide concentrations over many days or generations, hence the increasing attention to the long term sublethal effects (Stark & Banks, 2003). This paper reports the LC_{50} and the sublethal effects of imidacloprid on the adults of *Aulacophora foveicollis* (Lucas) (Coleoptera: Chrysomelidae). The report is focused on the biological performance upon exposure to sublethal concentration of imidacloprid and further qualifies the effects on longevity and fecundity of the pest.

Materials and Methods

Insect Culture

Aulacophora foveicollis adults were collected from a cucumber plot at the farm of Universiti Putra Malaysia (UPM) and mass-reared in the laboratory in plastic containers (20 x 30 x 17 cm) and maintained under the laboratory environment of 28±2ºC and 70±5% RH. The containers were covered with muslin cloth to provide better aeration. The colonies were fed daily with fresh pumpkin leaves and fresh cut ripe pumpkins, while moist soils in 9 cm Petri dishes were provided as oviposition substrate. The Petri dishes were replaced after every 12 days of incubation period. Subsequently, the larvae were carefully transferred to new containers and were provisioned with cucurbit seedlings in small shallow pots. These cultures were used for colony maintenance as well as the F_1 generation adults meant for this experiment.

Experimental Environment

All the experiments were conducted in the laboratory under the ambient environment of 28±2ºC and 70±5% RH with 24 h illumination (Duro-Test, 40-W True-Lite, Duro-Test International, Fairfield, New Jersey).

Contact Dose-Mortality Bioassay

A standard bioassay procedure following Snodgrass (1996) with a slight modification using a plastic container (8 cm diameter x 5 cm) instead of using glass-vials. Imidacloprid (Kendor® 18.3SL) was used in this study. Dilutions were prepared in distilled water with an initial concentration (company's recommended dosage) of 0.2 mg a.i. L^{-1} followed in decreasing order with 0.05, 0.02, 0.01, 0.005 and 0.001 mg a.i. L^{-1}. The control consisted only distilled water. The treatments were used immediately after the preparation in order to minimize any possible decomposition.

An initial cohort of 20 pairs of randomly selected young males and females from the stock culture were pooled to lay eggs overnight. These eggs were then transferred the following day to a container and raised until adulthood. From these, 25 one-day old F_1 adult females were selected randomly and placed in the treated plastic containers that had been sprayed earlier with 0.2 ml of the designated dilutions with the aid of a Sigma® hand atomizer. These treated containers were left to air dry in a fume chamber for 10 minutes. The containers were sealed with muslin cloth. This procedure was repeated with the rest of the treatments. The atomizer could deliver an even volume diameter droplet spray of 75 µm (VMD). The treatments were randomized with six replications.

Mortality was recorded 24 h after treatment. An individual was determined dead or moribund when it was unable to right itself or did not respond when probed with a soft brush. An estimate of LC_{50} and the regression equation with a 95% FL for the dose-mortality line was obtained using a probit programme based on the procedure of Finney (1971) (software EPA version 1.5, USA). The LC_{50} value was used for the sub-lethal treatment in subsequent study.

Effects of Sublethal Exposure of Imidacloprid on Survival and Fecundity

Eggs laid overnight from an initial cohort of 20 pairs of young males and females from the stock culture were raised to the adult stage. These F_1 adults were divided into two groups. All the adults in the first group were transferred to the plastic containers treated with the sublethal concentration of imidacloprid (0.005 mg a.i. L^{-1}). After 24 hours the surviving adults were separated and a pair was selected randomly and allowed to mate in a plastic container (15 x 8 x 9 cm). Cucumber seedlings and fresh cut ripe pumpkins were provided as foods. Moist soil in Petri dishes was provided as the oviposition substrate. The same procedure was followed for the second group of adults except that adults were transferred to containers treated earlier with distilled water to serve as the control. All together 30 pairs were observed for each treated and the control.

The longevity of females was recorded up to the death of the last female and the fecundity per female was determined by the daily egg counts. Unpaired t-test was used to determine statistical difference for the longevity and fecundity.

Results and Discussion

Contact Dose-Mortality Bioassay

The mortality of females increased with increasing concentration of imidacloprid. The highest concentration of 0.2 mg a.i. L^{-1} resulted in 100% mortality. Other mortalities were 98.7 % at 0.05 mg a.i. L^{-1}, 95.3% at 0.02% mg a.i. L^{-1}, 80% at 0.01, and 53.3% with 0.005 mg a.i. L^{-1}. At the highest concentration the beetle demonstrated rapid wing motion and leg tremors followed by disoriented movements, paralysis and eventually death. No mortality was recorded in the control. The probit analysis indicates that the regression equation for dose-mortality line is $Y = 10.61 + 2.39x$, while the median lethal concentration (LC_{50}) was determined to be 0.005 mg a.i. L^{-1} with 95% FL at 0.004-0.005 (Table 1). It was revealed that imidacloprid was relatively safe against predatory beetle *Coccinella septumpunctata* (Xue & Li, 2002), some predatory carabids, staphylinids (Kunkel *et al*., 1999; James & Vogele, 2001) and some predatory pentatomids, reduviids, and lygaeids (Elzen, 2001; James & Vogele, 2001; Hough-Goldstein & Whalen, 1993).

Sublethal Effects of Imidacroprid

Imidacloprid was found to be sublethally harmful on the longevity as well as fecundity of *A. foveicollis* (Table 2). The mean longevity of the treated females (38.1 days) was

Table 1 Probit analysis for imidacloprid on females of *Aulacophora oveicollis*

No. treated	a ± SE	b ± SE (mg a.i. L^{-1})	LC_{50}	95% F.L.
1050[a]	10.607±0.53	2.389±0.256	0.005	0.004-0.005

[a] 25 females per replicate, 6 replicates per concentration, and 6 levels of concentration per assay including a control

significantly shorter by 25.2 days when compared with the control (63.3 days) and this concurs with the results obtained by Kumar & Nadarajan (2008). It was observed that the time span of normal life for the untreated females before the first death was long with the number dropping drastically by the 58th day; conversely, the treated females began to die gradually from the 14th day after treatment. A study by Lashkari *et al*. (2007) also showed that sublethal dose of imidacloprid significantly reduced the adult longevity of the cabbage aphid *Brevicoryne brassicae*.

Table 2 Longevity and fecundity of female *Aulacophora foveicollis* upon exposure to sub-lethal concentration of imidacloprid

Number of adults	Mean longevity (Days)	Mean fecundity (Eggs female^{-1})
Control = 15	63.67±1.25 [a]	56.53±6.54 [a]
Imidacloprid = 15	36.06±2.63 [b]	0.0 [b]

Means within column followed by the same letter are not significantly different at $\alpha = 0.05$ as determined by Unpaired Wilcoxon-Mann-Whitney Rank Test

Significantly no oviposition was observed with the treated females as opposed to the untreated reproducing females which oviposited an average of 121.1 eggs per female throughout its life span. The lack of oviposition could be due to alteration in courtship and oviposition or deleterious effect on egg fertilization, oogenesis, ovulation, spermatogenesis and sperm mortality (Haynes, 1988). Due to the disturbance of the neurosecretary system, the reproductive physiology could be disrupted. Since fecundity is hormone-regulated, neurohormone imbalance upon insecticide poisoning could interfere with normal fecundity (Maddrell & Reynolds, 1972).

Besides reduction in life span and fecundity, sublethal effects may be expressed as reduction in developmental rate, egg hatching, nymphal survival and adult moulting rate (Stark & Banks, 2003).

Sublethal effects of imidacloprid upon natural enemies reported to date include reduction in prey consumption (Elzen, 2001) and altered locomotory behaviour (Vincent *et al*., 2000; Smith & Krischick, 1999). A beneficial sublethal effect was reported by James (1997) on the predatory mite *Amblyseius victoriensis* Womersley whereby an increase of up to 54% in egg production was recorded, however, conversely this response of hormoligosis could be highly disadvantageous in case of the pest mite, red spider mite *Tetranychus urticae*.

Conclusion and Recommendation

In order to fully assess the sublethal effects of imidacloprid on *A. foveicollis* under field condition, additional research is needed such that the sublethal effects could be related to the degradation dynamics of the insecticide under harsh tropical condition. Significantly shorter longevity and void of oviposition as the result of sublethal exposure of the females to imidacloprid implies that the compound may not induce physiological resurgence or hormoligosis, hence its suitability as a component in an integrated insect resistance management programme.

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