Jurnal Perlindungan Tanaman Indonesia, Vol. 21, No. 2, 2017: 80–86
DOI: 10.22146/jpti.24759

Research Article

Impact of Abamectin on *Anagrus nilaparvatae*, An Egg Parasitoid of *Nilaparvata lugens*

Dampak Abamektin terhadap Anagrus nilaparvatae*, Parasitoid Telur* Nilaparvata lugens

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Submitted May 3, 2017; accepted June 5, 2017

ABSTRACT

Anagrus nilaparvatae (Hymenoptera: Mymaridae) is an egg parasitoid potential for controlling the major pests on rice, the brown planthopper (*Nilaparvata lugens* [Hemiptera: Delphacidae]). Abamectin is one of insecticides registered for *N. lugens.* The research was aimed to investigate the impact of contact application of abamectin on the parasitism level of *A. nilaparvatae* under laboratory conditions. Adults of *A. nilaparvatae* and the first instars as well as adults of *N. lugens* were exposed to the residue of abamection inside the test tube. *A. nilaparvatae* was much more susceptible to abamectin compared to *N. lugens.* Application of abamectin at the recommended concentration (22.78 ppm) for 30 min caused 100% mortality, and it reduced to 85% when the concentration was decreased to 0.36 ppm. In contrast, the mortality for the first instar of *N. lugens* was only 15% at 22.78 and no mortality at 0.36 ppm. No *N. lugens* adults died even when they were exposed to 22.78 ppm. Furthermore, the parasitism test was conducted using 38 days after planting of IR-64 rice variety. Those plants were infested with 50 females of *N. lugens* for 2 days. *A. nilaparvatae* were exposed by contact to 0.02, 0.23, and 2.28 ppm of abamectin. The survivors were released to the rice plant containing eggs of *N. lugens*. Contact application of abamectin reduced parasitism level of *A. nilaparvatae* as much as 86.34, 70.01, and 28.43% with concentrations of 2.28 ppm, 0.23 and 0.02 ppm, respectively. In addition, the number of parasitoids emerged decreased with increasing concentration of abamectin. These results suggest that abamectin could be detrimental to *A. nilaparvatae* due to direct mortality, reduced the parasitism level, and decreased the number of progeny produced.

Keywords: egg parasitoid, emergence, insecticide, mortality

INTISARI

Anagrus nilaparvatae *(Hymenoptera: Mymaridae) merupakan salah satu parasitoid telur yang berpotensi untuk mengendalikan hama utama tanaman padi, wereng batang padi cokelat (*Nilaparvata lugens *[Hemiptera: Delphacidae]). Abamektin adalah salah satu insektisida yang terdaftar untuk pengendalian* N. lugens. *Penelitian ini bertujuan untuk mengetahui dampak aplikasi kontak abamektin terhadap suseptibilitas dan tingkat parasitasi* A. nilaparvatae *terhadap telur* N. lugens *pada kondisi laboratorium. Imago* A. nilaparvatae *serta instar satu dan imago* N. lugens *dipapar dengan residu abamektin di dalam tabung reaksi.* A. nilaparvatae *lebih peka terhadap abamektin dibandingkan* N. lugens*. Aplikasi abamektin pada konsentrasi anjuran (22,78 ppm) selama 30 menit menyebabkan mortalitas* A. nilaparvatae *100%, dan mengurangi sampai dengan 85% pada konsentrasi yang lebih rendah 0,36 ppm. Sebaliknya, mortalitas instar satu* N. lugens *hanya sebesar 15% pada 22,78 ppm dan tidak menimbulkan kematian pada 0,36 ppm. Konsentrasi 22,78 ppm tidak menimbulkan kematian imago* N. lugens*. Selanjutnya, uji parasitasi dilakukan menggunakan media tanaman padi varietas IR-64 umur 38 hari setelah tanam. Tanaman diinfestasi dengan 50 ekor betina* N. lugens *selama dua hari.* A. nilaparvatae *dipapar abamektin dengan metode kontak pada konsentrasi 0.02, 0,23, dan 2,28 ppm. Parasitoid yang mampu bertahan hidup dilepaskan pada tanaman padi yang telah diinfestasi telur* N. lugens. *Aplikasi kontak abamektin mengurangi tingkat parasitasi* A. nilaparvatae *sebesar 86,34, 70.01*, *dan 28,43% pada konsentrasi 2,28; 0,23; dan 0,02 ppm. Selain itu, jumlah parasitoid yang muncul semakin menurun dengan peningkatan konsentrasi abamektin. Hasil ini menunjukkan bahwa abamektin dapat merugikan secara langsung terhadap mortalitas serta mengurangi tingkat parasitasi dan jumlah keturunan* A. nilaparvatae*.*

Kata kunci: insektisida, kemunculan, mortalitas, parasitoid telur

INTRODUCTION

The rice brown planthopper (*Nilaparvata lugens* Stal.) (Hemiptera: Delphacidae) is the major insect pest of rice in Asia. In Indonesia, *N. lugens* was reported as pest on rice by Stal in 1894 and the first outbreak occurred in 1978−1979 causing damage of hundred thousand hectares of rice farms (Mochida Okada, 1979). In 2009, *N. lugens* received serious attention in rice producing countries in Asia, such as Vietnam, China, Indonesia, Korea, Japan, and Malaysia since its invasion simultaneously happened and expanded reaching million hectares. The hopperburn caused the yield loss up to 90% (Heong, 2009). In Indonesia, the peak of damage took place in 2011 with area of 223,606 ha (Directorate of Food Crop Protection, 2012). In addition, *N. lugens* could vector viruses causing the grassy and ragged stunt diseases. The development of *N. lugens* could be enhanced by the high humidity, non-uniform planting dates, use of susceptible variety, and inappropriate application of insecticides (Baehaki & Mejaya, 2014).

The application of wide spectrum insecticides damaged the role of natural enemies, left the residue on plant and environment, and resulted the resistance on target pest (Chelliah & Heinrichs, 1980). Furthermore, misuse of insecticide causing the sub-lethal dose influenced the behavior, interrupted physiological process, and reduced the fecundity of parasitoid which eventually decreased the parasitism level and effectiveness in controlling the target pest (Hardin *et al.*, 1995). Wang *et al.* (2008) reported that the application of dichlorvos orally for two hours caused 100% mortality of *Anagrus nilaparvatae*, while the residue of thiametoxam, triazophos, and fipronil at the 7th day after application could resulted in mortality between 50.0−67.5%. Furthermore, the application of deltametrin at the sub-lethal dose declined more than 50% of the fecundity of *A. nilaparvatae* (Hymenoptera: Mymaridae) (Meilin *et al*., 2012a).

Anagrus nilaparvatae is a potential parasitoid to control *N. lugens.* The parasitism level of *A. nilaparvatae* on eggs of *N. lugens* varied depending on the environmental conditions (Dupo & Barion, 2009). In Japan, parasitism level of *A. nilaparvatae* in May−June ranged between 11.3−29.6% and in September−November between 3.3−29.6% (Chiu, 1979). In Srilanka, parasitism level of *Anagrus* sp. could reach 75−82% (Fowler *et al.*, 1991) and in Taiwan parasitism level of *A. optabilis* was about 2.4−43.9% (Miura *et al.*, 1981). In Indonesia, parasitism level of egg parasitoids has been reported up to 53.45% (Atmaja & Kartohardjono, 1990) and 40% (Meilin, 2012).

Abamectin is an insecticide often used by farmers in Indonesia to control *N. lugens*. Abamectin is a contact and stomach poison with wide spectrum activity (Pfeifer, 1993; Ananiev *et al.*, 2002). Abamectin works at the nerves system by stimulating gamma aminobutyric acid (GABA) neurotransmitter causing an increased flow of chlorine which eventually resulted in the permanent paralysis and insect mortality (Pfeifer, 1993). Sub-lethal concentration of abamectin decreased the fecundity and longevity, and inhibited the development of *A. nilaparvatae* (Haryati, 2016). This research was conducted to determine the impact of abamectin on *A. nilaparvatae* when the adults got exposure to this insecticide through contact. The susceptibility of *A. nilaparvatae* was compared to that of *N. lugens* by exposing these insects at the recomended rate. Furthermore, adults of *A. nilaparvatae* were treated with low concentrations of abamectin to have survivors and the survivors were examined for their capacity in parasitizing eggs of *N. lugens*.

MATERIALS AND METHODS

Rearing of Nilaparvata lugens

Rearing of *N. lugens* was carried out using an established laboratory method in the Laboratory of Pesticide Toxicology, Faculty of Agriculture, Universitas Gadjah Mada. The rearing method has been practiced since 1985. Ciherang rice seeds susceptible to *N. lugens* were washed, soaked for 24 hours, air-drained and kept for 48 hours to germinate. The germinating seeds were transferred into rearing medium in the plastic jars (20 cm in diameter, 19 cm in height). The jars were covered with gauze. The 7−10 days rice seedlings (DAS) were used as natural feed and oviposition site of *N. lugens.* The founding population of *N. lugens* was obtained from the field in Ngestiharjo Village, Kasihan County in the District of Bantul, Special Province of Yogyakarta and reared. Oviposition of *N. lugens* took place between 3−8 days and eggs would hatched approximately 8 days later. Transfer of nymphs and feed substitution were performed by lifting up the old rice seedlings and putting them into a new rearing jar containing new rice seedlings. A buffering wire was used to support the old seedlings above the new one by placing it crossly on the surface of new rearing medium. Feed substitution

was carried out every week or when the leaves of old seedlings started yellowing. The population of *N. lugens* was adjusted to the needs for performing the bioassays.

Rearing of Anagrus nilaparvatae

The initial population of *A. nilaparvatae* was obtained by trapping (Haryati, 2017). One month-old of Ciherang rice plants were infested with 50 females of *N. lugens*. Two days after infestation, rice plants were randomly placed in the rice plantation for 3 days in Tanjungharjo Village, Nanggulan County in the District of Kulonprogo, Special Province of Yogyakarta. The rice plants were then brought to the laboratory. The leaves and roots were removed leaving the stem. The bottom part of stem was covered by water-wetted tissue, transferred into small pots and covered with the mica tube of 7 cm in diameter and 25 cm in height. Adults of *A. nilaparvatae* emerged from the rice stems were collected for further rearing using method previously developed by described Meilin *et al.* (2012b). Rearing of *A. nilaparvatae* was carried out in the laboratory at the temperature of 28.8−28.9°C and and the relative humidity of 59.9−64.9%.

Susceptibility Study

This experiment was set to differentiate the susceptibility of *A. nilaparvatae* and *N. lugens* against abamectin. Solution of abamectin (Demolish 18 EC, PT Dharma Guna Wibawa) in aceton as much of 0.1 ml with the concentration of 22.78 (the field recommended concentration), 5.80, 1.42, and 0.36 ppm were poured into the respective test tubes (0.5 cm in diameter, 10 cm in length) using a micropipette. Then, the tubes were rolled gently to evenly cover the inner tube surface with abamectin solution. The control tube was treated with acetone. One hour after treatment, ten *A. nilaparvatae* adults aged 1−2 days, first instars of *N. lugens* aged 1 day, or adults of *N. lugens* aged 1−2 days were released into the treated or control tubes. The observations were conducted at 30, 60, and 120 minutes after release to record the mortality. Each treatment was replicated 10 times.

Parasitism Study

Preparation of rice plants. The 30-day IR-64 rice variety obtained from the field in Triharjo Village, Sleman County in the District of Sleman, Special Province of Yogyakarta. IR-64 is the resistant variety mostly planted by farmers in endemic areas of *N. lugens*. Rice plants were transferred into plastic pots of 13.5 cm in diameter and 11.5 cm in height. Each pot was filled with 5 rice plants. Afterwards, the potted plants were covered with the cylinder made of plastic mica with the upper part was covered with gauze. Acclimatization was carried out in the green house for 8 days before use. The oviposition of *N. lugens* was conducted by infesting 50 females of *N. lugens* on each potted plant for 3 days, and then the surviving *N. lugens* were removed and the potted plants were used for parasitism study.

Parasitism test. The assay was performed using completely randomized design (CRD) with four serial concentrations of abamectin (22.78, 2.28, 0.23, and 0.02 ppm). The highest tested concentration was similar to the field recommended rate. Acetone was used as the control. The test was based on the contact method (Desneux *et al.*, 2006) with modification on the tested concentrations. Abamection solution (0.1 ml) from each concentration and the control was transferred into the respective test tubes (0.5 cm in diameter, 10 cm in height) using a micropipette. The tubes were rolled gently to evently distribute the solution to cover the whole inner surface of the tubes. An hour after treatment, 10 *A. nilaparvatae* adults were released into the control or treated tubes for 1 hour. The abamectin-exposed *A. nilaparvatae* were then infested on the potted rice plant containing eggs of *N. lugens*. The parasitoids were fed with 10% of honey solution (Madu Nusantara) by dripping the honey solution on the inner wall of plastic mica using a pipette. Each tested concentration was repeated for 10 times. The observation was carried out daily by counting the number of *A. nilaparvatae* and *N. lugens* emerged. Eggs of *N. lugens* being parasitized but no emergence and the number of non-parasitized and unhatched eggs of *N. Lugens* were recorded by dissecting the stems after 3 days in a row without any emergence. Parasitism level was calculated by dividing the number of parasitized eggs of *N. lugens* (*A. nilparvatae* emerged and parasitized but no emergence) with the number of *N. lugens* eggs laid (*N. lugens* nymphs + the number of emerged *A. nilaparvatae* + the number of unhatched eggs of *N. lugens* [parasitized and non-parasitized]).

Data Analysis

Analysis of variance for the data of parasitism was conducted using R version 3.3.3 following CRD. Mean differences were continued with LSD test at 5% level when significant differences among means existed.

RESULTS AND DISCUSSION

Susceptibility

Anagrus nilaparvatae was very susceptible to the contact application of abamectin. Increasing the concentration of abamectin increased and fastened the mortality (Table 1). At the concentration of 22.78 ppm, abamectin caused 100% mortality within 30 min. However, the same concentration only resulted 15% mortality of the first instars of *N. lugens* and no mortality for the adults even after being exposed for 120 min. At the lowest tested concentration (0.36 ppm), abamectin still caused 85% mortality of the parasitoid but no effect on the insect pest. These findings suggest that *A. nilaparvatae* was much more susceptible to abamectin than that of *N. lugens* nymphs and adults.

The application of an insecticide could eliminate the important parasitoids since they commonly had similar physiological system with the target pest (Desneux *et al.*, 2007). Similar situation could occur when abamectin was applied to control *N. lugens* in the rice field, particularly because of the high susceptibility of *A. nilaparvatae* in comparision with its host, *N. lugens*. Soitong and Escalada (2011) reported that abamectin had high toxicity to bees and parasitoid of Hymenoptera. In addition, the high mobility of *Anagrus epos* increased the amount of abamectin exposure (Corbett & Hofenheim, 1996). Mortality caused by application of abamectin against *A. nilaparvatae* reached 98.9% at 8 hours after treatment (Wang *et al.,* 2008). Furthermore, Haryati (2016) reported that the application of abamectin at the recommended concentration caused 76% mortality of *A. nilaparvatae*. Differences in the mortality of the parasitoid occured because of the differences in the origins of the parasitoid used for testting. These parasiotids might have received different level of selection pressure due to insecticide applications (Carvalho *et al.*, 2003).

Parasitism

Anagrus nilaparvatae survived from contact application of abamectin had lower capacity to parasitize the eggs of *N. lugens* (Table 2). Parasitism level decreased with increasing the concentration of abamectin. At the concentration of 2.28 ppm which was significantly lower than field recommended concentration (22.78 ppm), the reduction of parasitism reached 86.34% compared to that of the control.

Table 1. Differences in the level of susceptibility to abamectin between *Nilaparvata lugens* and its parasitoid *Anagrus nilaparvatae*

Tested insects	Abamectin (ppm)	Number of insects	Cumulative mortality after exposure (%)		
			30 min	60 min	120 min
A. nilaparvatae (adults)	22.78	40	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
	5.70	40	77.5 ± 2.63	92.5 ± 0.96	100.0 ± 0.00
	1.42	40	45.0 ± 1.73	70.0 ± 1.83	75.0 ± 2.08
	0.36	40	42.5 ± 2.99	75.0 ± 1.29	85.0 ± 0.58
	Control	40	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
N. lugens (first instars)	22.78	40	0.0± 0.00	2.5 ± 0.50	15.0 ± 0.58
	5.70	40	0.0 ± 0.00	0.0 ± 0.00	7.5 ± 0.96
	1.42	40	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	0.36	40	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	Control	40	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

Note: The same concentration of abamectin were tested on adults of *N. lugens* and no mortality was found.

Table 2. The effect of abamectin on the parasitism of *Nilaparvata lugens* eggs by *Anagrus nilaparvatae*

Abamectin (ppm)	Number of *N. lugens* eggs			Parasitism level (%)
	Laid	Parasitized	Non-Parasitized	
0.02	248.8 ± 13.88	96.4 ± 6.66 b	152.4 ± 12.68 c	38.79 ± 2.71 b
0.23	236.2 ± 14.39	38.2 ± 7.43 c	198.0 ± 17.64 b	16.25 ± 3.59 c
2.28	232.4 ± 14.31	17.2 ± 1.30 d	215.2 ± 13.55 a	7.41 ± 0.45 d
Control	245.6 ± 19.35	132.8 ± 8.04 a	112.8 ± 15.14 d	54.20 ± 3.24 a

Note: The females of *A. nilaparvatae* were exposed to the residue of abamectin and then exposed to *N. lugens* eggs. Means followed by similar letter in the same column were not significantly different at α=0.05.

At the concentrations of 0.23 and 0.02 ppm, parasitism level declined 70.01 and 28.43%, respectively. These findings and the adverse effect of abamection on *A. nilaparvatae* as previously reported by Haryati (2016) documented that exposing *A. nilaparvatae* to the sub-lethal concentrations of abamectin reduced their fecundity.

In addition to reduced parasitism, contact application of abamectin impacted on the emergence of *A. nilaparvatae*. Although the eggs of *N. lugens* were parasitized by *A. nilaparvatae*, not all parasitoids were able to emerge. The higher the concentration of abamectin, the lower the number of *A. nilaparvatae* emerged (Figure 1). Carvalho *et al.* (2003) reported that application of abamectin decreased the emergence of *Trichogramma pretiosum* up to 49.7%. It was suspected that abamectin had an ovicidal effect on eggs that affected the development of parasitoids. Abamectin and spinosad severely affected egg hatching and embryonic development of *Liriomyza trifolii* (Saryazdi *et al.*, 2012). Carbofuran and monocrotophos applied to the rice plants decreased egg hatching of *N. lugens* up to 17,9% (Senguttuvan & Gopalan, 1990). Reduction in the number of parasitoid progeny due to abamectin would decrease the service provided by *A. nilaparvatae* in the rice ecosystem in regulating the population of *N. lugens*. Disruption in the ecosystem services would lead inbalance between the population of *N. lugens* and its natural enemies which eventually could increase the probability of resurgence (Chelliah & Heinrichs, 1980; Kartohardjono, 2011).

The emergence time for *A. nilaparvatae* was slightly delay when they were exposed to abamectin (Figure 2). For the control, the emergence of *A. nilaparvatae* started from the 12th and ended on the

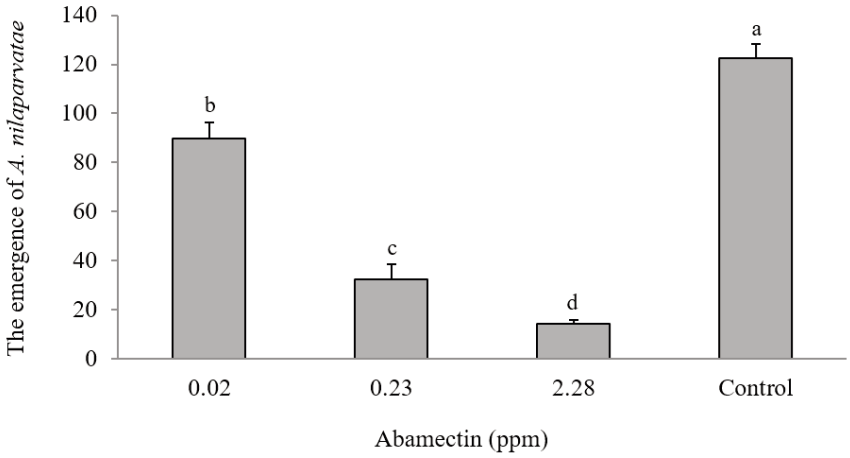

Figure 1. The effect of abamectin applied by a contact method on the emergence of *Anagrus nilaparvatae;* 10 females were released into 38 days after planting of IR-64 rice variety previously infested with 50 females of *Nilaparvata lugens* for two days

Figure 2. The effect of abamectin by a contact method on the daily emergence of *Anagrus nilaparvatae;* 10 females were released into 38 days after planting of IR-64 rice variety previously infested with 50 females of *Nilaparvata lugens* for two days

14th day. On the other hand, for the abamectin treatment the parasitoids emerged until the 15th day. These findings were not completely similar to the previous reports which documented that egg parasitoid emerged at the 7th until 14th day (Meilin *et al.*, 2012) and at the 9th until 13th day (Haryati *et al.*, 2016). These differences might be due to the differences in the fitness of parasitoids used for the experiments, particularly after being reared in the laboratory for many generations without adding field-collected population (Usmani, 2012).

CONCLUSION

Anagrus nilaparvatae was much more susceptible to abamectin than *N. lugens* when they were exposed by contact. Application as low as 0.36 ppm with the field recommendation rate of 22.78 ppm resulted 85% mortality after 120 min of exposure. Furthermore, when *A. nilaparvatae* adults were exposed to sub-lethal concentrations of abamectin, it reduced the parasitism level and the number of parasitoid progeny emerged. Exposure to 2.28, 0.23, and 0.02 ppm reduced the parasitism up to 86.34, 70.01 and 28.43%, respectively. Increasing the concentration of abamectin cause lowering the number of *A. nilaparvatae* emerged. Abamectin used to control *N. lugens* posed detrimental effects to the parasitoid which increasing the risk for outbreak of *N. lugens*.

ACKNOWLEDGEMENT

We would like to thank to Siti Haryati for the discussion on rearing techniques of the parasitoid, Sudarto for letting us to use of his rice field for parasitoid trapping, Nike Grace Hanjelina for identification of parasitoid, and Noviany for data analysis. This manuscript is part of master thesis entitled "*Dampak Aplikasi Abamektin terhadap* Anagrus nilaparvatae *(Hymenoptera: Mymaridae)* [Impact of Abamectin on *Anagrus nilaparvatae* (Hymenoptera: Mymaridae)]"

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