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Effect of Insecticide Residue and Spray Volume Application of Azadirachtin and Rotenone on *Trichogramma papilionis* (Hymenoptera: Trichogrammatidae)

MOGERET BINTI SIDI, MD. TOUHIDUL ISLAM, YUSOF IBRAHIM† AND DZOLKHIFLI OMAR[1]
Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
†Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia
[1]Corresponding author e-mail: zolkifli@putra.upm.edu.my

ABSTRACT

The effect of insecticide residue and spray volume application of the two botanical insecticides- azadirachtin (Neemix 4.5 EC) and rotenone (Rotenone 6.6 EC) were investigated against hymenopteran egg parasitoid *Trichogramma papilionis*, in comparison with synthetic insecticide- cypermethrin (Cyper 5.5 EC). The rates tested were based on the label of recommended dose (RD). Fresh solutions in distilled water were prepared for each treatment. Samples of treated leaves were taken repetitively for 1, 2 and 3 d after treatment. Each insecticides was sprayed in a pot using a knapsack sprayer fitted with the three different nozzles to deliver 100, 200 and 400 L/ha. The result demonstrated that the percentage of parasitism of *T. papilionis* was significantly reduced compared with control immediately after spraying by cypermethrin. Cypermethrin was the most toxic with 42.3% and 34.9% reduction in parasitism after 1 and 2 d of spraying, respectively. It yielded 100% mortality of *T. papilionis* at 400 L/ha. In case of 200 L/ha, rotenone yielded 59.5% mortality followed by cypermethrin (56.4% mortality). At 100 L/ha, cypermethrin yielded 82.6% mortality followed by rotenone (64.4% mortality); while azadirachtin yielded the lowest mortality (13.4%). This paper shows that azadirachtin is safer than cypermethrin and rotenone at all spray volume application rates. © 2012 Friends Science Publishers

Key Words: Biocontrol agent; Botanicals; *Corcyra cephalonica*; IPM; Maize; Parasitism capacity

INTRODUCTION

Pesticide impact on beneficial or non-target organisms based on databases depends heavily on dose-response relationship obtained in laboratory bioassays (Stark & Walter, 1995). Despite in natural situations, the natural enemies can be affected by the pesticide by multiple routes such as a long residual activity of insecticides (Banken & Stark, 1998). These residues may impose sublethal effects that can be expressed through the changes in physiological and behavioural processes in beneficial arthropods and severely reduce their performance as biological control agents (Desneux *et al*., 2007); and affect the reproductive potential of their offspring (Moscardini *et al*., 2008). Cypermethrin is a synthetic pyrethroid (Shukla *et al.,* 2002) which is used widely in the control of various agricultural pests belonging to the orders Lepidoptera, Coleoptera, Diptera, and Hemiptera. It was highly toxic to *Trichogramma pretiosum*; while it caused moderate toxicity on emergence of *T. cacoeciae* and *T. exiguum* Pinto and Platner (Suh *et al.,* 2000).

Sumner *et al*. (2000) compared three sprayer configurations with different volumetric application rates and droplet sizes and concluded that smaller droplet sizes tended to drift to the underside of cotton leaves and that larger droplet sizes were found deposited on the upper side of leaves. Mortality of budworm larvae resulting from vegetable oil sprays of permethrin increased with decreasing droplet size, but droplet size of permethrin sprays in water did not affect mortality (Smith & Luttrell, 1987). Omar *et al.* (1991), using Brussels sprout leaf bioassays of diamond back moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), larvae reported that smaller droplet sizes for both ultra low volume and water emulsion sprays of permethrin produced lower LD_{50} values than did larger droplets. The effect could not be explained completely by droplet density on the leaf surface and that the transfer of insecticide to the insect was greater with smaller droplets (Omar *et al*., 1991). Whitely, *Bemisia tabaci* control and insecticide deposition was similar for applications made by electrostatic sprayers and a controlled droplet applicator, as compared with deposits by hydraulic nozzles (Coates, 1996).

Volumetric application rate is a factor that is easily manipulated by a pesticide applicator. When good coverage is important, as in herbicide application, a high volumetric

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application rate is usually recommended. It is also often recommended for insecticide applications, but in the case of insect control, little documentation exists to verify the utility of high volume recommendations (Reed & Smith, 2001). Thacker *et al.* (1995) reported that topical bioassays of a pyrethroid and an organophosphate insecticide on aphids [*Myzus persicae* (Sulzer)] and a ground beetle [*Nebria brevicollis* (F.)] resulted in lower LD_{50} values with small droplets than with large droplets. Hall and Thacker (1994) demonstrated the same results with two pyrethroids topically applied to cabbage looper, *Trichoplusia ni* (Hubner). In both of these studies, higher concentration of the insecticide in smaller droplets was cited as the factor responsible for the increase in mortality in comparison with that of larger droplets with a lower concentration of insecticide per droplet.

Application technology can influence on the efficacy of pesticide sprays (Knoche, 1994; Matthews, 2000) and spray droplets have been shown to affect the biological effectiveness of pesticides (Ford & Salt, 1987). Locally made sprayers are mostly fitted with variable cone nozzles that produce infinitely variable range of droplet sizes and flow rates, and are arguably a contributory factor to reported poor or variable efficacy insecticides (Bateman & Chapple, 2000). While effect of different types of nozzle on some insecticides on crops have been conducted in the laboratory, information from the particular field conditions may give different results and have to be investigated (Hassan & Abdelgader, 2001). Currently, a great deal of research effort is directed towards the screening of insecticides for their selectivity so that chemicals which are less toxic to natural enemies can be recommended.

The information concerning sublethal effects of the residues of plant derived insecticides is very limited. Therefore, the present work was conducted to determine the residual effects of cypermethrin, rotenone and azadirachtin on parasitism capacity of *T. papilionis.* The degree of hazard on parasitism capacity and persistency of azadirachtin, rotenone and cypermethrin under field condition was evaluated.

MATERIALS AND METHODS

Study site: The experiment was conducted in the Laboratory of Toxicology, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The present study was carried out under laboratory conditions at 26 ± 2ºC, 70 ± 5% RH, and L12: D12 photoperiod.

Egg parasitoid: *T. papilionis* was originally collected from the maize field in Tanjung Karang, Selangor as embrios in parasitized eggs of Asian maize borer, *Ostrinia furnacalis* Guenée (Lepidoptera: Pyralidae) and kept in refrigerator at 4ºC.

Insect: The rice moth, *Corcyra cephalonica* (Pyralidae: Lepidoptera) was obtained from the Laboratory of Insect Ecology, Department of Plant Protection, Faculty of Agriculture, UPM. The rearing medium of *C. cephalonica* was consisted of an equal mixture of sterilized ground rice and maize placed in a clear plastic container (35 cm × 28 cm × 19.5 cm). The culture was maintained in an ambient environment of 26 ± 2ºC and 70 ± 5% RH. During the studies, every 3 to 4 months, fresh parasitized *O. furnacalis* eggs batches were collected from maize field in Tanjung Karang and mixed with the old laboratory colony of the parasitoid. Eggs of *C. cephalonica* were used as factitious host for rearing the egg parasitoid *T. papilionis*.

Pesticides: Two botanical insecticides- azadirachtin (Neemix 4.5 EC, Zeenex, Malaysia) and rotenone (Rotenone 6.6 EC, Saphyr, France); and one synthetic insecticide- cypermethrin (Cyper 5.5 EC, Hextar, Malaysia) were used in this experiment (Table I). Fresh solutions in distilled water were prepared for each treatment. The rates tested were based on the label of recommended dose (RD).

Maize plants: Maize plants were sown in poly bag and maintained until one month-old under glass house condition in the field under Faculty of Agriculture, UPM.

Experiment- 1: Effect of insecticide residue on parasitism capacity of *T. papilionis*: Insecticides were applied using a knapsack sprayer fitted with a cone nozzle (Lurmark 30HCX8) calibrated for an output of 100 L ha^{-1}. Immediately after application of the insecticides, fully expanded the upper third leaves were sampled. Two leaf sections (5 × 1 cm) were cut out from each leaf and individually placed in a vials (7.5 × 2.5 cm) containing 30 of less than 1 d old female *T. papilionis*. Fifty percent honey solution was painted within inner of vials (2 cm diameter). The vial was plugged with cotton wool and turned up side down to minimize the adults from entangled in the cotton wool. Samples of treated leaves were taken repetitively for 1, 2 and 3 d after treatment. The reduction in parasitism compared with controls was used to measure the persistency of the insecticides. The persistency was based on the reduction required for the pesticide residue to lose its effectiveness so that a reduction in parasitism of < 30% compared with the control was reached. The persistency of insecticides tested was classified according to the IOBC Scheme (Hassan *et al.*, 1998): 1, short lived (<5 days); 2, slightly persistent (5-15 days) 3, moderately persistent (6-30 days); and 4, persistent (>30 days). There were 3 insecticide treatments using the recommended dosages (Table I). The control pot was free of insecticide. Each treatment was consisted of 7 potted maize plants.

Experiment- 2: Effect of spray volume application rates on mortality of *T. papilionis*: Maize leaf samples were collected from one-month old maize plants. Following treatment, fully expanded the upper third leaves were sampled. Each leaf was cut into 5 × 2 cm. Each insecticides was sprayed using a knapsack sprayer fitted with three different nozzles to deliver 100, 200 and 400 L/ha (Table II). The treatments were made between 09:00 and 10:00 h to minimize the effects of exposure to UV ray. The treated

Table I: Duration of pesticides activities (persistence) on maize plants using the highest recommended dose of formulated products

Treatment	Conc. of a.i. (%) used	Evaluation category				Level of toxicity			
		0-day	1-day	2-day	3-day	0-day	1-day	2-day	3-day
Control	-	-	-	-	-	-	-	-	-
Azadirachtin	0.002	1	1	1	1	H	H	H	H
Rotenone	0.02	1	2	2	1	H	SH	SH	H
Cypermethrin	0.004	2	2	2	1	SH	SH	SH	H

H = harmless; SH = slightly harmful

Table II: Types of nozzle used in the effect of spray volume application rates on mortality of *T. papilionis*

Type of Nozzle	Swath width (m)	Flow rate (mL/min)	Forward Speed (m/min)	Application rate (L/ha)	Category
Lurmark 01-F110 (peach)	0.98	1.1	27.7	400	High
Lurmark 01-F110 (orange)	0.95	0.5	27.7	200	Medium
Lurmark 01-F110 (blue)	0.93	0.3	27.7	100	Low

The concentration of insecticides (azadirachtin, rotenone and cypermethrin) were used as same as Table I

leaves were immediately collected following treatment. Each sample of treated leaf (5 cm × 2 cm) was placed in a vial (7.5 cm × 2.5 cm) containing 10 adults of *T. papilionis*. Mortality was recorded after 1 d of spraying treatment was performed.

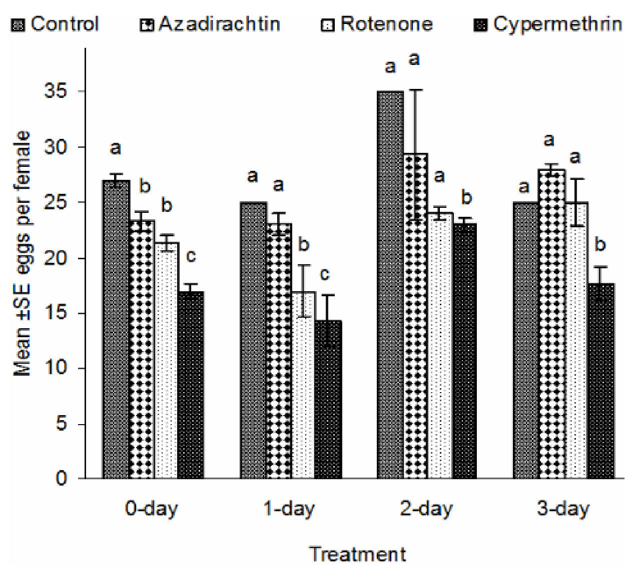
Statistical analysis: The experiments were replicated 3 times with freshly prepared insecticide suspension. The experiments were arranged in Randomized Completely Block Design (RCBD). Abbott's formula was used to correct for control mortality (Abbott, 1925) before subjecting mortality data to analysis of variance (ANOVA). Data were arcsine transformed and subjected to one-way ANOVA using the PROC GLM procedure (SAS Institute, 2001). Means were separated using honestly significant difference (HSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Experiment-1: Effect of insecticide residue on parasitism capacity of *T. papilionis*: The percentage of parasitism of *T. papilionis* was significantly reduced compared with control immediately after spraying by cypermethrin (Fig. 1). There was no significant difference between azadirachtin and rotenone and they were less toxic than cypermethrin. After 1 and 2 d of spraying, there were no significant difference in reduction of parasitism between azadirachtin and control (Fig. 2). Cypermethrin was the most toxic with 42.3% and 34.9% reduction in parasitism after 1 and 2 d of spraying, respectively (Fig. 2). After 3 d of spraying, there was no significant difference in the percentage of parasitism of *T. papilionis* between azadirachtin and rotenone compared with control (Fig. 1). However, parastisim capacity of *T. papilionis* significantly reduced by cypermethrin resulting 29.3% reduction in parasitism compared with control (Fig. 2).

As expected, azadirachtin (0.002%) in the current persistence test showed no effect on parasitism capacity of *T. papilionis*. This could be due to more rapid degradation of the active ingredients of neem that was hastened by sunlight, high temperature, and UV light (Kumar &

Fig. 1: Percentage of parasitized eggs/♀ of *Trichogramma papilionis* compared with their respective controls. Means with same letters within same column under similar insecticide are not significantly different (One-way ANOVA; HSD; P < 0.05)



Poehling, 2006). The disappearance of azadiracthin residues from sprayed conifer and deciduous foliage was rapid with 50% loss occurring after 17-22 d and the residual effect lasted for 4-8 d (Sundaram & Curry, 1994; Schumutterer, 1997). The neem products were safer compared with insecticides based on the rate of parasitization and emergence of adults from parasitized eggs (Thakur & Pawar, 2000). The azadirachtin formulations did not cause the significant mortality compared with control, when it applied at a low concentration of 0.00375% a.i to *T. minutum* (Lyons *et al*., 2003). Neemazal® at 3,000 ppm solution had relatively low residual toxicity to *T. cacoeciae* adults and was slightly harmful to the capacity of parasitism (Saber *et al*., 2005). Similarly, topical application of rotenone at the recommended dose to the adult egg predatory beetle, *Coleomegilla maculata* resulted in high mortality after 48 h (Hamilton & Lashomb, 1997). Neem seems not to be toxic to parasitoids for the ones that belong to the Hymenoptera order. The parameters usually measured to quantify the effect of neem on parasitoids are: level of parasitization, survival of adults (mortality), parasitoid development, adult emergence, longevity and antifeedancy. There are many cases where neem treatments do not affect parasitoids and can be compatible with Integrated Pest Management programmes (Cóndor_Golec, 2007). The lesser impact of rotenone on parasitism capacity of *T. papilionis* could be explained by short environmental persistence of rotenone that it is labile towards ultraviolet rays or solar irradiation, which affected the insecticidal activity of rotenone directly or indirectly (Xu *et al*., 2001). Short-lived insecticides demonstrated in this study are likely to have much less impact on natural enemies in the field and, therefore, more suitable for use in integrated plant protection (Malavolta *et al.,* 2002). Cypermethrin was more toxic to parasitoids than azadirachtin and rotenone.

Adult emergence, parasitization, mortality and antifeedancy are the most important parameters when assessing the effects of neem on parasitoids. There is a minimal impact of neem on parasitoids if applied in low doses or commonly field applied doses. In conclusion, though azadirachtin is slightly harmful to *T. papilionis* at the recommended dose, but synthetic pyrethroids might be replaceable by azadirachtin and it might also be the most suitable insecticide in IPM strategies (Depieri *et al.,* 2005; Mohan *et al*., 2007; Islam *et al.,* 2010a, b; 2011; 2012) for suppression of insect pest with minimum impact on the egg parasitoid.

Experiment-2: Effect of spray volume application rates on mortality of *T. papilionis*: In the current study, all applications were conducted at the same size area but applied with different nozzle cone tips that produce different volume application rates. At 400 L/ha of cypermethrin, the significant difference was observed on the mortality (100%) of *T. papilionis* compared with control (Fig. 3). There was no significant difference between rotenone and azadirachtin compared with control during present investigation. A similar phenomenon was observed for 200 L/ha of rotenone and yielded 59.5% mortality followed by cypermethrin (56.4% mortality) (Fig. 3). There was no significant difference in the mortality of *T. papilionis* between azadirachtin and control. At 100 L/ha, cypermethrin yielded 82.6% mortality followed by rotenone (64.4% mortality); while azadirachtin yielded the lowest mortality (13.4%). The results indicate that azadirachtin is safer than cypermethrin and rotenone at all spray volume application rates (Fig. 3).

The information concerning the effect of different spray volume application rates on the efficacy of biological pesticides is very limited. However, if pesticide applied inefficiently, it can cause unintended effects to beneficial organisms, especially among egg parasitoids which usually

Fig. 2: Percentage of reduction in parasitism of *Trichogramma papilionis*

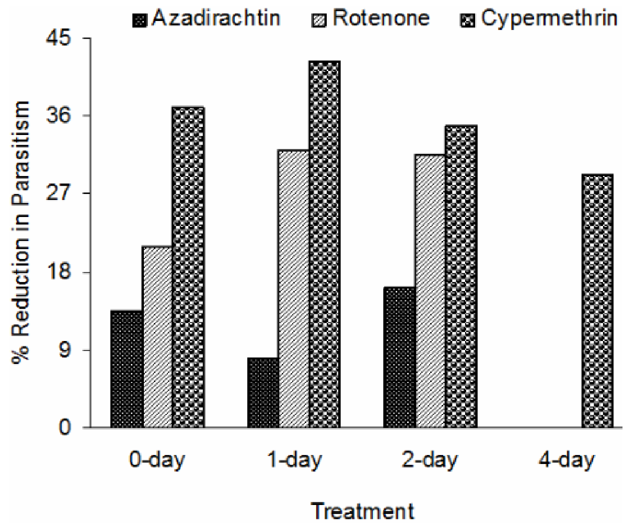
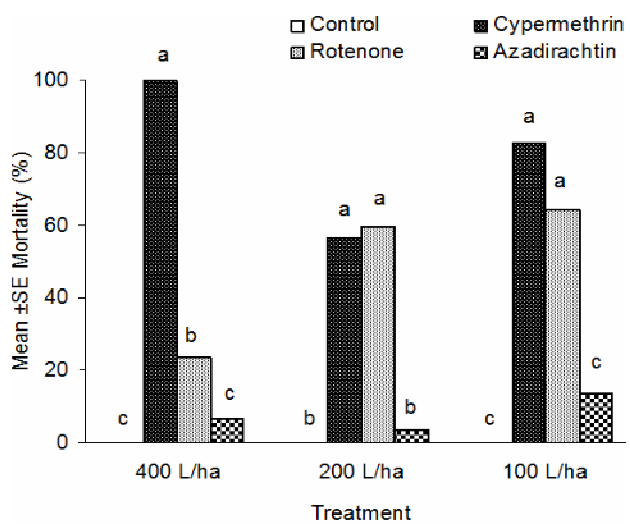


Fig. 3: Percentage of mortality of *Trichogramma papilionis* after spraying to different insecticides at different volume application rates compared with their respective controls. Means with same letters within same column under similar insecticide are not significantly different (One-way ANOVA; HSD; P < 0.05)



regulate pest populations under natural conditions (Knoche *et al*., 1998). Parasitoids and predators of pests are often susceptible to insecticides and hence can be severely affected (Vickerman, 1988). At spray volume of 400 L/ha, the mass application rate of active ingredient is higher and the spectrum of spray droplet size of the hydraulic nozzle ranges from coarse to medium. Cypermethrin was observed to give high mortality. As the spray volume was lowered the mass application of a.i. was reduced and the spray droplet spectrum of the nozzle range from medium to fine droplets. At 100 L/ha, most of the droplets were in the fine category and gave better coverage thus increasing the probability of the insect coming into contact with the a.i. Thus, cypermetrin was observed to give higher mortality. The effects of spatial structure, droplet size, droplets per area, and the amount of toxin per area on the efficacy of fipronil for control of cabbage looper on cabbage via a computer simulation model and bioassay were evaluated (Ebert *et al.,* 1999a, b). Results of their studies indicate that the interactions among the factors of deposit size, number of drops per unit area, and concentration affect efficacy more than any other single component. Droplet size and droplet concentration are approximately equal in their effect on efficacy, and that both together are more important than the number of droplets per unit area.

The integrated pest management practices require efficient use and judicious placement of agrochemicals to maximize natural control systems (Moritz & Martin, 1999). Moreover, successful integrated pest management practices rely on maintaining an active population of predators and other natural control agents. There has been considerable economic and ecological interest in increasing efficacy of spray application as often inefficient method for applying agrochemicals to crop plants (Hislop, 1987; Pimentel, 1991) and so much effort has been devoted to modifying and developing alternatives to chemical pesticides.

In conclusion, the result showed that variation in spray volume for botanical insecticides influenced their efficacy in the same manner as in case of synthetic insecticides.

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