



SAFETY OF NEWER MOLECULE DIAFENTHIURON 50 WP (NS) TO HYMENOPTERAN PARASITIDS UNDER LABORATORY CONDITIONS

*Ranjith, M., Krishnamoorthy, S.V. & Gajalakshmi, M.

Department of Agril. Entomology, Tamil Nadu Agril. University, Coimbatore- 641 003, Tamil Nadu, India

*Corresponding author email: entoranjith@gmail.com

ABSTRACT

Laboratory studies were carried out at toxicology laboratory, Tamil Nadu Agricultural University, Coimbatore to assess the safety of diafenthiuron 50 WP (NS) against three hymenopteran parasitoids viz., *Trichogramma chilonis* (Ishii), *Chelonus blackburnii* (Cameron) and *Bracon hebetor* (Say). The adult emergence study on *T. chilonis* revealed that diafenthiuron 50 WP (NS) and (ES) @ 400 g a.i. ha⁻¹ recorded an adult emergence of 75.96 and 71.14 % respectively and resulted in parasitisation of 70.12 and 66.30 % respectively. The field recommended dose of diafenthiuron 50 WP (NS) @ 400 g a.i. ha⁻¹ recorded a % mortality of 40.74 and 35.71 for *C. blackburnii* and *B. hebetor* respectively. Higher dose of diafenthiuron 50 WP (NS) @ 800 g a.i. ha⁻¹ was found toxic to all the three species. Standard checks Quinalphos 25 EC @ 150 g a.i. ha⁻¹ and phenthoate 50 EC @ 100 g a.i. ha⁻¹ recorded mortality % of more than 50.00 % and was found harmful to hymenopteran parasitoids. Untreated control resulted in least mortality for all three species and the order of toxicity of different treatments to three parasitoids are quinalphos 25 EC @ 150 g a.i. ha⁻¹ > phenthoate 50 EC @ 100 g a.i. ha⁻¹ > diafenthiuron 50 WP (NS) 800 g a.i. ha⁻¹ > diafenthiuron 50 WP (ES) 400 g a.i. ha⁻¹ > diafenthiuron 50 WP (NS) 400 g a.i. ha⁻¹ > 300 g a.i. ha⁻¹ > 200 g a.i. ha⁻¹. New Source (NS) Existing Source (ES)

KEY WORDS: Diafenthiuron, safety, parasitoids, adult emergence and mortality.

INTRODUCTION

In recent years, there has been considerable interest in development of plant protection programs that assure a more compatible use of chemical and biological methods of pest control. In these so-called 'integrated control programs' certain chemical control practices can destroy the pests without disrupting their effective natural enemies, thereby restricting subsequent pest increase. Among the most useful programs are those in which success depends upon the selection of pesticides that are less toxic to the most important natural enemies. With the use of more selective insecticides, that are less toxic to natural enemies, integration of biological and chemical applications may become helpful (Hulls and Beers, 1985). If a given insecticide or miticide kills a particular target pest or pests, why would it not kill a natural enemy? It is equally important to define what is meant by "compatibility?" Biorational insecticides and miticides are considered to be more selective to natural enemies and potentially more compatible than most conventional insecticides and miticides because they are active on a broad range of target sites or systems (Croft, 1990). Parasitoids wasps are important organisms in the natural and human modified environment. They are the natural enemies of arthropod hosts in natural ecosystem and are able to keep down the pest population, and can help prevent a pest outbreak (Hentz *et al.*, 1998). Egg parasitoids of the genus *Trichogramma* can be found worldwide in a diversity of crops and hosts preferentially in lepidopterans especially on agriculturally important pests (Hasan *et al.*, 1997). Trichogrammatids are one of the most important groups of biotic agents for the

suppression of many lepidopterous pests in India (Singh *et al.*, 1994). The braconid *Bracon hebetor* Say is a gregarious, idiobiont arrhenotokous ectoparasitoid that parasitizes lepidopteran larvae and is an important biological control agent for several stored product moth pests (Darwish *et al.*, 2003). *Chelonus* spp. are solitary egg-larval koinobiont endoparasitoids of Lepidoptera, ovipositing into the host egg and killing the host larva just before pupation (Shaw and Huddleston, 1997). In this regard, the present study aimed at incurring the harmful effect of diafenthiuron 50 WP (NS) against *T. chilonis*, *B. hebetor* and *C. blackburnii* under laboratory conditions.

MATERIALS & METHODS

Mass Rearing of Egg parasitoid, *Trichogramma chilonis* Ishii

Larvae of *Corcyra cephalonica* (Stainton) was reared in the laboratory as per the method described by (Navarajanpaul, 1973). The adult moths were allowed inside an oviposition cage of 21 x 25 x 20 cm size, with a wire mesh at the bottom for easy collection of eggs and lateral sides for ventilation. Adults were fed with 50 % honey solution. Eggs were collected from the oviposition cages daily up to four days and cleaned with sieves or egg separator. The cleaned eggs were sprinkled over half ground cumbu grains, at the rate of one cc per 2.5 kg of grains fortified with ten grams of yeast in a plastic basin (45 x 30 x 10 cm) and covered with muslin cloth. Care was taken to maintain the culture free of storage mites and diseases by mixing five grams of wettable sulphur (80 WP) and streptomycin sulphate 0.5 %, respectively. The emerged adults were collected and used

again for culturing both hosts (*C. cephalonica*) and parasitoid (*T. chilonis*). The culture was maintained at room temperature ($28 \pm 2^\circ\text{C}$ and $80 \pm 5\%$ RH).

The egg parasitoid, *T. chilonis* was mass cultured in the biocontrol laboratory on the eggs of rice moth, *C. cephalonica* as per the method described by (Prabhu, 1981). Fresh eggs of *C. cephalonica* were collected in the morning and sterilized under UV radiation of 15W capacity for 20 min at a distance of 15 cm to avoid the emergence of larvae and pasted on paper cards of 20 x 30 cm size having 30 (7 x 2cm) rectangles and placed in polythene bags along with nucleus card at 6:1 ratio for parasitisation by the egg parasitoids. The parasitised egg cards were cut into one sq. cm bits and three days old 100

% parasitised eggs (eggs appearing black and plumpy) were used for the study.

Adult emergence study

Three days old parasitised egg cards of one cm² was sprayed with insecticides using an atomizer at different concentrations viz., diafenthiuron 50 WP (NS) @ 200, 300, 400, 800 g a.i. ha⁻¹ and compared along with diafenthiuron 50 WP (ES) @ 400 g a.i. ha⁻¹ and standard checks quinalphos 25 EC @ 150 g a.i. ha⁻¹ and phenthoate 50 EC @ 100 g a.i. ha⁻¹ and replicated thrice. For untreated check, only distilled water was sprayed. The treated egg cards were shade dried for 10 minutes and then kept in a test tube of 10 x 1.5 cm size. The number of parasitoids emerged from each treatment was recorded after 24 hours of treatment and %emergence was worked out using the formula:-

$$\% \text{ emergence} = \frac{\text{No. of wasps emerged (eggs with emergence slit)}}{\text{Total no. of eggs in 1 cm}^2} \times 100$$

Parasitization study

Corcyra eggs pasted on 20 x 30 cm having 30 (7 x 2 cm) rectangles were treated with insecticide solution at different concentrations as mentioned above. The treated

eggs were provided to *Trichogramma* parasitoids at 6:1 ratio and the number of parasitized eggs (eggs appearing black and plumpy) were recorded after 48 h and the % parasitization was worked out using the formula,

$$\% \text{ parasitization} = \frac{\text{No. of parasitized eggs}}{\text{Total no. of } \textit{Corcyra} \text{ eggs}} \times 100$$

Chelonus blackburni (Cameron)

The dry film contact toxicity bioassay method described by (McCutchen and Plapp, 1988) for *Chrysoperla carnea* Stephens was adopted with slight modifications. Test insects were collected from biocontrol laboratory, Tamil Nadu Agricultural University, Coimbatore and different concentrations of insecticide solutions used for adult emergence study were prepared using water and acetone in the ratio 20: 80. Glass scintillation vials of 20 ml capacity

were evenly coated with 0.5 ml of insecticide dissolved in acetone and dried by rotating the tube horizontally on a table with palm. Adults of *C. blackburni* were released @ 10 per vial and covered with muslin cloth fastened with a rubber band. After 1h of exposure, they were released in test tubes (15 cm ht x 2.5 cm dia.) and honey solution was given as feed and observations on the mortality of the adults were made. % mortality was worked out as given below,

$$\% \text{ mortality} = \frac{\text{No. of insects dead}}{\text{Total no. of insects}} \times 100$$

Bracon hebetor Say

The bioassay method used for *C. blackburni* was used for *B. hebetor* also. Test insects were collected from biocontrol lab, TNAU, Coimbatore and the adults of parasitoid wasps were released into the vials @ 10 per vial and covered with muslin cloth secured with a rubber band. After 1h of exposure, they were released in test tubes (15 cm ht x 2.5 cm dia.) and honey solution was given as feed and observations on the mortality of the adults were made. % mortality was worked out as mentioned for *C. blackburni*.

RESULTS

Adult emergence and parasitization of *T. chilonis*

The effect of diafenthiuron on adult emergence and parasitization of *T. chilonis* was studied under laboratory conditions. *Trichogramma* parasitized *Corcyra* eggs sprayed with different doses of diafenthiuron showed a maximum adult emergence % up to 84.59. The emergence % was 75.96 and 71.14 % for diafenthiuron 50 WP (NS) and (ES) @ 400 g a.i. ha⁻¹, respectively. Diafenthiuron even at a higher dose of

800 g a.i. ha⁻¹ resulted in 69.35 % emergence. Quinalphos 25 EC @ 150 g a.i. ha⁻¹ and phenthoate 50 EC @ 100 g a.i. ha⁻¹ recorded the least emergence % of 48.02 and 51.51 respectively (Table 1). Diafenthiuron 50 WP (NS) was also found to have less effect on the parasitization of *T. chilonis*. The highest % parasitization was recorded in untreated check (90.87%). Among the different doses of diafenthiuron 50 WP (NS) the parasitization ranged between 62.34 and 82.06 per cent, in which 82.06 % was recorded in diafenthiuron 50 WP (NS) @ 200 g a.i. ha⁻¹ and the least 62.34 % from diafenthiuron 50 WP (NS) @ 800 g a.i. ha⁻¹.

Effect of diafenthiuron 50 WP (NS) on *Chelonus blackburni* Cameroon

The results on the bioassay conducted to test the contact toxicity of diafenthiuron 50 WP (NS) to *Chelonus* adults revealed that the highest dose of diafenthiuron 50 WP (NS) @ 800 g a.i. ha⁻¹ caused about 53.33 % mortality in about 6 HAT, whereas it was 33.33 % in diafenthiuron 50 WP (NS) @ 400 g a.i. ha⁻¹. Diafenthiuron (NS) and (ES) @ 400 g a.i. ha⁻¹ recorded 46.67 and 50.00% mortality 24 HAT respectively.

TABLE 1. Toxicity of diafenthuron 50 WP (NS) to *Trichogramma chilonis* Ishii

S. No	Treatments	Dose (g a.i. ha ⁻¹)	% adult		% parasitisation	% un parasitisation
			emergence	mortality		
1.	Diafenthuron 50 WP NS (0.8 g l ⁻¹)	200	84.59 (66.89) ^{ab}	15.41	82.06 (64.94) ^b	17.94
2.	Diafenthuron 50 WP NS (1.2 g l ⁻¹)	300	79.85 (63.33) ^{ab}	20.15	73.31 (58.89) ^{bc}	26.69
3.	Diafenthuron 50 WP NS (1.6 g l ⁻¹)	400	75.96 (60.64) ^{ab}	24.04	70.12 (56.86) ^c	29.88
4.	Diafenthuron 50 WP NS (3.2 g l ⁻¹)	800	69.35 (56.38) ^{bc}	30.65	62.34 (52.14) ^c	37.66
5.	Diafenthuron 50 WP ES (1.6 g l ⁻¹)	400	71.14 (57.50) ^{bc}	28.86	66.30 (54.51) ^c	33.70
6.	Quinalphos 25EC (1.2 ml l ⁻¹)	150	48.02 (43.86) ^d	51.98	46.53 (43.01) ^d	53.47
7.	Phenthoate 50EC (1ml l ⁻¹)	100	51.51 (45.87) ^{cd}	48.49	47.73 (43.70) ^d	52.27
8.	Untreated check	-	93.48 (75.21) ^a	6.52	90.87 (72.41) ^a	9.13

(Mean of three observations)

In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT

Figures in parentheses are arcsine \sqrt{P} transformed values**TABLE 2.** Toxicity of diafenthuron 50 WP (NS) to *Chelonus blackburni* Cameron

S. No	Treatments	Dose (g a.i. ha ⁻¹)	6 HAT		12 HAT		24 HAT	
			Percent mortality	Corrected mortality (%)	Percent mortality	Corrected mortality (%)	Percent mortality	Corrected mortality (%)
1.	Diafenthuron 50 WP NS (0.8 g l ⁻¹)	200	10.00 (18.43) ^{ab}	6.90	16.67 (24.09) ^{ab}	13.80	23.33 (28.88) ^b	14.81
2.	Diafenthuron 50 WP NS (1.2 g l ⁻¹)	300	20.00 (26.57) ^{bc}	17.24	26.67 (31.09) ^{bc}	24.14	36.67 (37.27) ^{bc}	29.63
3.	Diafenthuron 50 WP NS (1.6 g l ⁻¹)	400	33.33 (35.26) ^{cd}	31.04	40.00 (39.23) ^{cd}	37.93	46.67 (43.09) ^c	40.74
4.	Diafenthuron 50 WP NS (3.2 g l ⁻¹)	800	53.33 (46.91) ^d	51.73	66.67 (54.74) ^d	65.52	83.33 (65.91) ^d	81.48
5.	Diafenthuron 50 WP ES (1.6 g l ⁻¹)	400	36.67 (37.27) ^{cd}	34.49	43.33 (41.17) ^{cd}	41.38	50.00 (45.00) ^c	44.44
6.	Quinalphos 25EC (1.2 ml l ⁻¹)	150	60.00 (58.62) ^d	58.62	66.67 (54.74) ^d	65.52	90.00 (71.57) ^d	88.89
7.	Phenthoate 50EC (1ml l ⁻¹)	100	56.67 (48.83) ^d	55.17	63.33 (52.73) ^d	62.07	86.67 (68.58) ^d	85.19
8.	Untreated check	-	3.33 (10.52) ^a	-	3.33 (10.52) ^a	-	10.00 (18.43) ^a	-

(Mean of three observations)

HAT- Hours after treatment

In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT

Figures in parentheses are arcsine \sqrt{P} transformed value

ABLE 3. Toxicity of diafenthiuron 50 WP (NS) to *Bracon hebetor* Say

S. No	Treatments	Dose (g a.i. ha ⁻¹)	6 HAT		12 HAT		24 HAT		Corrected mortality (%)
			Percent mortality	Corrected mortality (%)	Percent mortality	Corrected mortality (%)	Percent mortality	Corrected mortality (%)	
1.	Diafenthiuron 50 WP NS (0.8 g l ⁻¹)	200	6.67 (14.96) ^{ab}	3.45	16.67 (24.09) ^b	13.80	23.33 (28.88) ^b	17.85	
2.	Diafenthiuron 50 WP NS (1.2 g l ⁻¹)	300	10.00 (18.43) ^{abc}	6.90	20.00 (26.57) ^b	17.24	30.00 (33.21) ^{bc}	25.00	
3.	Diafenthiuron 50 WP NS (1.6 g l ⁻¹)	400	16.67 (24.09) ^{bcd}	13.80	26.67 (31.09) ^{bc}	24.14	40.00 (39.23) ^{bcd}	35.71	
4.	Diafenthiuron 50 WP NS (3.2 g l ⁻¹)	800	26.67 (31.09) ^{de}	24.14	50.00 (45.00) ^d	48.28	60.00 (50.77) ^{def}	57.14	
5.	Diafenthiuron 50 WP ES (1.6 g l ⁻¹)	400	23.33 (28.88) ^{cde}	20.69	43.33 (41.17) ^{cd}	41.38	50.00 (45.00) ^{cde}	46.43	
6.	Quinalphos 25EC (1.2 ml l ⁻¹)	150	46.67 (43.09) ^e	44.83	53.33 (46.91) ^d	51.73	80.00 (63.43) ^f	78.57	
7.	Phenthoate 50EC (1ml l ⁻¹)	100	36.67 (37.27) ^{de}	34.49	46.67 (43.09) ^{cd}	44.83	73.33 (58.91) ^{ef}	71.43	
8.	Untreated check	-	3.33 (10.52) ^a	-	3.33 (10.52) ^a	-	6.67 (14.96) ^a	-	

(Mean of three observations)

HAT- Hours after treatment

In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT

Figures in parentheses are arcsine \sqrt{P} transformed values

Mortality 86.67% was recorded in phenthoate 50 EC @ 100 g a.i. ha⁻¹ exposed adult parasitoids which proved to be extremely harmful (Table. 2).

Effect of diafenthiuron 50 WP (NS) on *Bracon hebetor* Say.

The contact toxicity of diafenthiuron 50 WP (NS) on the parasitoid *B. hebetor* studied under laboratory conditions revealed that all the doses of diafenthiuron 50 WP (NS) caused less mortality to the parasitoid up to 12 HAT. Diafenthiuron 50 WP (NS) @ 400 g a.i. ha⁻¹ recorded 35.71 % mortality which was on par with diafenthiuron 50 WP (ES) @ 400 g a.i. ha⁻¹ (46.43 %). The higher dose of diafenthiuron 50 WP (NS) @ 800 g a.i. ha⁻¹ recorded 60.00 % mortality at 24 HAT. Quinalphos 25 EC @ 150 g a.i. ha⁻¹ and phenthoate 50 EC 100 g a.i. ha⁻¹ were found to be harmful causing mortality % of 80.00 and 73.33 per cent, respectively (Table. 3).

DISCUSSION

The findings falls in line with the studies of (Jyoti, 2006 and Halappa *et al.*, 2013) where diafenthiuron recorded more than 85 % of adult emergence in *T. chilonis* and diafenthiuron had no adverse effects on the parasitization of *B. tabaci* by *T. chilonis* in diafenthiuron sprayed fields (Lenli *et al.*, 2003). The results are in closer proximity of the studies conducted by (Javed and Matthews, 2002, Ootidobiga *et al.*, 2003) which proved the safety of diafenthiuron to braconid parasitoids. The insecticidal effect on non-target organisms are categorized according to the recommendations of the International Organisation for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group (Hasan 1989; Nasreen *et al.*, 2000) as harmless (< 50% mortality), slightly harmful (50 to 79% mortality), moderately harmful (80 to 89% mortality) and harmful (> 90% mortality) when tested at the field recommended dose. Based on this, the field recommended dose of diafenthiuron 50 WP (NS) @ 400 g a.i. ha⁻¹ was proved safer to all the three parasitoids tested and it can be used as a safer chemical in Integrated Pest Management practices.

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