SIDE EFFECTS OF INSECTICIDES ACTIVE INGREDIENTS DIMEHIPO AND FIPRONIL ON MORTALITY AND IMAGO INCIDENCE *Trichogramma* sp.

UNDERGRADUATE THESIS

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PENGARUH SAMPING INSEKTISIDA BERBAHAN AKTIF DIMEHIPO DAN FIPRONIL TERHADAP MORTALITAS DAN KEMUNCULAN IMAGO *Trichogramma* Sp.

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FOREWORD

Praise be to Allah Subhanahu Wa Ta'ala who has bestowed His grace and guidance so that the author can compile a thesis entitled "Side Effects of Insecticides with Active Ingredients Dimehipo and Fipronil on Mortality and Emergence of Imago *Trichogramma* sp.". The author would like to thank:

1. Dr. Ir. Wiwin Windriyanti, M.P. as main supervisor
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6. Mrs. Kholifah as chairman of PPAH Tani Makmur
7. Parents, siblings and friends, especially sasa, and bani aripin for their support while carrying out this thesis.

The writing of this thesis proposal aims to determine the potential side effects of insecticides made from the active ingredients dimehipo and fipronil on mortality and emergence of *Trichogramma* sp. It is hoped that this thesis can provide knowledge about the use of synthetic insecticides made from the active ingredients dimehipo and fipronil that are safe for the natural enemies of *Trichogramma* sp.

The author really hopes for constructive criticism and suggestions as an evaluation for the perfection of this thesis and hopefully can provide benefits for readers.

Surabaya, September 22, 2023

AUTHOR

Side Effects of Insecticides with Active Ingredients Dimehipo and Fipronil on Mortality and Emergence of Imago Trichogramma sp.

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ABSTRAC

The application of synthetic insecticides often accompanies biological control using parasitoids. The simultaneous application of control still experiences problems, namely the use of insecticides that kill the target pest and affect the presence of parasitoids that have an essential role in suppressing the development of pest populations. This study aims to determine the effect of the active ingredients dimehipo and fipronil on the mortality and emergence of T. chilonis. The test method used the Fresh Residue Contact test method. The propagation process of T. chilonis includes breeding the host C. cephalonica, preparing pias, and breeding the host C. cephalonica.

T. chilonis. Residual testing of insecticides with active ingredients dimehipo and fipronil used the lowest field recommended concentration of 0.75 ml/l each. Observation parameters in this study included mortality, imago emergence, and insect morphology after insecticide application. Mortality data were analyzed using variance analysis (ANOVA) and DMRT further test. The results showed that insecticides made from active ingredients dimehipo and fipronil hurt the mortality of T. chilonis imago. The percentage of appearance of T. chilonis imago treated with dimehipo and fipronil insecticides was 21.56% and 34.89%. Applying these insecticides resulted in the T. chilonis imago experiencing abnormalities in the shriveled wings, reduced tassels on the edges of the wings, and the insect's body appearing to shrink and dry out. The results showed that insecticides made from active ingredients dimehipo and fipronil are harmful to T. chilonis, reducing the effectiveness of biological control.

Keywords: Dimehipo, Fipronil, Insecticide Residues, Trichogramma chilonis

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I. INTRODUCTION

1.1 Background

Indonesian agriculture has developed the concept of Integrated Pest Management (IPM) since 1992 in accordance with government regulations as a basic policy for every crop protection program (Sembiring, 2007). The IPM system is a concept or way of thinking in an effort to control the level of pest attacks by applying various control techniques that are combined in one unit to prevent crop damage and the incidence of economic losses and prevent damage to the environment and ecosystems. Integrated pest management is also defined as controlling pests and plant diseases with an ecological approach that is multi- disciplinary in nature to manage pest and disease populations by applying various compatible control techniques (Yani, 2019). One aspect of IPM is biological control by utilizing natural enemies as controllers, which consist of parasitoids, predators and pathogens.

One of the parasitoids that are often used as biological agents in Indonesia is Trichogramma sp. The utilization of Trichogramma sp. is often used because Trichogramma sp. is an egg parasitoid that is polyphagous (generalist) and Trichogramma sp. is easy to propagate in mass. The utilization of Trichogramma sp. as a biological agent began to be recognized in 2010 as a biological control in rice plants. One example is Trichogramma chilonis which has a parasitation rate of 20% without choice and 24% with choice on Chilo supressalis eggs in laboratory experiments (Yang et al., 2016). Tang et al. (2017) added that T. chilonis in laboratory experiments was able to parasitize 40.7% ± 7.11% of Scirpophaga incertulas eggs and in field experiments T. chilonis was able to parasitize 15% ± 14.1% of S. incertulas eggs.

Biological control has a weakness in its ability to suppress pest populations, so it must be supported by the use of insecticides. However, the combination of the two controls still has problems, namely the use of insecticides that not only kill the target pest but also kill the pest.

The presence of natural enemies such as parasitoids has an important role in suppressing the development of pest populations. In accordance with the statement of Marwoto (2010), parasitoids will be more sensitive to insecticides than target pests. Trisnaningsih (2016) added that the use of insecticides has a direct or indirect effect on the killing of natural enemies due to the unwise use of insecticides by farmers. Some types of insecticide active ingredients recommended by the Ministry of Agriculture to control rice stem borers include chlorantraniliprole, fipronil, dimehipo and spinetoram.

Research on the effect of insecticides made from the active ingredients chlorantraniliprol and spinoteram has been conducted. Based on research by Mufida (2018), it was found that residual insecticides made from spinetoram were harmful to T. chilonis because they caused 100% mortality and 100% failure of imago emergence. In contrast, insecticides made from chlorantraniliprole at field recommended concentrations have no effect on the failure of imago emergence or mortality of T. chilonis imago. Another study conducted by Castro et al. (2013) showed similar results, namely chlorantraniliprole has the highest toxic effect on pests and a low toxic effect on predators.

Dimehipo and fipronil are two active ingredients registered with the Ministry of Agriculture and recommended to control S. incertulas stem borer as well as several other pests (Chairul et al, 2010). Dimehipo is an insecticide with contact, stomach poison and systemic properties. In Indonesia, dimehipo is marketed under various trade names in controlling brown planthoppers, stem borers, whiteflies, leaf flies and false whiteflies in rice plants. Fipronil is a systemic insecticide registered on rice plants to control stem borers, brown planthoppers, white back leafhoppers and false white pests (Chairul et. al., 2010). There is still little information on the effect of insecticides made from the active ingredients dimehipo and fipronil on the parasitoid Trichogramma sp. Therefore, this study was conducted to determine the effect of the two active ingredients of these insecticides on the mortality of imago and the emergence of imago Trichogramma sp.

1.2 Problem Formulation

The formulation of this research problem is as follows:

1. Can side effects of insecticides made from dimehipo and fipronil at field recommended concentrations affect the mortality of imago and emergence of Trichogramma sp.?
2. What is the morphological character of the parasitoid Trichogramma sp. after exposure to each residue of the active ingredient of the pesticide used?

1.3 Research Objectivees

The objectives of conducting this research are as follows:

1. To determine the side effects of insecticides made from dimehipo and fipronil at field recommended concentrations that can affect imago mortality and emergence of Trichogramma sp.
2. Observing the morphological characteristics of the parasitoid Trichogramma sp. after exposure to each residue of the active ingredient of the pesticide used.

1.4 Research Benefits

The benefits that can be obtained from this research are to provide knowledge and information about the side effects of insecticides made from active ingredients dimehipo and fipronil on Trichogramma sp. to support the implementation of Integrated Pest Management (IPM) that is safe for natural enemies.

II. LITERATURE REVIEW

2.1 Trichogramma sp.

2.1.1 Morphology of Trichogramma sp

Trichogramma spp. is an egg parasitoid insect that belongs to the class Insecta, order Hymenoptera and family Trichogrammatidae (Borror et al, 1992). This insect is small with a body length of approximately 0.4- 0.5 mm with a head width of 0.17-0.21 mm, has short cylindrical antennae consisting of 3-8 segments, including one ring segment. The antennae of male Trichogramma sp. insects are straight and overgrown with hairs, while the antennae of female insects are mace- shaped, short-haired and sparsely grown (almost hairless) (Figure 2.1). The front wing of Trichogramma sp. is rather wide, thin, with the number of trichia Rs1 (radial sector vein tract-1 st abscissa) as many as 7-10. The forewing is 0.45 ± 0.05 mm long and 0.22 ± 0.02 mm wide. Hind wing length 0.33 ± 0.04 mm and width 0.02 ± 0.01 mm. The edges of the fore and hind wings are covered with hairs. The hind tibia is 0.14 ± 0.02 mm long and the tarsi consist of 3 segments (Yunus, 2005).

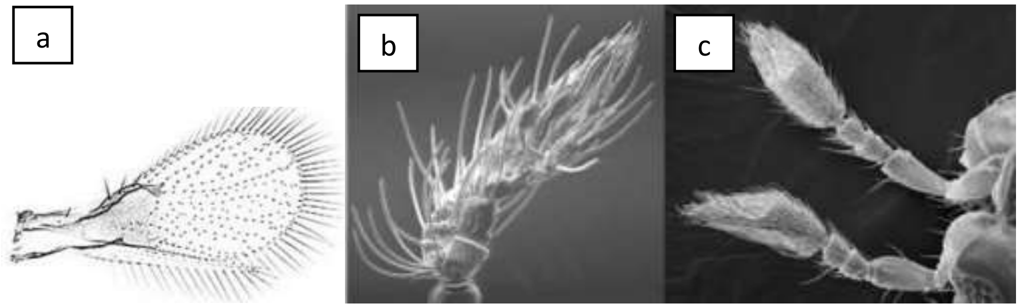

Figure 2.1. Morphology of *Trichogramma*. (a) *Trichogramma* wings and (b) male antennae (c) female antennae (Querino *et al.*, 2010).

Guidelines in identification in the Trichogrammatidae family, can see some morphological characters to distinguish the two genus Trichogramma and Trichogrammatoidea, namely in Trichogrammatoidea found two funicle segments on the antenna and 3 segments of the antenna club. Trichogrammatoidea does not have the RS1 line (radial sector vein tract-1st abscissa) on the forewing (Nagakartti & Nagaraja, 1977). On the other hand, the genus Trichogramma has a distinctive The main RS1 line and antennae of male Trichogramma lack 3 funicle segments (Nagakartti & Nagaraja, 1977).

2.1.2 Life Cycle of Trichogramma spp.

The pre-imago phase (egg-larva-pupa phase) develops inside the host egg, feeding on nutrients and damaging or killing the host (Yunus, 2005). One day after the host egg is parasitized by Trichogramma sp., first instar larvae are formed and then develop into second instar larvae on the second day after parasitization. The larvae develop rapidly by feeding on nutrients from the host eggs. On the fourth day, Trichogramma sp. develops into pre-pupae and pupae on the fifth day after parasitization. On the fourth day, the host egg will turn grayish and then black. The black color arises from the secretion of labial glands in the larvae (Consoli et al., 1999). The eighth day the imago will come out of the host egg, in the imago phase the parasitoid lives freely outside the host body, carrying out feeding, mating, and egg laying activities (oviposition). The parasitized host egg will turn black due to the formation of granules on the inner surface of the chorion. The life cycle length of the parasitoid T. japonicum in the pre-imago is 8.2 ± 0.3 days and the imago is 1.8 ±.7 days on the host C. cephalonica (Yunus, 2005). The utilization of Trichogramma sp. is very beneficial to be used as a biological agent in controlling rice stem borer pests, considering that S. innotata attacks occur throughout the growth of rice plants, so that parasitoids will develop continuously to reach 9-10 generations during one rice growing season.

2.1.3 Behavior of Trichogramma spp.

Trichogramma sp. is an egg parasite whose imago searches for host eggs using the sense of smell. Generally, the host being sought will emit an odor that can lure the female Trichogramma sp. imago to come to the host. When the female imago finds the host egg, it will be examined using its ovipositor or antennae to determine whether the host egg to be selected is fresh, healthy, and not parasitized by other female imago (Romli & Desta, 2006). T. chilonis will insert its eggs into the selected host eggs and then develop into larvae and take nutrients inside the host eggs. so that the host egg dies. The host eggs will turn black on the fourth day indicating that they have been parasitized by Trichogramma sp. The appearance of the imago is characterized by a crack or crack in the eggshell (chorion) of the host then from the crack the imago will emerge. The imago slowly moves out, starting with the two antennae breaking through the chorion, followed by other body parts. Generally, the males emerge first followed by the females. On the eighth day, the imago will emerge from the host egg, then the imago will stay for a while to adapt to the environment, and then Trichogramma sp. will clean itself using its limbs and antennae. The newly emerged female imago is immediately approached by the male who has come out first. The female does not fly away immediately, but actively walks and is followed by the male. The pair then immediately copulates and the imago goes in search of liquid honey (Yunus, 2005).

Generally, parasitoids are only active during the day because they cannot find the host in the dark. The ability of Trichogrammatoidea bactrae to parasitize the host is more effective in the morning than in the afternoon. Other genera such as Trichogramma sp. females tend to move towards the light source. The dispersal power of Trichogramma sp. parasitoids can reach 50 m from the release point, but effectively parasitize the host only within a radius of 5 m (Marwoto, 2010). In the research of Nagakartti & Nagaraja (1977) showed the results in Indonesia only found the species T. australicum which was later corrected to T. chilonis (Nagakartti & Nagaraja, 1979). According to Polaszek (2010), there are various species distributed in Erurasia and some Trichogramma species found in Indonesia are T. chilonis, T. chilotraeae, and T. japonicum. Research by Buchori et al. (2010) added that some species of Trichogramma sp. found in Java are T. japonicum, T. chilonis, and T. australicum.

2.1.4 Trichogramma spp. as Biocontrol Agents

Parasitoid insects have an important role in nature and agricultural ecosystems because they can regulate or influence the population density of parasitoid hosts. Parasitoids are used in the control of several agricultural pests, especially Lepidoptera and Hemiptera (Garcia, 2011). Egg parasitoids are one of the natural enemies that can control pests in rice crops. The effectiveness of parasitoids is highly dependent on the ability to find hosts (host finding) and handle their hosts in certain environmental conditions, such as temperature, humidity, rainfall, as well as the quality, number and density of hosts (Godfray, 1994).

Trichogramma is the largest genus in the Trichogrammatidae family, with 210 species distributed worldwide (Querino et al., 2010). The genus Trichogramma can be found worldwide with 145 species (Pinto & Stouthamer, 1994). Trichogramma sp. have been used as biological control agents on at least 32 million hectares of agricultural and forestry land in the world, with the former Soviet Union ranking as the top user, followed by China and Mexico (Li, 1994; Lenteren & Bueno, 2003). In Australia, it is estimated that less than 6000 hectares are treated with Trichogramma sp. to control Helicoverpa spp. and other insect pests (Li, 1994; Seymour et al., 1994). Trichogramma sp. used in biological control include T. minutum Riley for control of Cydia pomonella (Linnaeus) moth on apple, and the species groups T. pretiosum Riley, T. australicum Girault, Trichogrammatoidea flava Girault, T. carverae Oatman & Pinto, T. bactrae Nagarajan, and Tnr. Brassicae Bezdenko to control H. armigera and H. punctigera (Wallengren) in cotton (Scholz & Murray, 1994). T. chilonis is also an egg parasitoid that attacks various Lepidoptera species, such as pests of corn, rice, cotton, vegetables, fruit crops and sugarcane in Hawaii, Pakistan, India, and Indonesia (Vargas & Nishida, 1982; Rasool et al., 2002; Hussnain et al., 1997; Masod et al., 2011; Muhammad et al., 2021); Krishnamoorthy A, 2012); Tang et al., 2017).

One of the advantages of using Trichogramma sp. as a pest biological control agent is that this parasitoid can be cultured easily using alternative hosts. Various hosts that can be used for mass breeding of Trichogramma sp. include Chinese oak silkworm eggs, Antheraea pernyi (Guérin-Méneville), silkworm eggs, Samia cynthia ricini Boisduval, rice moth eggs Corcyra cephalonica Stainton, Angoumois grain moth eggs (Sitotroga cerealella) as well as artificial host eggs (Wang et al., 2011), 2014). In Indonesia, Trichogramma sp. is usually mass propagated using C. cephalonica eggs (Nurindah et al., 2016; Yunus, 2018; Sharma et al., 2020).

2.2 Insectisides

Insecticides are materials that contain toxic chemical compounds that can kill all types of insects such as, insect pests attacking plants to obtain food in various ways, according to the type of mouth or other insects that are accidentally exposed to insecticides. Insecticides consist of a formulation containing active ingredients and other ingredients. The government has explained in Government Regulation No. 107/2015 that the active ingredients in insecticide formulations are synthetic chemicals or natural ingredients that have toxicity and biological effects on target organisms. The way insecticides enter the body of target insects can be divided into three groups of insecticides, namely stomach poison, contact poison and respiratory poison.

The active ingredient dimehipo is an insecticide with a class of neristoxins that work systemically through contact and stomach poisons that can be absorbed and transported to all parts of the plant, so that insect pests that eat every part of the plant that has been sprayed will die. Insect pests will also die if they are directly exposed to the spray liquid or come into contact with the surface of the leaves or other parts of the plant being sprayed. In Indonesia, dimehipo is marketed under various trade names aimed at controlling brown planthoppers, stem borers, white pests, leaf flies and false white pests in rice plants. Research by Sianipar Sianipar et al., (2020), explained that insecticides made from dimehipo are used in controlling rice pests such as the stem/leaf sucker group, including brown planthoppers. In addition, Yurista et al. (2014) added that the use of insecticides made from dimehipo can reduce the attack of Liriomyza chinensis on Palu Valley shallot plants.

Fipronil is a broad-spectrum insecticide that belongs to the phenylpyrazole class. Fipronil is registered as an insecticide for the control of pests in rice plants such as stem borers, brown planthoppers, white-backed planthoppers, and false white pests. Fipronil in the IRAC (Insecticide Resistance Action Committee) classification belongs to group 2B phenylpyrazole (Deptan, 2002). Fipronil is an inhibitor for GABA receptors that can affect the stimulation of the nervous system such as excessive activity, irritation ability, trembling with possible convulsions. Insecticides made from the active ingredient Fipronil insecticide is an insecticide that plays a dual role, besides being an insecticide it can also act as a plant growth substance. The form of the insecticide is in the form of concentrated SC (Suspension Concentrate), EC (Emulsifiable Concentrate) and GR (Granule).

Many studies have shown that the use of pesticides not only controls pest organisms, but can also destroy various other living things. Pesticides are not only killers of target pests, but also other organisms in the ecosystem, including natural enemies such as parasitoids and predators that act as pest controllers in nature. Maramis et al. (2011), added that the population abundance of Trichogramma sp. parasitoids in rice fields will vary due to differences in insecticide applications and the types of insecticides used in paddy rice plants. The application of ecologically selective insecticides can be done based on the bioecology of parasitoids that are most resistant to the effects of insecticides. Ecologically selective insecticides are expected to kill pests but are safe for natural enemies (Marwoto, 2010).

2.3 Hypotesis

1. Dimehipo and fipronil insecticides had the highest effect on Trichogramma sp. imago mortality at 0 hours residue after spraying and had an effect on imago emergence.
2. There were changes in the morphology of Trichogramma sp. due to exposure to residual dimehipo and fiproni insecticides.

III. RESEARCH METHODS

3.1 Time and Place of Research

The research was conducted from May to July 2023. Testing of imago mortality and imago emergence was carried out at the Plant Health Laboratory, Faculty of Agriculture, National Development University "Veteran" East Java. Trichogramma sp. propagation was carried out at the Tani Makmur Biological Agency Service Center (PPAH), Pasuruan.

3.2 Tools and Materials

3.2.1 Tools

The tools used in this study include jars, pans, stoves, test tubes, plastic boxes, funnels, brushes, UV lamps, manila paper, black cloth, rubber bands, gauze, microscopes, pipettes, beakers, needles, tea filters, tape, staples, brushes, trays, handcounters, thermorhygrometers, stationery and cameras.

3.2.2 Materials

The materials needed in this study include distilled water, Trichogramma sp., Corcyra cephalonica eggs, ground corn, bran, povinal glue, insecticides made from dimehipo and fipronil.

3.3 Research Methods and Implementation

3.3.1 Insecticides

The insecticides used were insecticides made from dimehipo and fipronil. The insecticide made from dimehipo used the brand name Sidatan Xr 525 SL (dimehipo 525 g/l), while the insecticide made from fipronil used the brand name Fipros 55 SC (fipronil 50 g/l). The concentrations used in the application of insecticides were in accordance with the field recommended concentrations for yellow rice stem borer (Scirphopaga incertulas) stated on the insecticide package label. The field recommended concentration for dimehipo is 0.75 ml/l, while the field recommended concentration for fipronil is 0.75 ml/l.

3.3.2 Trichogramma propagation

Trichogramma sp. was obtained from the Tani Makmur Biological Agency Service Center (PPAH), Pasuruan. Propagation of Trichogramma sp. used a surrogate host C. cephalonica obtained from PPAH Tani Makmur. The propagation process included breeding the host C. cephalonica, preparation of pias and breeding Trichogramma sp.

a. Host breeding of C. cephalonica

Breeding of C. cephalonica begins with preparing the feed first, namely bran and ground corn in a ratio of 2:1. The bran and ground corn that have been prepared are first roasted so that they are not contaminated with other pests. The mixed feed is then placed into breeding jars with a size of 5 liters as much as 3 kg per jar. Approximately 2500 C. cephalonica moth eggs were placed into the breeding jars and reared for 5 weeks (Figure 3.1). C. cephalonica moths can be collected after five weeks and transferred into nesting tubes, using test tubes (Figure 3.1). The nesting tubes are made of paperboard in the shape of a tube and then the top and base are covered using gauze. One nesting tube contains a maximum of 250 moths. The nesting tubes were placed in an upright position on a paper-bottomed tray. The nesting time for C. cephalonica moths was 24 hours. C. cephalonica eggs attached to the gauze are collected using a brush and then placed on a tray, to clean the dirt attached to the C. cephalonica eggs are sieved using a tea strainer (Brotodjojo, 2022).

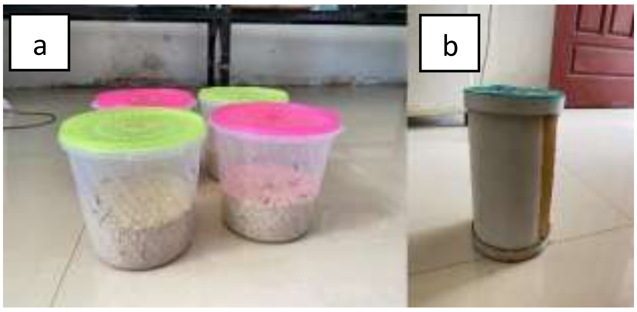

Figure 3. 1. Host culture of C. cephalonica. (a) Breeding jar, (b) Nesting tube.

(Source: Personal Documentation)

b. Preparation of pias

Pias are the implementation of manila paperboard on which a number of C. cephalonica host eggs are attached as a habitat for the propagation process of Trichogramma sp. Pias preparation is carried out by preparing three kinds of pias, namely propagation pias, starter pias and test pias. The propagation pias were prepared for the propagation of Trichogramma sp. The pias were made of manila paperboard measuring 2 cm x 10 cm. The ends of the propagation pias were smeared with 2 cm x 2 cm of povinal glue, then previously cleaned C. cephalonica eggs were spread on the surface of the pias. Pias were then sterilized using UV light for 20 minutes. Propagation of Trichogramma sp. was carried out by inserting the propagation pias into tubes containing starter pias. This starter pias is a pias that has been parasitized by Trichogramma sp. The test pias is a pias used for testing the appearance of Trichogramma sp. imago, which usually consists of ± 50 C. cephalonica eggs that have been parasitized by Trichogramma sp. The model of propagation pias, starter pias and test pias can be seen in the figure below. 3.2. The characteristics of pias that have been parasitized by Trichogramma sp. and are ready for testing are blackish in color.

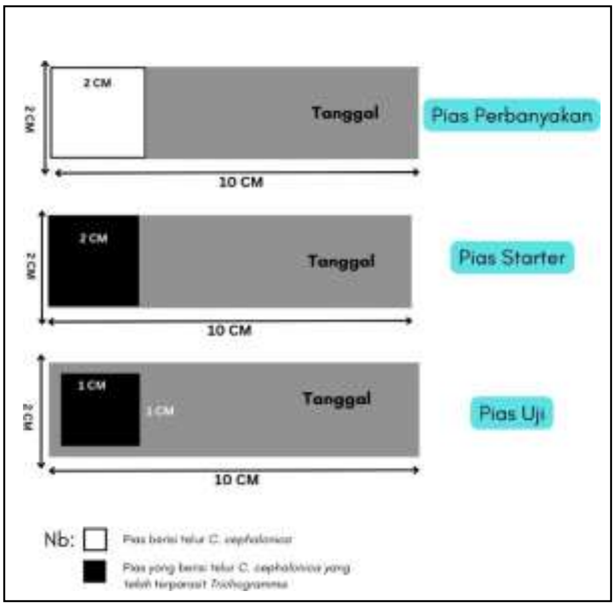

Figure 3.2. Model of Propagation Pias, Starter Pias, Test Pias. (Source: Personal Documentation)

c. Breeding of Trichogramma sp.

The breeding of Trichogramma sp. began by putting 1 parasitoid starter Trichogramma sp. and 5 parasitoid C. cephalonica eggs into a plastic bottle, then the plastic bottle was closed using a white cloth and tied using a rubber band. The second day the Trichogramma sp. eggs hatched in the C. cephalonica eggs which were used as a food source and living medium for the Trichogramma sp. larvae. The fourth day the C. cephalonica eggs parasitized by Trichogramma sp. were blackish in color. On the fifth day, the eggs turned completely black, indicating that the eggs had been parasitized and Trichogramma had developed well. Pias containing parasitized C. cephalonica eggs can be kept refrigerated to delay hatching. When testing the pias are removed from the refrigerator and placed in plastic bottles. Imago Trichogramma sp. will hatch on the second day. Imago that have hatched are ready to be used for testing. During the Trichogramma sp. propagation process, the humidity of the rearing room was monitored using a thermohygrometer to determine room humidity and room temperature because Trichogramma sp. is best maintained at a temperature of 27°C and RH = 70 - 80%.

Figure 3.3. Breeding of Trichogramma sp. (Source: Personal Documentation)

3.3.3 Testing Phase

a. Fresh Residue Contact Test against Trichogramma sp. Imago Stadia

Fresh Residue Contact Test against Trichogramma sp. Imago Stadia Testing fresh residue contact Trichogramma sp. stadia imago adopting the research method of Mufida (2018). This fresh residue contact test The test was used to determine the side effects of contact insecticides on Trichogramma sp. The test began by preparing the insecticides Sidatan Xr 525 SL (dimehipo 525 g/l) and Fipros 55 SC (fipronil 50 g/l) dissolved in distilled water. The concentration of the insecticide solution used was at the field recommended concentration of 0.75 ml/l of each insecticide. The insecticide solution was then taken using a micropipette as much as 0.5 ml, after which it was leveled in the test tube by manually twisting the test tube. The control treatment was carried out by dripping distilled water in a test tube as much as 0.5 ml. The test tube was then aerated for 1 hour. Trichogramma sp. that were less than 12 hours old were then transferred into test tubes as many as 20 individuals per tube. The time of transferring Trichogramma sp. into the tube is 0, 3, and 6 hours after the application of the insecticide solution in the test tube. The test insects were incubated for four hours. The test insects were then transferred to another clean test tube. Observations and recording of the number of deaths of Trichogramma sp. imago were carried out at 1, 3, 6 hours after treatment. The treatments were then arranged based on the design plan as shown in Figure 3.4 using a group randomized design with 4 replications, according to the treatments in Table 3.1.

Table 3.1. Mortality Test Treatments on *Trichogramma* sp. in the Laboratory

No.	Code	Treatment
1	PK0	Control treatment (distilled water)
2	PD1	*Trichogramma* sp. + dimehipo insecticide residue 0 hours
3	PD2	*Trichogramma* sp. + dimehipo insecticide residue 3 hours
4	PD3	*Trichogramma sp.* + dimehipo insecticide residue 6 hours
5	PF4	*Trichogramma sp.* + fipronil insecticide residue 0 hours
6	PF5	Trichogramma sp. + fipronil insecticide residue 3 hours
7	PF6	*Trichogramma* sp. + fipronil insecticide residue 6 hours

PK0U1	PD3U 2	PD2U 3	PF6U4
PD1U1	PK0U 2	PD3U 3	PF4U4
PD2U1	PF4U 2	PK0U 3	PD1U4
PD3U1	PF5U 2	PF4U 3	PD2U4
PF4U1	PF6U 2	PF5U 3	PD3U4
PF5U1	PD1U 2	PF6U 3	PF5U4
PF6U1	PD2U 2	PD1U 3	PK0U4

Figure 3.4. Plan of Randomized Group Design

b. Fresh Residue Contact Test against Trichogramma sp Pre-imago Stadia

Fresh residue contact tests were carried out using insecticides made from active ingredients dimehipo and fipronil with field recommended concentrations according to the insecticides used. This fresh residue contact test refers to the research of Novita et al. (2017) which was carried out on the pre-imago stadia of Trichogramma sp. which is still in the host egg of C. cephalonica which is characterized by black pias (4-5 days). Each pias contained 50 parasitized eggs of Trichogramma sp. The insecticides used were dimehipo and fipronil at the field recommended concentration of 0.75 ml/l each. Application of the insecticide treatments was done by dipping in the insecticide solution for 1 second. The control treatment was carried out by dipping the test paper in distilled water for 1 second. The chias were then dried for 10 minutes. The chias that have been dried are put into a test tube and then covered using gauze and tied using a rubber band. This test consists of 3 treatments according to table 3.2. Where each treatment was carried out as many as 9 replicates.

Table 3.2. Test treatments for imago emergence on *Trichogramma* sp.

No.	Code	Treatment
1	K0	*Trichogramma* sp + distilled water
2	K1	*Trichogramma* sp. + dimehipo
3	K2	*Trichogramma* sp. + fipronil

3.3.4 Observation Stage

3.4 Data Analysis

IV. RESULTS AND DISCUSSION

4.1 Identification of Trichogramma sp.

The genus Trichogramma is a group of small parasitoid insects belonging to the family Trichogrammatidae. The morphological characters typical for members of this genus include several important features used to identify and classify species. According to Nagarkatti & Nagaraja (1977) the genus Trichogramma has a special characteristic, namely there is an RS1 line on the front wing and the male antenna does not have 3 funicle segments. Based on the observations, the characteristics of the RS1 line (radial sector vein tract-1st abscissa) on the forewing were found (Figure 4.1B), which shows the suitability of the genus Trichogramma.

Identification of the genus Trichogramma can be done by using supporting characteristics such as body color, antenna hair and antenna shape. Based on the results of the identification that has been carried out, there are morphological characteristics such as the RS1 line on the front wing (Figure 4.1B), mace-shaped antennae with little hair (Figure 4.1A), a distinctive body color that is yellowish with brownish lines on the abdomen (Figure 4.1C) and has a striking red eye color (Figure 4.1D). Based on these morphological characteristics, it can be confirmed that the insect used in this study is Trichogramma chilonis (Hymenoptera: Trichogrammatidae). Supported by Borror et al. (1992) that the imago of T. chilonis has special characteristics, namely the presence of fine hairs on the wings, while the tarsis is three-pronged. According to Chan & Chou (2000), T. chilonis has a male body length of 0.57-0.79 mm, females 0.73-0.79 mm, with the number of antenna hairs in males less than 50. Has a brownish yellow body color, with prominent red eyes that are striking. The wings of T. chilonis imago display an interesting structure, with various tassels, the longest of which are located on the edge of the wings, giving T. chilonis a distinctive character. In addition, the antennae of female T. chilonis are mace- shaped, short-haired and sparsely grown, while the antennae of male T. chilonis are straight and overgrown with hairs (Yunus, 2005).

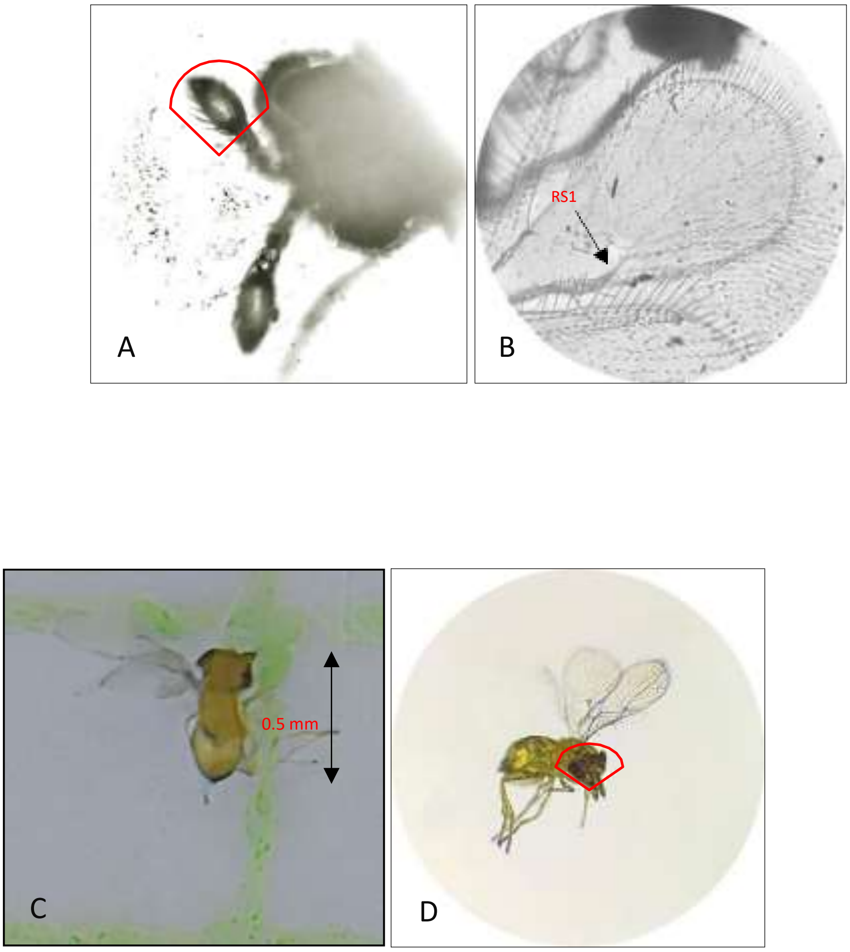

Figure 4.1. Morphological characters of T. chilonis identified; (A) Antennae; (B) Forewings; (C) Parasitoid size; (D) Striking red eyes. Photographs of olympus CX300 microscope with 1000x magnification and endoscope camera digital microscope with 500X magnifier.

4.2 Morphology og T. chilonis after Insecticide Application

Trichogramma is a group of egg parasitoids that have an important role in agricultural pest control. Successful control of pest populations with Trichogramma depends on a number of factors, including environmental conditions and the biological quality of the parasitoids themselves. One factor that may potentially affect the biological quality of Trichogramma is exposure to insecticides. The results showed that the application of insecticides made from the active ingredients dimehipo and fipronil had an effect on the morphology of Trichogramma.

T. chilonis. T. chilonis imago after insecticide application had abnormalities in the wings. Wings exposed to insecticide residues appear shriveled (abnormal) and the tassels on the wing edges are sparse (Figure 4.2B). These conditions will reduce the flying and dispersal ability of T. chilonis. In addition, the size of the imago also appeared to shrink after exposure to residues of the insecticide (Figure 4.2A).

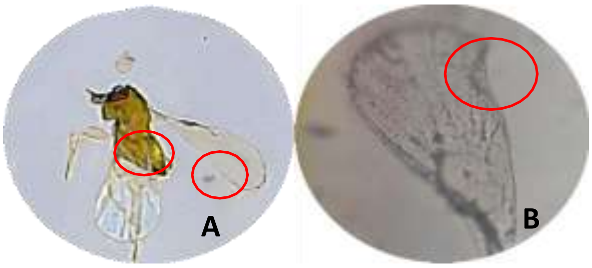

Figure 4.2. Morphological changes of T. chilonis. (A) Shriveled body of T. chilonis imago and abnormalities in the wings of T. chilonis; (B) Abnormal wings (shriveled) of T. chilonis observed using an olympus CX300 microscope at 1000x magnification.

Insecticide spraying affected parasitoid survival resulting in the failure of T. chilonis to emerge from C. chepalonica host eggs. T. chilonis that failed to emerge can be seen from the condition of black eggs, still intact, and there are no tears that penetrate the egg chorion (Figure 4.3.(a)). In addition, there are T. chilonis able to penetrate the host egg chorion but unable to come out completely (Figure 4.3 (b)). In accordance with the opinion of (Campbell et al., 2015), the failure of T. chilonis emergence is because the insecticide applied to C. chepalonica eggs is able to enter the egg through micropyles (egg fertilization site) so that T. chilonis dies in the pupal phase and the insecticide used has an ovicidal effect that can affect the death of insects in the egg. Successful T. chilonis emergence is characterized by a crack or tear in the eggshell of C. chepalonica (Figure 4.3.(c)). The process of imago emergence is characterized by a gap or crack in the host eggshell (chorion), which is the position where the imago will emerge. Slowly, the imago moves out, starting with the two antennae breaking through the chorion and then followed by other body parts (Yunus, 2005). Based on these results, it shows that the effect of insecticides on Trichogramma emergence is significant. Insecticides can damage the development and survival process of the parasitoid, inhibit the emergence of the imago, as well as the emergence of Trichogramma.
contributes to the failure of agricultural pest control through Trichogramma.

Figure 4.3. Test frames after exposure to insecticides. (a) C. chepalonica eggs intact; (b) C. chepalonica eggs with tears; (c) T. chilonis imago failing to exit the host egg (d) C. chepalonica eggs not parasitized by T. chilonis. Observed using a 500X magnifier digital endoscope camera microscope.

4.3 Effect of Insecticides on T. chilonis Imago

The effect of residual insecticide application on the mortality of T. chilonis parasitoids using the fresh residue contact method obtained results as presented in table 4.1. The observation results at 1 JSA (Hours After Application) showed that the highest mortality was found in the PD1 treatment which reached 93.75%. The PD2 and PD3 treatments did not show significant differences with the PD1 treatment. The lowest mortality was found in the PF6 treatment. The results of 3 JSA observations showed that the treatments PD1, PD2, PD3 and PF4 were not significantly different. Treatments PD1, PD2, PD3 and PF4 are categorized as dangerous because they have mortality rates between 80% - 99%. The results of the 6 JSA observation showed that the highest mortality was found in the PD1 treatment with 100% mortality, so that the residue in the PD1 treatment was categorized as very dangerous to the parasitoid T. chilonis in the 6 JSA observation. Meanwhile, in other treatments, namely the residues of treatments PD2, PD3, PF4, PF5 and PF6, the mortality rate of T. chilonis reached 96.25%, 96.25%, 96.25%, 95%, and 92.5%, respectively, categorized as insecticides harmful to natural enemies in the 6 JSA observations.

Hassan (1985) defined four categories of results of evaluating the side effects of pesticides in the laboratory on mortality of natural enemies, namely safe (mortality of <50%), moderate (50% - 79% mortality), harmful (80% - 99% mortality) and very harmful (>99% mortality). The results showed that the application of dimehipo insecticide on T. chilonis imago resulted in faster and higher mortality compared to the use of fipronil insecticide. This is because the insecticidal properties of the active ingredient dimehipo have a mode of action as a contact and stomach poison, so that the T. chilonis parasitoid dies faster, so that in 1 JSA observations this insecticide has been categorized as harmful to natural enemies. In addition, Chen (2017) states that the active ingredient dimehipo will be stable in water and degrade very slowly, the residue of the active ingredient dimehipo is more difficult to hydrolyze than the residue of the active ingredient fipronil. The treatment of fipronil residues in 1 JSA observations is still categorized as moderate in 0 hour and 3 hour fipronil residues. Meanwhile, 6 hours of fipronil residue is categorized as safe against natural enemies. Ratna, et al. (2022) mentioned that fipronil insecticide is a phenylfrizone group with the mode of action of blocking GABA (Gamma Amino Butyric Acid) gated chloride channels in the central nervous system. The result is paralysis and then death of the insect, so that the residue of fipronil is slower in affecting the death of T. chilonis imago. It can be seen that in the observation of 6 JSA, fipronil residue treatment reached a high mortality rate.

Table 4.1. Percentage Mortality of T. chilonis Imago after Insecticide Application.

Table 4.1. Percentage Mortality of *T. chilonis* Imago after Insecticide Application.

Treatment	Mortality of Imago Trichogramma chilonis		
	1 JSA	3 JSA	6 JSA
PD1	93.75d	95.00c	100.00b
PD2	91.25d	93.75c	96.25a
PD3	91.25d	95.00c	96.25a
PD4	78.75cd	95.00c	96.25a
PD5	58.75c	66.25b	95.00b
PD6	37.50b	66.25b	92.50b
PD0	1.25a	1.25a	8.75a

Notes: Numbers followed by the same letter in the same treatment group show no significant difference according to Duncan's multiple range test at P = 0.05.

4.4 Insecticide Efficacy

Based on mortality observation data, the efficacy value of each treatment of dimehipo and fipronil insecticide residues can be seen in table 4.2. showed that the highest efficacy value in the PD1 residue treatment was 100%, class IV (hazardous) based on IOBC, while in the PD1 and PD2 residue treatments were grouped in class III (medium hazardous). The results of the efficacy value of PF4, PF5 and PF6 residual treatments do not have a large difference with values of 95.89%, 94.325% and 91.78% respectively (medium hazardous).

Residual testing of both insecticides is harmful to the presence of T. chilonis parasitoids because regardless of the residual insecticide exposed, T. chilonis will experience a high mortality rate. This is because the activity of T. chilonis is limited to the test tube used in the test. This limitation causes the parasitoid insect

T. chilonis to be continuously exposed to insecticide residues on the test tube surface. The impact of this continuous exposure is an increase in the mortality rate of T. chilonis insects.

Each insecticide gives different symptoms of death, dimehipo insecticide more quickly causes the death of T. chilonis because the active ingredient dimehipo is a class of nereistoxins that are gastric poisons with a mechanism of action that blocks the front nerve cells until insect nerve paralysis occurs, besides this dimehipo insecticide is a contact poison so that T. chilonis exposed to its residue will die faster. Meanwhile, the active ingredient fipronil from the phenylpyrazoles group has contact properties with a mechanism of action that disrupts the central nervous system of insects by blocking GABA and glutamate- gated chloride (GluCl) channels resulting in hyperexcitation of insect nerves and muscles (IRAC, 2018). Thus, the symptoms of death are slower, the imago will appear to be silent for a while then return to walking again then the imago has convulsions g.

Table 4.2. Efficacy Value (%) in Residual Test of Each Treatment

4.5 Effect if Insecticides on the Emergence of T. chilonis

Biological control using parasitoids is often accompanied by the application of synthetic insecticides, so maintaining the survival of the pre-imago stadia of T. chilonis parasitoids still inside the host eggs is necessary to maintain the sustainability of biological control. The insecticides used are likely to affect the survival of parasitoids as biological agents. The results of residual testing of pre- imago stadia parasitoids can be seen in table 4.3.

Table 4.3. Effect of Insecticide Residues on the Percentage of T. chilonis Imago Emergence

Treatment	Average	Imago emergence (%)
K0	47.22	94.44
K1	10.78	21.56
K2	17.44	34.89

The results showed that the percentage of emergence of T. chilonis imago treated with dimehipo (K1) and fipronil (K2) insecticides was smaller than the percentage of emergence in the K0 treatment, which were 94.44%, 21.56%, and 34.89%, respectively. The occurrence of T. chilonis in the fipronil active ingredient treatment was higher than the occurrence of T. chilonis in the dimehipo active ingredient treatment. This shows that dimehipo insecticide has a higher toxicity. High toxicity will affect the formation of incomplete chitin so that the active ingredient will be able to enter the eggs of C. chepalonica and kill the pre- imago stadia that are still in the eggs. Therefore, there are conditions where eggs that have been parasitized by T. chilonis fail to emerge from the host eggs. Supported by research by Nurhudiman et al. (2018) reported that the Plutella xylostella egg insecticide test, the botanical active ingredient babadotan is able to damage egg development due to the high level of toxicity to lepidoptera eggs and has an effect as an ovicide. Therefore, the use of insecticides made from the active ingredients dimehipo and fipronil has a side effect on their application in the field. The negative effect of insecticide application on T. chilonis is to reduce the effectiveness of biological control.

V. CLOSING

5.1 Conclusion

The conclusions obtained from the results of the study on the side effects of insecticides made from active ingredients dimehipo and fipronil on mortality and emergence of T. chilonis imago, among others:

1. Residues from the application of insecticides made from active ingredients dimehipo and fipronil at field recommended concentrations significantly affect the mortality of T. chilonis imago with the highest mortality of 100% in the 0 hour dimehipo residue treatment and 96.25% in the 0 hour fipronil residue treatment.
2. Application of dimehipo and fipronil insecticides resulted in T. chilonis imago experiencing abnormalities in the wings, namely the wings appear shriveled and reduced tassels on the edges of the wings and the insect's body appears to shrink and dry out.

5.2 Advice

Exposure to insecticides has the potential to affect the presence of Trichogramma, which in turn affects the efficiency of biological pest control. Further research is needed to understand the mechanism of interaction between insecticides and Trichogramma sp. and its effect on biological control strategies in the field. This will provide important insights in supporting sustainable agricultural approaches that consider ecosystem dynamics and pest control effectiveness.

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APPENDIX

Appendix 1. Variance of Mortality of *T. chilonis* After Application

Table 1.1. Variance of mortality of *T. chilonis* 1 JSA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29669.643a	9	3296.627	16.361	.000
Intercept	117003.571	1	117003.571	580.697	.000
Treatment	29008.929	6	4834.821	23.996	.000
Repeat	660.714	3	220.238	1.093	.377
Error	3626.786	18	201.488		
Total	150300.000	28			
Corrected Total	33296.429	27			

Table 1.2. Variance Analysis of Mortality of *T. chilonis* 3 JSA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28388.393a	9	3154.266	23.040	.000
Intercept	153772.321	1	153772.321	1123.207	.000
Treatment	28271.429	6	4711.905	34.417	.000
Repeat	116.964	3	38.988	.285	.836
Error	2464.286	18	136.905		
Total	184625.000	28			
Corrected Total	30852.679	27			

Table 1.3. Variance Analysis of Mortality of *T. chilonis* 6 JSA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26350.000a	9	2927.778	66.468	.000
Intercept	195557.143	1	195557.143	4439.676	.000
Treatment	26242.857	6	4373.810	99.297	.000
Repeat	107.143	3	35.714	.811	.504
Error	792.857	18	44.048		
Total	222700.000	28			
Corrected Total	27142.857	27			

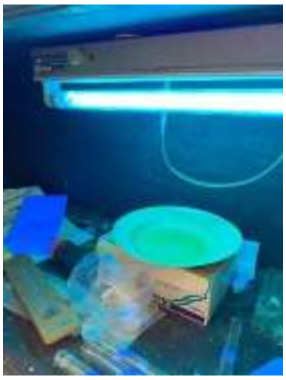
Documentation of *T. chilonis* propagation and residual testing

Propagation of *C. cephalonica*

Figure 2. *C. cephalonica* nesting tube

Transfer of *C. cephalonica* to the nesting tube

Collection of *C. cephalonica* eggs



UV light irradiation on *C. cephalonica* eggs

Laying *C. cephalonica* eggs on paperboard

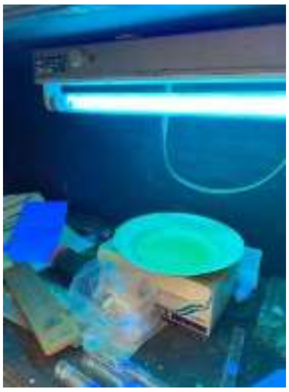


Figure 7. Starter chassis

Figure 8. *T. chilonis* propagation pias

Figure 9. Test frame

T. chilonis propagation tube

Preparation of insecticide solution

Residual testing on test tubes

Figure 13: Transfer of *T. chilonis* imago to test tubes

Figure 14. Testing the occurrence of *T. chilonis*

Figure 15: Residual testing on *T. chilonis* imago

Figure 16: Appearance of *C. cephalonica* eggs parasitized by *T. chilonis*

Figure 17. *T. chilonis* after exposure to insecticides

Figure 18: Appearance of *T. chilonis* imago that failed to emerge.

Key to Determination of Trichogramtidae

Characteristics of Insects (*Insecta*)

a. The body can be divided into 3, namely head, thorax and abdomen.
b. Its oral apparatus is used for biting, chewing, sucking and licking
c. The shape of the foot changes according to its function
d. On the head there is one pair of facet eyes (compound), single eye (occellus) and one pair of antennae which are used as a touch tool
e. Lives on land and in freshwater
f. Open circulatory system
g. Separate genitals (male and female)
h. Its digestive apparatus consists of the mouth, esophagus, cache, stomach, intestines, rectum and anal passage.
i. The mouth consists of the back jaw (mandibular), front jaw (maxillary), = upper lip (labrum) and lower lip (labium).
j. Respiratory system with tracheal system

KEY TO THE DETERMINATION OF INSECT ORDERS

(Boror *et al*., 1992)

1 Wings present, the front pair of wings often hard or membranous……………2
1'Has only one pair of membranous wings……………………………………….3
2 Has two pairs of wings………………………………………………………..4
2'Partially thick or hard wing pairs……………………………………………5
3 Reduced hind wing pair **order Diptera**
3'Membrane-shaped hind wings are not noisy……………………………….6
4 Two pairs of membranous and scaly wings **order Lepidoptera**
4' Two pairs of membranous wings **order Hymenoptera**
5 Front wings harden as a whole **order Coleoptera**
6 Front wings partially hardened **order Hemiptera**
7 Forewing hardened above costal margin **order Orthoptera**

KEY TO THE DETERMINATION OF THE FAMILY TRICHOGRAMMATIDAE

(Boror *et al*., 1992)

The determination key of the family Trichogrammatidae is 1b-13b-14a-15b-22a- 23b- 46a-47b-51a, as follows:

1b. Base of abdomen narrowed; abdominal tergum inserted into thorax functionally so that 3 pairs of spiracles and posterior pair are clearly visible dorsally; hind wings closed or fewer 488

13b. Antennae not elbowed, protonum variable 491

14a. Has wings. 491

15b. Pronotum without rounded lobes on each posterior side, if lobes are present it reachestegula 494

22a. Slight venation; forewing with 5 closed cells, hindwing without closedcells…………………………………..496

23b. Pronotum lateral or square and not reaching tegula; 3 closed cells on forewing, jugallobe on hindwing……………496

46a. Venation lacking, hind wings without incision with vannalobe shape; antennae elbowed; mesoma with distinct prepectus; trochanters generally 2-branched...501

47b. The hind femora are not swollen and have no teeth underneath or only 1 or 2 teeth……………………….501

51a. Tarsi 3-branched; wings pubescence arranged in small insects…………………………………………...502

KEY TO THE DETERMINATION OF THE GENUS TRICHOGRAMMA

(Querino *et al*., 2010)

1. Antennas with more than 1 postannual segment 2

2. Antenna with funnel available or not and stick minimum2- segment……………………………...6

3. Antenna with funnel 7Sinuate venation of anteriorwing, greatest curvature distally where the marginal vein (MV) gradually curves away from the wing marginanteriorthe stigmal vein(SV)……………………...8

4. Antennae clearly segmented into 3 segments; funicular segments sub- quadrateor only slightly transverse. Forewing withmarginal(MV) reachingthe anterior margin of the wing……………………………………………..9

5. Forewings with RS1 present; pre-marginal (PM) vein with 2 internodes. Male genitalia with dorsal lamina (DLA)……………………………………...*Trichogramma*

KEY TO THE DETERMINATION OF THE GENUS TRICHOGRAMMA

(Chan & Chou, 2000)

1. DLA is usually triangular or subtriangular, the aedeagus is not wide, with lateral margins not indented………………………….2
2. DLA with prominent lateral lobe………………………………...3
3. DLA with narrowly rounded lateral lobes at base; antennal flagellum of males containing less than 50 hairs; IVP extending to 0.38-0.44 ……………..…………......... *ADchilonis* Ishii