Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (3): 143-147

Received: 19-04-2015
Accepted: 17-05-2015

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Toxicity of selected insecticides against the zig zag ladybird beetle *Menochilus Sexmaculatus*

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Abstract
Selective insecticides may improve conservation of natural enemies and therefore, contribute to the success of integrated pest management (IPM) programs. With regard to significance of *Menochilus sexmaculatus* (Fab.) as a predator in the management of various pests, the basic studies on toxicity of four commonly used insecticides, Cypermethrin, Emamectin benzoate, Spinosad, and Neem oil against different stages of *M. sexmaculatus* were evaluated. The results showed that among the selected insecticides, Cypermethrin exhibited high level of toxicity by causing maximum egg, larva, pupa, and adult (66.67%, 83.33%, 76.67% & 86%) mortality, respectively. Emamectin benzoate proved to be the best one with significantly least level of toxicity against egg, larva, pupa, and adult (26.67%, 20%, 20%, & 30%) mortality, respectively. Spinosad followed Emamectin benzoate in toxicity against different stages of *M. sexmaculatus* (46.67%, 50%, 40%, & 44%) mortality, respectively. The results further revealed that Neem oil was highly toxic by causing maximum egg and pupa (80% & 86.67%) mortality, respectively. Thus, results show that there is a potential to use Emamectin benzoate in an IPM program.

Keywords: Insecticides, Toxicity, Ladybird beetle

1. Introduction

The success of biological control may be enhanced by preventing the careless use of pesticides by having direct and indirect toxic effects on natural enemies. These adverse effects on the bio-agents can be minimized by considering and implementing some tactics which may play important role in expanding the function of biological control. The goal of IPM is to select such chemicals that are having compatibility. Keeping in mind this, the studies about toxicity against natural enemies can provide valuable information.

The order Coleoptera ranks first regarding diversity and significance of biological control. The family "Coccinellidae" of the order Coleoptera is exclusively predaceous except subfamily Epilachnae. Coccinellids, popularly known as ladybird beetles are the most successful group of insects, playing their important role in looking for the aphids and other soft bodied insects in field. The insecticides applied on target pests also influence the non-target insects such as parasites and predators prevailing in natural ecosystem [19].

The beetle, *Menochilus sexmaculatus* (Fab.) is predaceous coccinellid with wide distribution in Pakistan, Indonesia, the Philippines, and South Africa as the most common and efficient aphid feeding species. *M. sexmaculatus* had been found feeding on various numbers of preys including aphids, thrips, brown plant hoppers, corn borers, coccids, jassids, lepidopteran insects, scale insects, mealy bugs, white backed plant hoppers, white flies and other soft bodied insects infesting large number of cultivated crops [13].

The adult body of *M. sexmaculatus* is nearly rounded and glabrous. Head is yellowish brown, not visible from above, completely covered by pronotum. Pronotum yellowish brown with a transverse black-brownish band in middle near the posterior margin and another smaller black transverse band anterior to first one and connected to each other in the centre. Colour of elytra highly variable but generally brownish yellow. Spots black and they may be in the form of transverse zig zag patches [14].

A female lays 165 eggs with incubation period of 4-7 days. Larval period is 6 days and pupal life lasts 10-11 days. Its 3rd and 4th instar larvae are more voracious. Predatory potentials of females are more voracious than males and laboratory reared adults are also more voracious than the field collected. The total developmental period is 18 days for many aphid hosts. At Faisalabad, it is present in sugarcane fields throughout the year with maximum number in July and on maize and sorghum in August-October. The consumption of male is 22-29 and of female 28-38 aphids per 48 hours [7].

Insect pests cause up to 56% losses of crops in Pakistan [2]. Insecticides that are broadly used in the insect pest management have toxic affects against both pests and their natural enemies and the overuse of insecticides can result in the pest resurgence and occurrence of secondary pests due to the abolition of natural enemies. The concept of integrated pest management (IPM) supports both chemical and biological control strategies. However, bio-agents are usually difficult to be maintained along with the application of pesticides for the control of pests because of their sensitivity to insecticides as compared to the pests. Therefore predators and parasitoids having resistance and tolerance to various groups of pesticides should be preferably used in IPM systems.

The insecticides role in Integrated Pest Management (IPM) is significant regarding their effects on bio-agents and the IPM goal can be achieved through proper selection of insecticides that can be used in IPM in combination with bio-agents. The present research has been planned to screen out the insecticides having minimum toxicity on the predator, *M. sexmaculatus* and are also more affective in lowering down the pest population. Therefore, experiments were carried out to investigate the insecticides toxicity against different stages of *M. sexmaculatus* for safer use in IPM.

2. Materials and Methods

Insect rearing

The adults of *Menochilus sexmaculatus* (Fab.) were brought from National Agricultural Research Center (NARC), Islamabad and reared in laboratory of Plant Protection Department, Khyber Pakhtunkhwa Agricultural University, Peshawar during summer 2010. Population was reared in the laboratory for 5 generations without exposure to insecticides. Adults were kept at temperature of 27 ± 2°C, relative humidity of 60 ± 5% and a photoperiod of 12:12 (L: D) hours in a rearing chamber (L: 36cm, W: 25cm, H: 35cm). Chambers were covered with muslin cloth from the top. Aphids, *Rhopalosiphum padi* (L.) and *Myzus persicae* were maintained on cereal as a food to offer to *M. sexmaculatus*. The egg batches laid on the leaves were collected and placed in another chamber for hatching. Aphids were offered daily to the hatched larvae. After passing through different developmental stages, the larvae turned into pupa. Pupae were collected in Petri dishes and placed in another empty chamber for adults' emergence. The emerged adults were shifted to rearing chamber. The adults were reared in a similar manner as mentioned above for further multiplication.

Insecticides preparation and application

The candidate insecticides (Table-1) were tested against the different stages of *M. sexmaculatus* under laboratory condition (27± 2ºc temp and 60±5 RH). Stock solutions of formulated insecticides were prepared according to the maximum recommended dose in tap water. A control treatment of water application was included in each test to assess hatching and mortality of the test insect.

Table 1: List of insecticides with their common names, trade names, mammalian toxicity and dose per hectare.

Common name	Trade name(mg kg^{-1})	Acute Oral LD50-Rat (mg kg^{-1})	Dose ha^{-1}
Cypermethrin	Ripcord®10 EC	250	617.5ml
Emamectin Benzoate	Emamectin Benzoate® 1.9 EC	1516	494ml
Spinosad	Tracer® 240 SC	>5000	247ml
Neem oil	—	non-toxic	4940ml
Control	—	—	—

Treatment of eggs

Completely randomized design (CRD) was used to perform the experiment with five treatments, each repeated ten times.

The egg batches of *M. sexmaculatus* (Fab.) laid on leaves were obtained from rearing chamber and were dipped in the prepared insecticides solution for 3 seconds. The treated eggs were placed on paper tissue to absorb excessive insecticide solution. The eggs were placed in Petri dishes. Aphids 15-20 were released in Petri dishes, for hatching larvae as food to avoid cannibalism. The number of eggs hatched was noted after 12, 24, and 48 hrs of treatment.

Treatment of larvae

Treatments were applied in the plastic Petri dishes (5cm dia.) with the help of micro liter syringes (100cc). The Petri dishes were slowly rotated to get even spreading of the chemicals and then dried at room temperature. Aphids 10-15 were offered to *M. sexmaculatus* (Fab.) larvae as a food in each Petri dish. Ten, 2^{nd} instar larvae of *M. sexmaculatus* per treatment were individually placed in the treated Petri dishes with the help of camel hair brush. Mortality was recorded after 12, 24, and 48 hrs of treatment and larvae were considered dead when they no longer moved after being touched with brush.

Treatment of pupa

Three-day old pupae were collected from rearing chambers. Ten, pupae per treatment each repeated ten times were dipped in prepared insecticides solution for 3 seconds. The treated pupae were placed on tissue papers to absorb excessive insecticides solution. Pupae were considered dead when they did not emerge after 7 or 8 days of treatment.

Treatment of adults

Ten pairs of adults were released in the jars treated with help of atomizer. And each treatment was repeated five times. Adults' mortality was recorded after 12, 24, and 48 hrs. The overall effects of test substances were judged on the basis of the following parameters:

1. Insect's mortality (for larval and adult stages) levels were determined on the basis of principles laid down by IOBC/WPRS working group [6]. A test was considered valid if natural mortality in control does not increase beyond 12.5 %. The insecticides were categorized as; non-toxic (< 50% mortality); slightly toxic (50-79% mortality); moderately toxic (80-89% mortality) and toxic (>90%mortality).
2. In case of egg and pupal stages,

$$\frac{\text{No. of eggs hatched}}{\text{Total no. of eggs treated}} \times 100$$

And

$$\frac{\text{No. of adults emerged}}{\text{Total no. of pupae treated}} \times 100$$

were determined respectively.

Statistical Analysis

Data was assessed for analysis of variance and difference among the treatments by using computer software MSTATC.

3. Results and Discussion

Treatment of predator eggs

Toxicity results of selective insecticides against different stages of *M. sexmaculatus* are shown in Tables I-IV. The eggs

hatching of *M. sexmaculatus* after treating with insecticides were recorded and are shown in Table I.
The Table-I revealed that toxicity of different insecticides varied from 1 to 1.2 with a mean of 1.04; from 1 to 1.9 with a mean of 1.5 and 1.2 to 2.6 with a mean of 2.08 after 12hrs, 24hrs and 48hrs, respectively of the treatment. Similarly, the insecticides toxicity after 12hrs showed the highest toxicity with a mean of 1.04, followed by 24hrs (1.5) and 48hrs (2.08).

Table I: Toxicity of different insecticides against egg hatching of *Menochilus sexmaculatus* (Fab.)

Treatments	**Egg hatched after**			**Mean**	**% Mortality**
	12hrs	**24hrs**	**48hrs**		
T1= Cypermethrin	1.0000d	1.0000d	2.000b	1.3330cd	67.33
T2= Emamectin Benzoate	1.0000d	1.6000bc	2.600a	1.7330ab	27.33
T3= Spinosad	1.0000d	1.6000bc	2.000b	1.5330bc	47.33
T4= Neem oil	1.0000d	1.4000cd	1.2000cd	1.2000d	80
T5= Control (water)	1.2000cd	1.9000b	1.600a	1.9000a	10
Mean	1.04000c	1.5000b	2.08a		

(LSD $_{0.05}$ for Treatment, Intervals, & Treatments/Intervals are 1.2639, 1.2044, & 1.4570, respectively). Means followed by different letter(s) are significantly different from each other (LSD's test, $P < 0.05$)

The toxicity data (Table I) of different insecticides against egg hatching of *M. sexmaculatus* showed that Neem oil differed significantly from rest of the treatments by registering minimum hatching of eggs. Cypermethrin followed Neem oil with no significant difference by causing minimum egg hatching. Significantly high egg hatching was revealed in case of Control and Emamectin benzoate. Spinosad revealed slightly maximum egg hatching which did not differ significantly from Emamectin benzoate and Cypermethrin.
Our findings regarding Cypermethrin are in conformity with the report of Tank *et al.* (2007) [19] who reported that Cypermethrin proved toxic to the eggs of *Cheilomenes sexmaculata* Fab. (*Menochilus sexmaculatus*). However, the report of Kaethner (1991) [10] regarding Neem oil deviated from our findings who categorized Neem oil harmless for the eggs of *Coccinella septempunctata*. However, our findings about Emamectin benzoate are in conformity with Sechser *et al*. (2003) [16] who reported Emamectin benzoate relatively safe for the eggs of two ladybird beetles, *Scymnus* spp. and *Coccinella undecimpunctata* L.

Treatment of predator larvae

The Table-II revealed that the larval mortality varied from 1 to 1.6 with a mean of 1.22; from 1.1 to 1.7 with a mean of 1.36 and from 1.2 to 2.2 with a mean of 1.56 after 12hrs, 24hrs and 48hrs, respectively. Hence showed the highest larval mortality (1.56) after 48hrs, followed by 24hrs (1.36) and 12hrs (1.22).

Table II: Toxicity of different insecticides against larvae of *Menochilus sexmaculatus* (Fab.)

Treatments	**Larval mortality after**			**Mean**	**% Mortality**
	12hrs	**24hrs**	**48hrs**		
T1= Cypermethrin	1.6000bc	1.7000b	2.200a	1.8330a	83.333
T2= Emamectin Benzoate	1.1000de	1.4000bcde	1.2000cde	1.2330c	23.30
T3= Spinosad	1.3000bcde	1.5000bcd	1.7000b	1.5000b	50
T4= Neem oil	1.1000de	1.1000de	1.4000bcde	1.2000c	20
T5= Control (water)	1.0000e	1.1000de	1.3000bcde	1.1330c	13.333
Mean	1.2200b	1.3600ab	1.5600a		

(LSD $_{0.05}$ for Treatment, Intervals, & Treatments/Intervals are 1.2599, 1.2013, & 1.4501, respectively). Means followed by different letter(s) are significantly different from each other (LSD's test, $P < 0.05$)

The toxicity data (Table II) of different insecticides against larvae recorded after treatment revealed that maximum larval mortality was found in the treatment of Cypermethrin and it differed significantly from the rest of the treatments. Control, Neem oil, and Emamectin benzoate registered significantly least mortality of larvae. Larval mortality recorded after treatment revealed that slightly maximum mortality was observed in case of Spinosad followed Cypermethrin.
Our results relating to Cypermethrin are in concurrence with Tank *et al*. (2007) [19] who reported Cypermethrin toxic for the larvae of *Cheilomenes sexmaculata* (*Menochilus sexmaculatus*). Regarding Spinosad, our findings are in agreement with Galvan *et al*. (2005) [4] who examined the effect of Indoxacarb and Spinosad on development, survival and reproduction of multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) by spraying first instars and found that Spinosad lessened the survival of treated larvae. While we differ somewhat in regard to Spinosad from the observations of Ghosh *et al*. (2010) [5] who revealed Spinosad safe for the larvae of *M. sexmaculatus* and with the results of Jalai *et al*. (2009) [8] who observed that Spinosad showed no effect on larvae of two-spot ladybird, *Adalia bipunctata*. Our findings with respect to Neem oil are in agreement with Markandeya and Divakar (1999) [12] who reported Neem formulation safe for larvae of *M. sexmaculata*. Likewise, Flavia and Martinez (2004) [3] observed that Neem seed oil aqueous solution did not affect larvae survival of Coccinellid predator, *Cycloneda sanguinea* (L.) The findings of Sechser *et al*. (2003) [16] are in conformity with our findings who monitored the predator funa after the application of Emamectin benzoate and found Emamectin benzoate relatively safe for larvae of two ladybird beetles, *Scymnus* spp. and *Coccinella undecimpunctata* L.

Treatment of predator pupae

The Table-III revealed that the adult emergence from pupae varied from 1.3 to 2.9 with a mean of 2.180 and from 1.1 to 1.8 with a mean of 1.42 after 7 days and 8 days, respectively. Hence showed that the adult emergence was high after the interval of 7th day (2.18), followed by 8th day (1.42).

Table III: Toxicity of different insecticides against adult emergence from pupae of *Menochilus sexmaculatus* (Fab.)

Treatments	Adult emergence from pupa after		Mean	% Emergence
	7 days	8 days		
T1= Cypermethrin	1.5000cd	1.2000cd	1.3500c	23.333
T2= Emamectin Benzoate	2.900a	1.5000cd	2.200ab	80
T3= Spinosad	2.300ab	1.5000cd	1.9000b	60
T4= Neem oil	1.3000cd	1.1000d	1.2000c	13.333
T5= Control (water)	2.900a	1.8000bc	2.350a	90
Mean	2.180a	1.420b		

(LSD $_{0.05}$ for Treatment, Intervals, & Treatments/Intervals are 1.4473, 1.44, & 1.6326, respectively). Means followed by different letter(s) are significantly different from each other (LSD's test, P < 0.05)

The toxicity data (Table III) of different insecticides against adult emergence from pupae of *M. sexmaculatus* showed that Neem oil differed significantly from the rest of the treatments by causing minimum adult emergence from pupae. Cypermethrin closely followed Neem oil by registering minimum adult emergence from pupae. Significantly maximum adult emergence from pupae was revealed in case of Control followed by Emamectin benzoate. Spinosad revealed slightly maximum adult emergence from pupae.

Our findings about Neem oil are in conformity with the observations of Lowery and Isman (1995) [11] who studied the application of three different concentrations of Neem seed oil and observed that Neem seed oil totally prevented adult eclosion of larval of Coccinellids, *Coccinella undecimpunctata* (L.). The observations of Sechser *et al*. (2003) [16] regarding Emamectin benzoate are parallel to our findings who concluded that the insecticide was harmless to pupae of Ladybird beetles, *Scymnus* spp, & *Coccinella undecimpunctata* L. irrespective of concentrations or method of treatments. We differ from the observations made by Galvan *et al*. (2005) [4] respecting Spinosad who studied the effect of Indoxacarb and Spinosad against *Harmonia axyridis* and observed that the Spinosad increased the time for first instars to develop into adults while according to our findings Spinosad is non-toxic for adult emergence from pupae.

Treatment of predator adults

The Table-IV revealed that the adult mortality varied from 1 to 3 with a mean mortality of 1.68; from 1.4 to 4.2 with a mean of 2.36 and from 1.6 to 4.4 with a mean of 2.8 after 12hrs, 24hrs and 48hrs, respectively.

Table IV: Toxicity of different insecticides on adult mortality of *Menochilus sexmaculatus* (Fab.)

Treatments	Adult mortality after			Mean	% Mortality
	12hrs	24hrs	48hrs		
T1= Cypermethrin	3.000c	4.200ab	4.400a	3.867a	86
T2= Emamectin Benzoate	1.4000def	2.200cde	2.400cd	2.000bc	30
T3= Spinosad	1.8000def	2.400cd	3.200bc	2.467b	44
T4= Neem oil	1.2000ef	1.6000def	2.400cd	1.7330cd	22
T5= Control (water)	1.0000f	1.4000def	1.6000def	1.3330d	10
Mean	1.6800b	2.360a	2.800a		

(LSD $_{0.05}$ for Treatment, Intervals, & Treatments/Intervals are 1.6504, 1.5038, & 2.127, respectively). Means followed by different letter(s) are significantly different from each other (LSD's test, P < 0.05)

The toxicity data (Table IV) of different insecticides on adult mortality of *Menochilus sexmaculatus* showed that Cypermethrin differed significantly from rest of the insecticides by registering high level of adult mortality. Neem oil revealed minimum adult mortality. Significantly least adult mortality was observed in case of Control followed by Neem, Emamectin benzoate and Spinosad.

Our results on the subject of Cypermethrin are in agreement with Tank *et al*. (2007) [19] who reported Cypermethrin toxic insecticide for adult of *Menochilus sexmaculatus*. The findings of Abdullah *et al*. (2001) [1] are likewise in conformity who found less number of *M. sexmaculatus* in the treated plots of Cypermethrin. Swaran *et al*. (1995) [18] also reported Cypermethrin toxic for adults of *M. sexmaculatus*. The results of Sharma and Kaushik (2010) [17] are also in accordance with our findings who reported Cypermethrin toxic for the ladybird beetles. Our findings in connection with Spinosad are in conformity with Ghosh *et al*. (2010) [5] who evaluated the bio-efficacy of Spinosad against tomato fruit borer (*Helicoverpa armigera* Hub.) and its natural enemies and reported Spinosad safe for the adults *M. sexmaculatus*.

The observations made by Jalali *et al*. (2009) [8] are parallel to our findings who reported that Spinosad had no effect on adults of two-spot ladybird, *Adalia bipunctata*. The results of Jyoti & Basavana (2008) [9] and Willams *et al*. (2003) [20] are likewise in accordance with our findings who reported Spinosad 45 SC safe for Coccinellids.

Our findings with respect to Neem oil are in accordance with the observations of Abdullah *et al*. (2001) [1] who reported significantly higher number of *Menochilus sexmaculatus* in the treated plots of Neem extract. The results of Markandeya and Divakar (1999) [12] are likewise who reported Neem formulation safe for adults of *M. sexmaculatus*. Sakthivel and Qadri (2010) [15] observations are parallel to our findings who recorded Neem oil safe for predatory Coccinellid beetles. Our findings about Emamectin benzoate are in agreement with Jyoti and Basavana (2008) [9] who reported that Emamectin benzoate 5 SG did not affect the natural population of Coccinellids. The findings of Sechser (2003) [16] are likewise in conformity with our findings who reported that Emamectin benzoate was safe for the adults of ladybird beetles, *Scymnus* spp and *Coccinella undecimpunctata* (L.). We differ from the observations made by Sharma and Kaushik (2010) [17] who reported Emamectin benzoate toxic for ladybird beetles.

4. Conclusion

Among the selected insecticides, Cypermethrin revealed high level of toxicity by causing maximum mortality to all stages of *Menochilus sexmaculatus.* Emamectin benzoate proved to be the best one with significantly least level of toxicity against all stages of *M. sexmaculatus.* Spinosad followed Emamectin benzoate in toxicity, while the botanical extracts, Neem oil was observed to have maximum toxicity against egg and pupa stages.

On the bases of the observed results, it is recommended that the use of Emamectin benzoate in IPM programs should be encouraged while Cypermethrin, due to deleterious effects on *M. sexmaculatus* should be dropped out of the IPM tool kit.

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