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Impact of Insecticide Application Containing Active Ingredients Emamectin Benzoate and Deltamethrin on the Mortality and Emergence of *Trichogramma japonicum* Imagoes

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ABSTRACT

The use of chemical insecticides remains the primary solution to agricultural pest problems. The use of insecticides does not entirely solve the problem, but can disrupt the existence of non-target organisms (parasitoids). This research aims to determine the effects of the active ingredients emamectin benzoate and deltamethrin on the mortality, morphological behavioral changes, and the emergence rate of T. japonicum imagoes. Testing was conducted using fresh residual and fresh residual contact methods. The process of rearing T. japonicum imagoes included breeding the host C. cephalonica, preparing the substrate, and rearing T. japonicum. Fresh residual and fresh residual contact tests were conducted using field concentrations of 2 ml/L and 3 ml/L, respectively for active ingredients emamectin benzoate and deltamethrin. Observation parameters included mortality rates, morphological changes, behavioral changes, and the emergence rate of T. japonicum imagoes. Mortality data were analyzed using a normality test. If the data were not normally distributed, the Kruskal-Wallis test (non-parametric) and Mann-Whitney test were employed, along with probit analysis of the lethal time 50 (LT50). The research results showed that the active ingredients emamectin benzoate and deltamethrin were harmful to the mortality and emergence of T. japonicum imagoes, with average LT50 values of 84 minutes and 46 minutes, respectively. Exposure to insecticides caused T. japonicum imagoes to exhibit abnormalities in wing folding and behavioral changes, resulting in reduced activity.

1. INTRODUCTION

Indonesia is a large country with a land area of more than 191 million hectares, of which at least 95.90 million hectares are potentially suitable for agricultural use (Gultom & Harianto, 2022). Optimizing the agricultural sector is a significant challenge due to numerous obstacles, including pest attacks that can cause losses. The use of chemical pesticides, especially insecticides, remains the primary solution to agricultural pest problems. Insecticides on the market have various brand names and types of active ingredients, including emamectin benzoate and deltamethrin.

Emamectin benzoate is classified as an avermectin insecticide with a mechanism of action that kills pests via contact and stomach poisoning (Sinyong *et al*., 2024). Deltamethrin is classified as a pyrethroid insecticide with a broad spectrum (Apriana, 2023). The use of chemical insecticides does not entirely solve agricultural problems in Indonesia, as it causes many negative impacts. The use of insecticides and herbicides pollutes water, soil, and air, damaging ecosystems (Hammada, 2024). Ibrahim & Sillehu (2022) add that the direct and inappropriate use of chemical insecticides can disrupt the survival of non-target organisms (parasitoids).

Parasitoids are insect organisms that utilize larger living hosts to complete their life cycles (Junaedi *et al*., 2016). Martuti & Anjarwati (2022) write that insect parasitoids originate from the order Hymenoptera, living as parasites by inserting their eggs into the eggs of other insects. There are 86 families of parasitoids in the world, originating from 6 orders, including the order Hymenoptera with the family *Trichogrammatidae* and genus *Trichogramma*. The species *Trichogramma japonicum* has been widely bred. *T. japonicum* is widely used as a biological control agent because it can parasitize various species of the order Lepidoptera. The use of parasitoids in agriculture is highly recommended because they are classified as biological control agents that do not pollute the environment. The release of *Trichogramma* sp. can reduce sugarcane borer infestation by up to 95.69% (Ismail *et al*., 2022). Muhammad *et al*. (2021) in Switzerland, China, Canada, and the former Soviet Union found that releasing *Trichogramma* sp. can reduce infestation by 70-92%.

The high impact and lack of information regarding the effects of active ingredients emamectin benzoate and deltamethrin on *T. japonicum* survival remain a concern. Therefore, investigation on the effects of the active ingredients emamectin benzoate and deltamethrin on mortality, morphological changes, and the emergence rate of imagoes is highly required.

2. RESEARCH MATERIALS AND METHODS

2.1. Research Location

This research was conducted in two stages at two locations. The first stage was propagating *T. japonicum* at the Biological Agency Service Center (PPAH) Tani Makmur in Pasuruan, East Java. The second stage involved testing and observation at the Plant Health Lab., Faculty of Agriculture, Universitas Pembangunan Nasional Veteran, Jawa Timur.

2.2. Equipment and Materials

The equipment and materials used included an Olympus CX33 microscope, a tray, a UV lamp, a brush, a funnel, a sprayer, and test tubes. The materials required are Corycra cephalonica starter eggs, *T. japonicum* starter, insecticides with the active ingredients emamectin benzoate (Amacel 30) and deltamethrin (Decis 25 EC), and distilled water.

2.3. Propagation of *T. japonicum*

Propagation was carried out according to the procedure developed by Muliani & Srimurni (2022), using *C. cephalonica* as a substitute host and its eggs. The feed medium consists of ground corn and corn bran in a 2:1 ratio. The medium is sterilized in an oven at 50 °C for 3 h (Herlinda *et al*., 2004). The medium is placed in a 5-liter jar until 1/3 full, and *C. cephalonica* starter eggs are placed inside. The jars were stored in a room at 28-30 °C for 40-50 days. *C. cephalonica* imagoes were harvested and placed in a tube container with a mesh base and lid. The trapped eggs were then harvested and placed under a UV lamp for 20 min to slow down or kill the development of *C. cephalonica*. *C. cephalonica* eggs are spread on 2×1 cm filter paper. Filter paper containing *C. cephalonica* eggs and *T. japonicum* starter is placed together in a test tube and covered with a cloth. *T. japonicum* propagation is successful if the *C. cephalonica* eggs turn black.

2.4. Testing of *T. japonicum*

The testing was conducted in two stages: fresh residual testing to determine the effect of insecticides on *T. japonicum* mortality, and fresh residual contact testing to determine the effect of insecticides on imago emergence. This testing stage was adopted from Irawan *et al*. (2017) and modified using concentrations based on field recommendations: 2 mL/L of emamectin benzoate and 3 mL/L of the active ingredient deltamethrin.

2.4.1. Fresh Residual Testing

Fresh Residual testing was conducted by spraying 1 mL onto the reaction tube surface and drying it at room temperature (27-29 °C) for 1 hour. Exactly 20 *T. japonicum* specimens were placed in reaction tubes according to the treatment. Seven treatments (Table 1) were arranged in a completely randomized design (CRD) in triplicates.

Table 1. Fresh residual testing

No	Code	Treatment
1	K0	Control (Aquades)
2	AB1	*T. japonicum* + emamektin benzoat 0 hours
3	AB2	*T. japonicum* + emamektin benzoat 6 hours
4	AB3	*T. japonicum* + emamektin benzoat 12 hours
5	D1	*T. japonicum* + deltrametrin 0 hours
6	D2	*T. japonicum* + deltrametrin 6 hours
7	D3	*T. japonicum* + deltrametrin 12 hours

2.4.2. Fresh Residual Contact Testing

Fresh residual contact testing was adopted from Irawan *et al.* (2017) and conducted using 50 *C. cephalonica* eggs parasitized by *T. japonicum*, aged 4-5 days and/or still within the egg (black in color). Filter paper was sprayed with 1 mL of aquades solutions of emamectin benzoate and deltamethrin, dried at room temperature for 10 min, and then placed in a sterile test tube.

2.5. Observation

2.5.1. Observation of Morphological and Behavioral Changes

Morphological changes were observed by examining abnormalities in the shape of *T. japonicum* imagoes and eggs exposed to insecticide residues. Behavioral changes were observed by comparing movement activity between the control and insecticide-exposed treatments.

2.6. Fresh Residual Observation

Observations were made at 0.5, 2, and 3 hours after application (HAA). The analysis consisted of mortality calculations and lethal time 50 (LT50). Values were determined by comparing the control using:

$$E\ (\%) = \frac{a}{a+b} \times 100\% \tag{1}$$

with E is mortality rate (%), a is number of dead imagoes, b is number of living imagoes. The mortality is classified into several levels based on the Internasional Organization of Biological Control (IOBC), namely E < 30% (class 1 = not harmful), 30% < E < 79% (class 2 = moderately harmful), 80% < E < 99% (class 3 = harmful), and E > 99% (Class 4 = highly dangerous) (Blibech *et al.*, 2015). The LT50 value was determined based on the formula:

$$LT50 = \frac{\text{Probit } 50(0) - \text{slope}}{\text{Intercept}} \tag{2}$$

2.7. Fresh Residual Contact Observation

Fresh residual contact observation was conducted on days 7 to 8 (when imago appeared), using the following formula:

$$\text{Appearance } (\%) = \frac{c}{c+d} \times 100 \tag{3}$$

where c is number of emerged *T. japonicum*, and d is number of *T. japonicum* that died and failed to emerge.

2.8. Data Analysis

Data analysis was performed using SPSS Statistics 25. Normality tests were conducted; if the data were not normally distributed, the Kruskal-Wallis test (Nonparametric) was performed. Further testing was performed using the Mann-Whitney test at the 5% level, and significant differences were reported. The LT50 test was performed using Excel.

3. RESULTS AND DISCUSSION

3.1. Morphological and Behavioral Changes after Pesticide Application

The parasitoid *T. japonicum* belongs to the family *Trichogrammatidae*, order Hymenoptera, and genus *Trichogramma*

(Oktaviani *et al*., 2021). Morphological identification of *T. japonicum* shows a length of 0.5 mm, a black thorax (Figure 1B), red eyes (Figure 1C), and numerous trichomes on the wing remigium (Figure 1A). This is in line with Yunus (2005), that *T. japonicum* has a body length of 0.4-0.5 mm, red eyes, numerous trichae on the wings, and a brownish-black thorax.

Observations showed morphological changes caused by exposure to insecticide residues. There were differences in the morphology of the wings of normal *T. japonicum* (Figure 2A). Observations showed folded wings (Figure 2B). The abnormalities were caused by pesticide residues that did not dry during testing and resulted in direct contact with *T. japonicum*. The change in shape (folded wings) significantly affected the life of *T. japonicum*, inhibiting the flying ability of *T. japonicum* imagoes (Wahyudin *et al*., 2024). In addition, exposure to residues altered the movement behavior of *T. japonicum* imagoes. *T. japonicum* imagoes in the control group had high walking and flying activity, while *T. japonicum* imagoes exposed to the active ingredients emamectin benzoate and deltamethrin had slower movement activity. The slowing of movement activity was caused by the pungent odor of the active ingredients, which disrupted the insects' respiratory systems.

The second danger of the active ingredients, aside from causing abnormalities, is that they can prevent *T. japonicum* from emerging from its host eggs. Observations made as a result of direct exposure to insecticides caused *T. japonicum* imagoes to be unable to emerge or to emerge imperfectly, as indicated by the absence of cracks (holes) in the host eggs, the imagoes' inability to penetrate the chorion wall, and only the head of *T. japonicum* emerging (Figure 2C). Wahyudin *et al*. (2024) wrote that the process of *T. japonicum* emerging from the egg is marked by cracks in the eggshell (chorion), which is where the *T. japonicum* imago emerges.

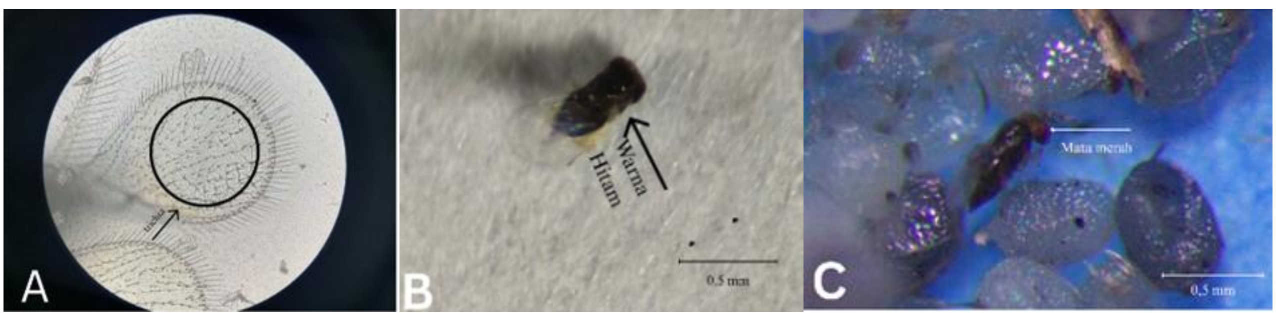

Figure 1. (A) Trichia on the wings; (B) Black color on the thorax; (C) Red eyes

Figure 2. (A) Normal wing of *T. japonicum*; (B) abnormal wing of *T. japonicum*; (C) *T. japonicum* failed to emerge

3.2. Fresh Residual Testing Observation

Based on fresh residue testing, the results are shown in Table 2. Observation of ½ HAA of the active ingredient emamectin benzoate at 0 hours showed a significant difference compared to the 6-hour and 12-hour treatments. However, the 6-hour and 12-hour treatments did not differ significantly, unlike the three deltamethrin treatments (0, 6, and 12 hours), which did not differ significantly. The highest percentage level in the ½ HAA observation was 36.7%.

The three treatments (0, 6, and 12 hours) in the 2 HAA observation showed no significant differences between the active ingredients, but there were significant differences between the treatments of the two active ingredients. The deltamethrin treatment achieved an average of 97.2% and a maximum of 98.3%, and was classified as a highly hazardous active ingredient. The sixth treatment at 3 HAA did not show any significant differences, with the 0-hour and 12-hour deltamethrin treatments reaching the highest percentage of 100%.

Deltamethrin exhibits a faster lethal toxicity than emamectin benzoate. At 2 HAA emamectin treatment had an average mortality rate of 78.9%. While, deltamethrin treatment had an average mortality rate of 97.2%. Deltamethrin is very dangerous because it is a broad-spectrum pyrethroid that targets the axon system and binds to voltage-gated sodium channels (VGSC) proteins, causing insects to become hypertensive and die more quickly (Martins & Valle, 2012). The effectiveness of deltamethrin is supported by Gabbar *et al*. (2020), who found that uncontrolled use of deltamethrin and prolonged direct exposure cause toxicity reactions and leave residues on non-target organisms. The toxicity of emamectin benzoate is relatively high. Its mechanism of action involves activating GABA (Gamma-Aminobutyric Acid) transporters and altering GABA levels, leading to paralysis and death (Kandil *et al*., 2020).

Table 2. Mortality rate of *T. japonicum* due to fresh residual testing

Treatment	Average Mortality Rate of *T. japonicum* Imago (%)			Class
	½ HAA	2 HAA	3 HAA	
Control	0	0	0	-
Emamectin Benzoate 0 hours	21.7a	80a	96.7a	III (Hazardous)
Emamectin Benzoate 6 hours	13.3b	78.3a	93.3a	III (Hazardous)
Emamectin Benzoate 12 hours	8.3b	78.3a	96.7a	III (Hazardous)
Deltamethrin 0 hours	36.6c	98.3b	100a	IV (Very hazardous)
Deltamethrin 6 hours	36.7c	96.7b	98.3a	III (Hazardous)
Deltamethrin 12 hours	30ac	96.7b	100a	IV (Very hazardous)

Note: Letters assigned to numbers in similar groups indicate no significant difference based on the Mann-Whitney test at the 5% level.

The time required for each treatment to kill 50% of the test insect population was calculated using probit analysis by determining the Lethal Time 50. The results in Table 3 show that the LT50 time for each emamectin benzoate treatment (0, 6, and 12 h) was 77, 87, and 87 min, respectively. The toxicity level of deltamethrin with LT50 for each treatment (0, 6, and 12 h) was 46, 48, and 44 min, respectively. The difference in the time required to kill 50% of the total insects may be due to sublethal effects of the emamectin benzoate treatment, as this active ingredient readily degrades at high temperatures and under direct UV light. The lethal effect and pungent aroma of the Decis brand deltamethrin further contribute to the immobilization of *T. japonicum* imago activity. Vinha *et al*. (2021) reported a decrease in physiological activity after 3 h of exposure to the fall armyworm (*Spodoptera frugiperda*). Chemically, deltamethrin is relatively stable; it does not readily decompose or evaporate (Dyanitha *et al*., 2024).

Table 3. Probit values of lethal time 50

Concentration (Treatment)	Total	Deathh 3	Slope	Intercept	Probit LT50 Analysis Values (Minutes)
Control	60	0	-	-	-
Emamectin Benzoate 0 jam	60	58	0,02	-1,34	77
Emamectin Benzoate 6 jam	60	56	0,02	-1,57	87
Emamectin Benzoate 12 jam	60	58	0,02	-1,91	87
Deltamethrin 0 jam	60	60	0,02	-0,98	46
Deltamethrin 6 jam	60	59	0,02	-0,95	48
Deltamethrin 12 jam	60	60	0,02	-0,93	44

3.3. Fresh Residual Contact Testing

Fresh residual contact testing aims to determine the percentage of *T. japonicum* imago emergence after direct exposure. The percentage of emamectin benzoate treatment reached 87.65%, which was close to the control treatment (95.23%), whereas the deltamethrin treatment reached 25.71% (Table 4). There was a significant difference between the control and emamectin benzoate treatments. Microscopic analysis showed that in the control treatment, *T.*

japonicum was able to live and move. In contrast, in the emamectin benzoate treatment, no living *T. japonicum* was observed, and several *T. japonicum* imagoes emerged abnormally. The deltamethrin treatment resulted in many eggs failing to hatch and *T. japonicum* imagoes emerging with morphological abnormalities. The high percentage in the control treatment was due to the absence of toxic active ingredients to kill insects, whereas the emamectin benzoate and deltamethrin treatments, which contained toxic active ingredients that inhibit development and kill insects.

Table 4. Percentage of *T. japonicum* imago appearance rates

Treatment	Average Occurrence (%)
Control	95.23
Emamektin Benzoat	87.65
Deltamethrin	25.71

The sublethal properties of emamectin benzoate treatment enable *T. japonicum* imagoes to penetrate host eggs and inhibit the death of *T. japonicum* imagoes. This is align with Yang *et al*. (2020) showing that the sublethal properties of emamectin benzoate do not significantly affect pupal development, but in the imago phase, it exhibits high toxicity. Deltamethrin treatment is lethal and can directly inhibit egg development.

4. CONCLUSION

Based on the results of observations, the active ingredients emamectin benzoate and deltamethrin are classified as hazardous to non-target organisms. Fresh residual testing showed mortality rates were high. For emamectin benzoate at 0.5 h, 2 h, and 3 h, reaching 96.7%, 93.3%, and 96.7% respectively, and for deltamethrin reaching 100%, 98.3%, and 100% respectively for 0.5 h, 2 h, and 3 h. Fresh residual contact testing showed an emergence rate of 87.65% for emamectin benzoate and 25.71% for deltamethrin. Morphological changes occurred in *T. japonicum* imagoes, characterized by folded wings and reduced movement.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
HMA	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
NR	✓	✓		✓	✓									
HN			✓	✓	✓									

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

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