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THE EFFECTS OF DELTAMETHRIN APPLIED AT SUBLETHAL CONCENTRATIONS ON THE ADULTS OF *Anagrus nilaparvatae* (HYMENOPTERA: MYMARIDAE)

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ABSTRACT

Anagrus nilaparvatae is one of major parasitoids for *Nilaparvata lugens* eggs. Deltamethrin is a pyrethroid insecticide that is still used by rice farmers. This research aimed to investigate the effects of deltamethrin on the longevity, development time, emergence rate of progeny, actual and potential fecundicity of *A. nilaparvatae.* The insecticide was applied to the parasitoid adults at sublethal concentrations using the contact method in a test tube. The tested concentration was 0, 023 ppm (LC_{10}) and 2, 235 ppm (LC_{40}); and the control was treated with acetone. Each parasitoid surviving from deltamethrin treatment was exposed into *N. lugens* eggs in the rice seedlings for 24 hours. The seedling was then removed and substituted with new seedling until the parasitoid died. Each treatment was repeated 10 times. The application of deltametrin at sublethal concentrations decreased the longevity of adults, increased the development time of progeny, a decreased the actual and potential fecundicity, but no effect on the emergence rate. These findings suggest that the application of deltametrin to rice plants could reduce the potenty of *A. nilaparvatae* as a biological control agent of *N. lugens*.

Keywords: *Anagrus nilaparvatae*, deltamethrin, sublethal, parasitoid.

INTRODUCTION

Nilaparvata lugens Stal. (Homoptera: Delphacidae) has long been known as a major pest on rice in Indonesia. Increasing damage due to this pest recently occurred at the beginning of the rainy season in 2009/2010 in the Provinces of East Java, Central Java, and West Java (Untung and Trisyono, 2010). The population growth of *N. lugens* is influenced by the existence of natural enemies, cultural practices, use of insecticides, and climatic conditions.

Natural enemies play an important role in controlling *N. lugens* population in nature. It has been reported that there are 79 species of natural enemies of *N. lugens*, consisting of 42 parasitoids and pathogens and 37 predators (Chiu, 1979). The level of parasitization of *Anagrus* sp. in *N. lugens* eggs ranged from 15.7 to 35.7% with an average of 24.9% (Yaherwandi and Syam, 2007), and Maryana (1994) reported 11.3%. The parasitization capacity of *Anagrus* reached 38.2% in *N. lugens* living on rice plants and 64.1% in *N. lugens* residing on grass (Atmadja and Arifin 1990). This shows that *Anagrus* sp. is a potential natural enemy for controlling *N. lugens.*

The potential of biological control would decrease as a result of the unjudicious use of insecticide (Geiger *et al.*, 2010). Dichlorvos caused 100% mortality of *Anagrus nilaparvatae* only within two hours after oral treatment, whereas seven-days residues of thiamthoxam, triazophos, and fipronil caused 50-67, 5% mortality. The oral toxicity of the IGRs (buprofezin, chlorfluazuron, hexaflumuron, and JS 118) to *A. nilaparvatae* was considerably low. However, JS 118 and chlorfluazuron reduced the longevity and fecundity of *A. nilaparvatae* female parasitoid (Wang *et al*., 2008). Seven-days residue of imidacloprid caused up to 80.7% mortality of *A. nilaparvatae* (Wang *et al*., 2008). It also reduced the performance of *A. nilaparvatae* as shown in the weakened ability to seek the host resulting in reducing the parasitization capacity (Liu *et al*., 2010).

Deltamethrin is one of synthetic pyrethroid insecticides commonly used by farmers in Indonesia to control *Leptocorixa acuta* Thumb. (Prajnanta, 2008). Pyrethroid insecticide was especially destructive to parasitoid adults (99% mortality), but less toxic to parasitoids that were still in the host (larva or pupa) with 30-70% mortality (Sterk *et al*., 1999). Furthermore, deltamethrin was very capable of lowering the parasitoid's ability to parasitize the eggs of its host (Bastos *et al*., 2006), and the parasitoid population (Longley *et al*., 1997). According to the standard method of the International Organization Biological Control (IOBC), deltamethrin is classified as an insecticide that is destructive to parasitoid adults and slightly destructive to *Trichogramma* pupae (Hassan, 1994 *cit* Bayram *et al*., 2010).

The studies of insecticides on non-target insects including natural enemies should cover lethal and sublethal effects (Desneux *et al.*, 2007; Stark and Banks, 2003). The sublethal effect is very important as a reference for risk analysis (Stapel *et al.*, 2000; Stark and Banks, 2003). This research aimed to determine the effects of deltamethrin applied at sublethal concentrations on the adults of *A. nilaparvatae* and its subsequent life.

MATERIALS AND METHODS

Nilaparvata lugens

The initial population of *N. lugens* was obtained from the laboratory of Toxicology, Faculty of Agriculture, University of Gadjah Mada (Yogyakarta, Indonesia). This population is extremely susceptible to insecticide. Since 1985, it has never been exposed to any insecticide and there has been no addition of *N. lugens* population from the field. *N. lugens* has been reared using an established laboratory procedure utilizing rice seedling of Cisadane variety.

Approximately 100 pairs of *N. lugens* adults were taken from the stock population (the 360th generation) with an aspirator as starter or initial population. The starters were put into a breeding jar (with an upper diameter of 20 cm and a lower diameter of 17 cm, 20 cm hight) containing rice seedlings (7 days after germination) for egg-laying. After 3-4 days, the females laid eggs and about 5-7 days later the eggs hatched, producing nymphs. The nymphs were reared in the rice seedlings until they became adults. When the rice plants turned yellow, changing with the new rice seedlings was done by lifting the old plants and putting them over the new plants by turning them. The old plants were supported with wire to prevent them from covering the new plants. *N. lugens* moved from the old to the new plants. Three days later the old plants were removed. The plants used for feeding were 7-day-old rice seedlings after germination.

Anagrus nilaparvatae

The initial parasitoid population was obtained from the rice field in Bantul (Yogyakarta, Indonesia) by trapping (Trisyono, 1991; Maryana, 1994; Yaherwandi and Syam, 2007). The rearing of *A. nilaparvatae* was done by using a plastic box, which is a cage measuring 14x18.5x18.5 cm made of mica plastic (0.6 mm thick) and a test tube (10 cm long, 1 cm in diameter) was attached in the middle of the front side. This tube served to facilitate parasitoid harvest, because newly emerged parasitoids flew toward the tube. The harvest was done by substituting the old tube containing the parasitoids with the new one. On the other side (opposite the test tube), a screen cloth (6x10 cm) was provided for aeration. At the bottom of the plastic box was a plastic tray (15x20x3 cm) containing soil with a thickness of 1 cm and rice seedlings of the *Cisadane* variety aged one week containing *N. lugens* eggs. These eggs were produced by 100 gravid females of *N. lugens* per box. Honey was applied to each wall of the plastic box to provide feed for the parasitoids. Fifty female parasitoids were released into each box and their progeny emerged 9-13 days later.

Insecticide

The insecticide used was deltamethrin (technical grade) containing 97% active ingredient provided by PT Agricorn (Jl. Siliwangi No. 68 Bogor, Indonesia).

Toxicity of deltametrin to *Anagrus nilaparvatae*

A bioassay was carried out to determine the LC_{10} and LC_{40} values of deltamethrin on *A. nilaparvatae*. This was done by using the method developed by Desneux *et al*. (2006) with modification on the concentrations used. Based on the result from the preliminary test, six concentrations (starting from 0.015625-16 ppm in acetone) were chosen to obtain 5-95% parasitoid mortality. The series of concentrations were prepared by four times dilution. Each treatment used 10 adults, and each treatment was repeated four times. Acetone was used on the control. The contact method was adopted using a test tube (1.3 cm in diameter, 10 cm in length). The test tube was wetted with 0.1 ml solution of each concentration. The test tube was rolled so that the whole inner surface was covered with deltamethrin solution and then was allowed to evaporate for one hour. Ten female parasitoids were put into the test tube and allowed to be exposed to the insecticide for one hour. The surviving parasitoids were then moved to the clean test tube containing a rice seedling which had been infested with *N. lugens* eggs aged two days and had 10% honey solution on the aluminium foil (0.5x4 cm). The test tube was capped with fine gauze cloth. The mortality of parasitoid was observed 24 hours after treatment. A probit analysis (Finney, 1971) was performed using Software SAS 9.3.1 Portable. The data would be analyzed if the mortality of the control was <20%.

The effects of deltamethrin applied at sublethal concentrations on the adults of *Anagrus nilaparvatae* and its subsequent life

A. nilaparvatae adults were treated with deltamethrin applied at sublethal concentrations (LC_{10} and LC_{40}) using the contact method, whereas the control was treated with acetone. The contact method used was the same as the one used in the testing for the toxicity of deltamethrin to *A. nilaparvatae.*

The plants used in this research were one-week-old rice seedlings. A one-week-old rice seedling was enclosed with tissue paper measuring 1x3 cm (4 layers) at the root and then wrapped in an aluminium foil of the same size. The tissue paper was immersed in water to keep the plant fresh. The edge of the seedling was cut to make its length the same as the length of the test tube (10 cm). One seedling was put into the test tube with its root at the bottom of the tube. The seedlings were prepared according to the number of treatments.

Three female adults of *N. lugens* that were about to lay eggs were put into the test tube that contained plants, and then it was capped with gauze cloth stuck with duck tape. The *N. lugens* adults were allowed to lay eggs on the rice plants for two days and then taken out. One female adults of *A. nilaparvatae* that was still alive after treatment of LC_{10} and LC_{40} was put into each test tube. The parasitoids were fed with 10% honey solution by applying it to the aluminium foil (0.5x4 cm) placed in the test tube. The rice seedlings were replaced daily by new seedlings that also contained *N. lugens* eggs. This

replacement was done until the female parasitoids died. Each treatment was repeated 10 times.

Observation was made on the parasitoid's longevity, developmental time, emergence rate of new progeny, and fecundity. The longevity was determined from the number of days since the adults emerged, treated with insecticide, given to the host of *N. lugens* eggs until the parasitoid died. The developmental time was obtained by adding up the result of the multiplication between the day of the parasitoid's emergence and the number of adults that emerged on that day divided by the total number of emerging adults. The emergence rate was determined by counting the percentage of parasitoids that emerged per day divided by the total of all emerging parasitoids. The fecundity observed was both actual and potential fecundity. The actual fecundity was determined by adding up the progeny of the parasitoids that emerged and those that failed to emerge (i.e., still in the eggs) by dissecting the rice plants tissue at the end of observation. The potential fecundity (egg-production potential) was the summation of actual fecundity and the remaining eggs in the ovary after the parasitoid died.

Analysis of Variance (ANOVA) was performed using Completely Random Design employing SAS 9.1.3. Portable. Analysis was continued with LSD test when significant differences existed (Gomez and Gomez, 1995). The data were analyzed using software SAS 9.1.3. Portable.

RESULTS AND DISCUSSIONS

Toxicity of deltamethrin to *Anagrus nilaparvatae*

LC_{10} and LC_{40} of deltamethrin with the contact method on *A. nilaparvatae* were 0.023 ppm and 2.235 ppm (Table-1). These values were then used to determine the sublethal effects of deltamethrin on *A. nilaparvatae* adults and their progeny.

Table-1. The toxicity of deltamethrin to newly emerged adults of *Anagrus nilaparvatae* employing the contact method*.

Parameter	Value
Number of test insects	360
LC_{10} (95% FL) (ppm)	0.023 (0.002 - 0.064)
LC_{40} (95% FL) (ppm)	2.235 (0.962 -12.172)
LC_{50} (95% FL) (ppm)	6.935 (2.331 - 81.901)
Slope ± SE	0.51 ± 0.05

***** Parasitoids were released into the deltamethrin-treated test tube for an hour

LC_{10} (0.023 ppm) and LC_{40} (2.235 ppm) of deltamethrin on *A. nilaparvatae* were lower than the recommended concentration mostly used in the field. The lowest recommended concentration for controling *Spodoptera litura* and *Leptocorixa acuta* in the rice ecosystem is 12.5 ppm (Anonymous, 2011a; Anonymous, 2011b). This shows that deltamethrin could be potentially destructive to *A. nilaparvatae.*

The effects of deltamethrin applied at sublethal concentrations on the adults of *A. nilaparvatae* and its subsequent life

The longevity and developmental time of *A. nilaparvatae* were not significantly different between those of the control and the treatment of deltamethrin with sublethal concentrations. However, the longevity of parasitoid tended to shorten and the developmental time tended to increase with the increasing concentrations of deltamethrin (Table-2). The effect of insecticide on longevity depends on insecticides, the species of parasitoid, and the method of application (Bayram *et al*., 2010). Deltamethrin reduced the longevity of *Telenomus busseolae* after treatment at LC_{25} (Bayram *et al*., 2010), the longevity of *Aphidius ervi* (Desneux *et al*., 2006), and the longevity of *Habrobracom hebetor* (Sarmadi *et al*., 2010). Conversely, reduced longevity did not happen to *Trissolcus grandis* when treated with deltamethrin (Saber *et al*., 2005). The emergence rate of *T. grandis* adults from the host's egg reduces up to 34.4% (Saber *et al*., 2005) and the emergence rate of the adults of *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae) up to 30% (Vieira *et al*., 2001).

Table-2. The effects of deltamethrin applied at sublethal concentrations on newly emerged adults of *Anagrus nilaparvatae* and its subsequent life.

Concentration	Longevity (days)	Developmental time (days)	Fecundity	
			Actual (offsprings/female)	Potential (eggs/female)
Control	2.0 a	10.0 a	26.8 a	40.4 a
LC_{10} (0.023 ppm)	1.8 a	10.1 a	19.3 b	23.7 b
LC_{40} (2.235 ppm)	1.5 a	10.2 a	13.7 b	15.7 c

Note: Parasitoid adults were treated individually using the contact method. Each treatment was repeated 10 times. The averages followed by the same letters in the columns were not significantly different at 5% level with the LSD test.

The actual fecundity of *A. nilaparvatae* in the control was significantly higher compared with those treated with deltamethrin at LC_{10} and $LC_{40.}$ Increasing the concentration of deltamthrin tended to decrease in the actual fecundity of *A. nilaparvatae*. In the same way, the potential fecundity of the control was higher compared with those treated with deltamethrin. The higher the sublethal concentration applied, the lower the potential fecundity of *A. nilaparvatae*. The percentage of the emerging female parasitoids was extremely high (> 95%) and the application of insecticide did not affect the emergence of female parasitoids. The actual fecundity of *A. nilaparvatae* decreased up to 50% as a result of the sublethal effect (LC_{40}) of deltamethrin. Deltamethrin applied at high concentrations also reduced potential fecundity >50%. Reduced fecundity after exposure to deltamethrin also occurred to *Trichogramma pretiosum* (Bastos *et al*., 2006). *Diaeretiella rapae* and *Aphidius ervi* (Desneux *et al*., 2006 ab), and *Habrobracon hebetor* (Samadi *et al*., 2010). In contrast, Gracia *et al*., (2006) showed that the fecundity of *T. cordubensis* was not affected by deltamethrin if it was applied to pupa, but it reduced the fecundity when applied to adults. These results show that the stadia of parasitoid exposed to insecticide affect the final fecundity of parasitoid.

The negative effects of an insecticide on parasitoid will eventually make the performance of parasitoid less effective as an agent of biological control in the field (Poletti *et al.,* 2007). The results of this research clearly show that sublethally exposed *A. nilaparvatae* produced fewer progeny, which will affect its ability to control the population growth of *N. lugens*. Information on the lethal and sublethal effects of an insecticide to natural enemies is useful to determine the selectivity and compatibility of the insecticide with biological control agents (Desneux *et al*., 2007).

CONCLUSIONS

Application of deltamethrin at sublethal concentrations could reduce the potency of *A. nilaparvatae* in controlling the population of *N. lugens* because it decreased the fecundity of *A. nilaparvatae* even though had no affect on the longevity as well as development time of their progeny.

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