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Toxic and Behavioral Effect of Pesticides on Aphidophagus Predator, *Coccinella septempunctata* (Linnaeus, 1758) (Coleoptera: Coccinellidae) Under Laboratory Conditions

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ABSTRACT

Eco-friendly natural enemies have received increasing attention in recent years due to their contribution in reducing the use of chemical pesticides. However, the deleterious effects of pesticides on these natural enemies remain to be understood. In the present study, toxic and behavioral effect of eight most commonly used pesticides in different orchards of Kashmir were tested against aphidophagus predator, *Coccinella septempunctata* Linnaeus, 1758 under the laboratory conditions. According to IOBC classification, alphamethrin was moderately harmful to the adults of *C. septempunctata* whereas, other pesticides were slightly harmful under direct exposure of pesticides. However, all the pesticides resulted harmful to the immature stages of *C. septempunctata*. Under the residual effect of pesticides on adults of *C. septempunctata*, thiamethoxam and dimethoate caused highest mortality. All the insecticides were observed to be toxic to the grubs of *C. septempunctata*, whereas, fungicides were moderately harmful to the grubs of *C. septempunctata*. The present results also showed that alphamethrin altered the behavior of the predator and reduced its predation rate, while as fungicide had minor behavioral effects.

Keywords: Environment, Pest control, natural enemies, pesticides, toxicity, behavior.

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INTRODUCTION

Natural enemies are vital components of agroecosystem and are consistently used as biological control agents. A wide variety of insect pest infests fruits in the Kashmir valley and cause damage to the fruit, reducing its yield and quality. Among these insect pests, scales, aphids and mites are most destructive sucking sap from the bark, foliage and fruit. They are the most recognized pests of fruit crop in Kashmir valley. In order to control the pest infestation, farmers have adopted the use of chemical pesticides and synthetic fertilizers (Bhanti & Taneja, 2007). The improvement in crop yield which is fostered by pesticides and fertilizers application is sometimes associated with the occurrence and persistence of pesticide and nutrient residues in the soil and water (Ware & Whitacre, 2004). The increasing use of these pesticides in agricultural areas have polluted aquatic environment through direct run off, discharge, careless dumping of empty containers and washing equipment directly from the rivers (Milidas, 1994). The use of pesticides is the most common, economical and easiest available method. However, the problems associated with these pesticides such as bioaccumulation, pollution and resistance are usually ignored. Also, one of the major concerns is the development of pesticide resistance. In recent years, pesticides are being used in a multipronged strategy to control insect pests (Azimizadeh, Ahmadi, Imani, Takalloozadeh, & Sarafrazi, 2012; Sánchez-Bayo, 2021). One of the important challenges of pest control with pesticides is to kill the target pests and minimizing the mortality of beneficial insects at the same time. Biological control agents such as insect predators, mite predators and hymenopterous parasitoids are usually more sensitive to pesticides than the target pests (Theiling & Croft, 1988). The effect of pesticides on natural enemies are associated with either direct effect (mortality) or indirect effects (Stapel, Cortesero, & Lewis, 2000; Elzen, 1990; Haseeb, Liu, & Jones, 2004; Borgemeister, Poehling, Dinter, & Holler, 1993). Indirect effects may be more chronic that inhibit the ability of beneficial insects to establish populations, suppress the predation and decrease reproduction (Croft, 1990; Van de Veire & Tirry, 2003; Grafton-Cardwell, Lee, Stewart, & Olsen, 2006). Biological control occupies a central position in integrated pest management (IPM) programmes, because biological control agents for pests have enormous and unique advantage, it is safe, permanent and economical. Among different insect predators Coccinellidae is the largest family of order Coleoptera and its members are commonly known as ladybird beetles, ladybugs or Coccinellid beetles. *Coccinella septempunctata* Linnaeus, 1758 is one of the most important beetle belonging to this family. The Coccinellidae are an important group of beetles from both economic point of view as their use in biological control and in their diversity and adaptation to a number of differing habitats. *C. septempunctata* is extremely diverse in their habitats and play important role as biological control attacking different pests such as aphids, coccids and other soft bodied insect pests. Thus, the aim of the present investigation was to study the lethal and behavioral effects of the most commonly used pesticides in the Kashmir valley, on predatory lady bird beetle *C. septempunctata.*

MATERIALS AND METHODS

Experimental Setup

The experiment was conducted in the entomology laboratory University of Kashmir. The adults and immature stages of *C. septempunctata* used in the experiment were laboratory reared under controlled conditions (28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity). Experiment was initiated by collecting adults of *C. septempunctata* from fields. Mating pairs were kept in glass jars covered with muslin cloth. They were provided with abundant supply of food in the form of infested twigs of cabbage aphids, *Brevicoryne brassicae* (Linnaeus, 1758) until oviposition. Dry twigs were replaced with fresh ones after every 24 hrs in order to avoid contamination. The newly emerged adults and immature stages of *C. septempunctata* were used for further experimentations.

Pesticides

Pesticides tested in this study include six insecticides such as alphamethrin, chlorpyrifos + cypermethrin, dimethoate, quinalphos, thiamethoxam, dichlorvos and two fungicides such as myclobutanil and flusilazole. These pesticides are locally used by the farmers against different pests in various crops and their use has been increased during recent years in Kashmir, India (Table 1). These insecticides were tested at a single rate of application, corresponding to their maximum recommended label rate which is generally used by the farmers (Handbook, 2015, 2017 and 2018). Adult and immature stages of *C. septempunctata* were exposed to pesticide through double exposure method: directly exposed to spray droplets and residually through walking on sprayed leaf. A double exposure method signifies the best condition that insects are likely to experience in the field.

Table 1. List of pesticides used in the present study. All pesticides were used at their highest label rates against predators.

Active ingredient	Commercial name	Mode of Action	Target Pests
Alphamethrin 10% EC	Stop 10 EC	Contact and Stomach Action. It acts on Nervous system.	Aphids
Chlorpyrifos50% +Cypermethrin 5%	Cyclone	It is non-systematic insecticide of organophosphorus and synthetic pyrethroids group with contact and stomach action.	Chewing and sucking insect pests
Dimethoate 30%EC	Rogor	Contact and ingestion type of systematic insecticide. It is Acetylcholinesterase inhibitor.	Mites, hoppers, bugs, borers, thrips, white flies and aphids.
Thiamethoxam 25% WG	Tara	Systematic insecticide having Contact and stomach action and belonging to Neonicotinoid group.	Borers, aphids, thrips, hoppers, caterpillars
Quinalphos 25% EC	Krush	Non-Systematic insecticide. Contact and stomach action belonging to Organothiophosphate group.	Paddy pests, wooly aphids, red gram pests.
Dichlorvos 76%	Nuvan	Organophosphate insecticide Acetylcholinesterase inhibitor.	Army worm, leaf eating pests and caterpillars.
Myclobutanil 10% WP	Index	Triazole group of Systematic fungicide. Steroid demethylation inhibitor.	Used against Ascomycetes, powdery mild dew, dollar spot, brown patch and scab.
Flusilazole 40% EC	Governor	Triazole group of Systematic fungicide. It is an organosilicon compound.	Powdery mild dew and scab.

Source: PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/ 49833, 3082, 5821911, 26124, 3039, 6336, 73675, 2730, 2912).

Direct exposure of pesticides

In this experiment, adult individuals of *C. septempunctata* were placed in petri dish (9 cm diameter) in a group of 10. The top of petri dish was covered with fine muslin cloth tightened with rubber band and were maintained at 28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity. They were also provided with food (cabbage aphids, *Brevicoryne brassicae).* Distilled water was used as control. The spray volume of pesticide solution and distilled water per application was 1 ml, using small calibrated hand sprayer (1liter capacity) equipped with a nozzle suited to low volume spray application (Martinou, Seraphides, & Stavrinides, 2014) (Table 2). The experiment was replicated thrice. The mortality was recorded after 24 hr, 48 hr, 72 hr and 96 hr. Moribund individuals were classified as dead. Similar method was applied to first and fourth immature stages of *C. septempunctata*.

Table 2. Direct exposure of Pesticide treatments used against the predator *Coccinella septempunctata* using petri plate assay under laboratory conditions (28.55 ± 2.83◦ C and 75.15 ± 6.06% relative humidity).

Sample No.	Group*	No. of Petri dishes in each group/ Individual	Treatments	Spray Volume (ml/Petri dish)
1	G1	3/30	Alphamethrin	1
2	G2	3/30	Chlorpyrifos + Cypermethrin	1
3	G3	3/30	Dimethoate	1
4	G4	3/30	Quinalphos	1
5	G5	3/30	Thiamethoxam	1
6	G6	3/30	Dichlorvos	1
7	G7	3/30	Flusilazole	1
8	G8	3/30	Myclobutanil	1
9	G9	3/30	Distilled water	1

*Each group represents three petri dishes with 10 individuals subjected to 9 treatments.

Residual effect of pesticides

In this study, fresh kale leaf discs (90 mm in diameter) sprayed with pesticide solution and distilled water as control was used (three replicates). The spray volume per application was 1 ml for each pesticide solution and distilled water, using small calibrated hand sprayer (1 litre capacity). Treated leaves were dried at room temperature for 2 hr and then placed on the bottom of clean petri dishes (9 cm diameter). In each petri dish 10 adult individuals of *C. septempunctata* were released using camel- hair brush. Cabbage aphids, *Brevicoryne brassicae* were provided as food and the top of petri dish was covered with fine muslin cloth. The petri dishes were maintained at 28.55 ± 2.83˚C and 75.15 ± 6.06% relative humidity. Moribund individuals were classified as dead (Martinou et al, 2014). Mortality was recorded after 24 hr, 48 hr, 72 hr and 96 hr (Table 3). Similar method was applied to first and fourth immature stages of *C. septempunctata*.

Behavioral effect of pesticides

In a second assay, behavioral effect of pesticides, that showed highest and lowest toxicity in previous experiment were evaluated. For this experiment young kale plant, *Brassica oleracea* var. *acephala,* Linnaeus with aphid infestation (five-week-old, height 15 cm, 5 leaves /plant) was sprayed with pesticide with the help of calibrated handheld sprayer and distilled water for control. Treated plant was dried at room temperature for 2 hours, after that adult *C. septempunctata* was placed at the bottom of the main stem of plant with the help of camel-hair brush. The adult predator was observed for 11 min with two magnifying glass held at 45° angle and at 30 cm distance. The duration for each of the following behaviors were recorded: feeding, preening, walking and resting. The experiment was carried out in 7 days between 10.00 am to 14.00 pm with three replicates of each treatment per day.

Table 3. Residual effect of pesticide treatments used against the predator *Coccinella septempunctata* using petri plate assay under laboratory conditions (28.55 ± 2.83◦ C and 75.15 ± 6.06% relative humidity).

Sample No.	Group*	No. of Petri dishes with treated leaf in each group/ Individual	Treatments
1	G1	3/30	Alphamethrin
2	G2	3/30	Chlorpyrifos + Cypermethrin
3	G3	3/30	Dimethoate
4	G4	3/30	Quinalphos
5	G5	3/30	Thiamethoxam
6	G6	3/30	Dichlorvos
7	G7	3/30	Flusilazole
8	G8	3/30	Myclobutanil
9	G9	3/30	Distilled water

*Each group represents three petri dishes with 10 individuals subjected to 9 treatments.

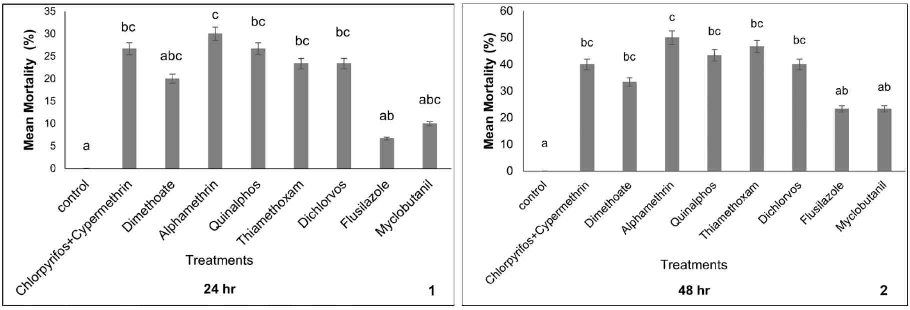
Data Analysis

The mortality results obtained after 24, 48,72 and 96 hours were adjusted for mortality that occurred in control (water) using the Abbot's formula (Abbot, 1925). The corrected mortalities of adults and immature stages were evaluated by the IOBC toxicity rating scale for pesticides under laboratory conditions: 1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79% Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality) (Hassan et al., 1994). For statistical comparison of the mortality of adults, the data was subjected to one-way analysis of variance (ANOVA). Post hoc Tukey HSD tests (Tukey HSD) were used for multiple pair wise comparison. The significance difference between the means was determined at $P \leq 0.05$. Behavioral data were expressed as time in "minutes" allocated to each different activity during the 11 minutes observation period.

RESULTS

Direct exposure of pesticides on adults of *C. septempunctata*

We observed differential degree of toxicity for the pesticides used against adults of *C. septempunctata*. After 24 hr exposure to the insecticides, the mortality caused by alphamethrin, quinalphos and chlorpyrifos+ cypermethrin was 30%, 26.66% and 26.66%, respectively. Mortality of *C. septempunctata* due to thiamethoxam and dichlorvos was found to be in the same range of 23.33%. Mortality caused by the treatments flusilazole and myclobutanil was found 6.66% and 10%, respectively. One-way ANOVA results showed no significant difference in mean percentage mortality among alphamethrin, quinalphos, thiamethoxam, dichlorvos, chlorpyrifos + cypermethrin, dimethoate and myclobutanil treatments (Fig. 1). Moreover, no mortality was observed in control treatment. Flusilazole and myclobutanil caused lowest mortality (23.33%) of *C. septempunctata* after 48 hr duration. However, 50% mortality was observed with treatment alphamethrin. Thiamethoxam and dimethoate caused second and third highest mortality. Alphamethrin showed significant difference from flusilazole and myclobutanil mortality (Fig. 2). Again, control was found with no mortality.



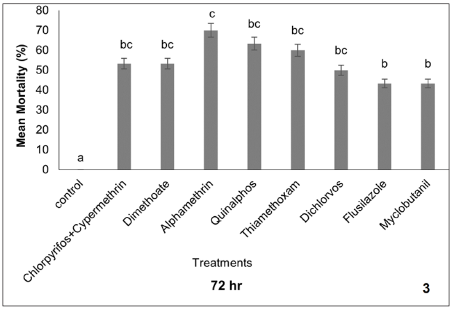
Figures 1-2. Mean percentage mortality of *Coccinella septempunctata* after 24 hr and 48 hr by direct exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.

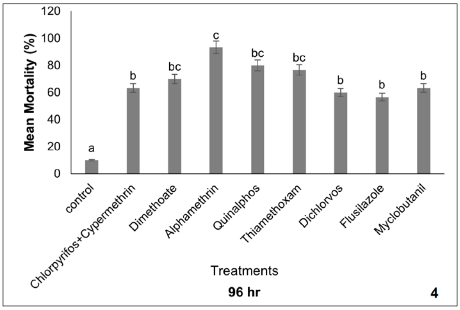
After 72 hr duration, alphamethrin again showed highest mortality of 70% in *C. septempunctata* followed by quinalphos 63.33%. ANOVA results showed that mortality caused due to alphamethrin differ significantly at p≤0.05 from control, flusilazole and myclobutanil mortality. However, there was no significant difference between the mortality caused by flusilazole and myclobutanil. Similar results were shown by quinalphos, thiamethoxam, dimethoate, dichlorvos and chlorpyrifos + cypermethrin (Fig. 3). After 96 hr of exposure, highest mortality was observed in alphamethrin (93.33%) followed by Quinalphos (80%) and Thiamethoxam (76.66%) against *C. septempunctata*. We observed 10% mortality of *C. septempunctata* in the control group. Mortality produced by alphamethrin was significantly (p≤0.05) higher than control, chlorpyrifos+cypermethrin, dimethoate, flusilazole and myclobutanil (Fig. 4). The corrected mortality of *C. septempunctata* under different treatments after 96 hr is shown in table 4. The corrected mortalities of adult *C. septempunctata* was evaluated by IOBC toxicity rating scale for pesticide under laboratory conditions. According to IOBC rating for laboratory assays, alphamethrin was observed moderately harmful to *C. septempunctata* after 96 hr treatment while others are slightly harmful during the present study.

Table 4. Mean and Corrected Mortality of *Coccinella septempunctata* after 96 hr direct exposure to Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)	Corrected Mortality (%)	IOBC* Rating
Insecticides	Alphamethrin	9.33±0.57	92.58	3
	Quinalphos	8.00±1.00	77.77	2
	Thiamethoxam	7.66±0.57	74.06	2
	Dimethoate	7.00±1.00	66.66	2
	Chlorpyrifos +Cypermethrin	6.33±0.57	59.25	2
	Dichlorvos	6.00±0.00	55.55	2
Fungicides	Myclobutanil	6.33±1.15	59.25	2
	Flusilazole	5.66±1.52	51.84	2

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).





Figures 3-4. Mean percentage mortality of *Coccinella septempunctata* after 72 hr and 96 hr by direct exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.

Direct exposure of pesticides on immature stages of *C. septempunctata*

The newly hatched first instar grubs and fourth instar grubs of *C. septempunctata* were used in this experiment. The results showed 100% mortality after 24 hr exposure to the treatments alphamethrin, thiamethoxam, dimethoate, dichlorvos, quinalphos and chlorpyrifos+ cypermethrin in first instar grubs of *C. septempunctata*. Whereas, myclobutanil and flusilazole caused 96.66% and 93.33% mortality, respectively. However, after 48hr 100% mortality was observed due to fungicides. On the other hand, 6.66% and 16.66% mortality were observed in control after 72 and 96 hr respectively (Table 5).

Similarly, in fourth instar grubs of *C. septempunctata*, 100% mortality was observed after 24 hr exposure to the treatments alphamethrin, thiamethoxam, quinalphos and chlorpyrifos +cypermethrin. Dimethoate and dichlorvos caused 93.33% and 96.66% mortality after 24 hr, respectively. On the other hand, both treatments caused 100% mortality after 72 hr. The mortality of fourth instar grubs due to treatments myclobutanil and flusilazole were 90% and 86% respectively after 24 hr and 100% after 72 hr in both the treatments. Moreover, in control 3.33% and 6.66% mortality was observed after 72 hr and 96 hr respectively during the present observations (Table 6). All the pesticides were found harmful to both the grubs of *C. septempunctata* according to IOBC rating for laboratory assay (Table 5 and 6).

Table 5. Mean Mortality of First instar grubs of *Coccinella septempunctata* after direct treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24hr →	48hr →	72hr →	96hr	
Insecticides	Alphamethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	6.66±0.00	16.66±0.00	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

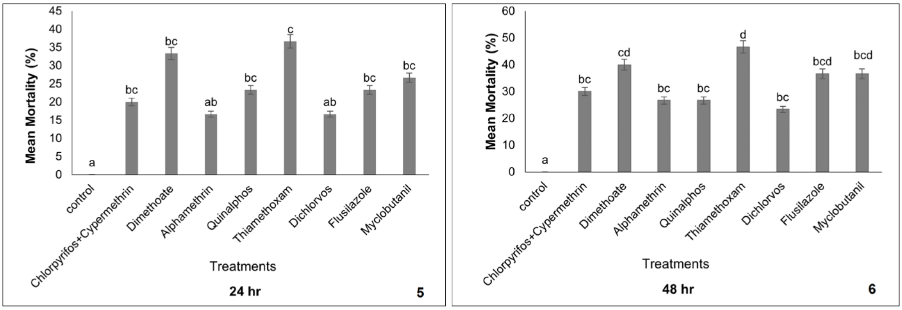
Table 6. Mean Mortality of Fourth instar grubs of *Coccinella septempunctata* after direct treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24hr	48hr →	72hr	96hr	
Insecticides	Alphamethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	93.33±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	96.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	90.00 ±0.00	93.33 ±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	86.66 ±0.00	90.00 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	3.33±0.00	6.66±0.00	1

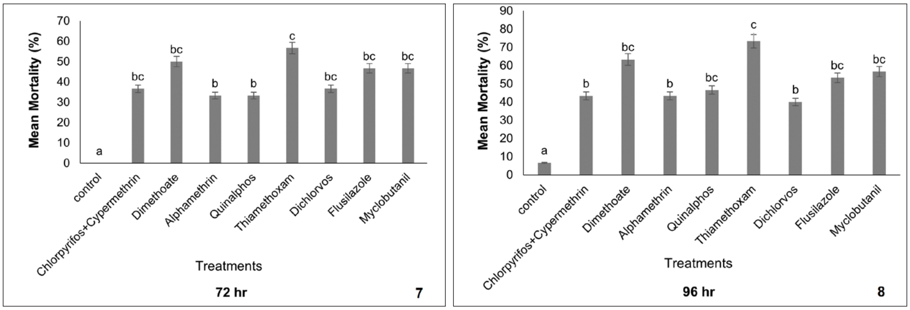
*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Residual effect of pesticides on adults of *C. septempunctata*

Under the residual effect after 24 hr duration, insecticide thiamethoxam and dimethoate was found to cause highest mortality of adult *C. septempunctata*. One-way ANOVA results showed that thiamethoxam caused significantly high mortality (36.66%) at $P \leq 0.05$ than other treatments. There was no significant difference at $p<0.05$ between the percentage mortalities caused by chlorpyrifos+cypermethrin, dimethoate, quinalphos, flusilazole and myclobutanil (Fig. 5). After 48 hr duration, the observed mortality of 46.66% was caused by thiamethoxam, 40% with dimethoate, 36.66% with flusilazole and myclobutanil and 30% with chlorpyrifos +cypermethrin against adult *C. septempunctata*. ANOVA results showed no significant difference at $p \leq 0.05$ between the mortality caused by chlorpyrifos+cypermethrin, alphamethrin, dimethoate, quinalphos, flusilazole and myclobutanil (Fig. 6). After 72 hr duration, the mortality of 56.66% and 50% was observed by thiamethoxam and dimethoate respectively against *C. septempunctata*. There was no significant difference amongst the mortality caused by flusilazole, myclobutanil, dimethoate, chlorpyrifos +cypermethrin and dichlorvos. Till 72 hr, no mortality was observed in control (Fig. 7). After 96 hr, the mortality of *C. septempunctata* reached to a maximum of 73.33% with treatment thiamethoxam, 63.33% with dimethoate and 56.66% in case of myclobutanil. The mean percent mortality of *C. septempuctata* in control was observed 6% during the present study. There was no significant difference at $p \leq 0.05$ between the mortalities caused by alphamethrin, dichlorvos and chlorpyrifos+cypermethrin (Fig. 8). The corrected mortality of *C. septempunctata* after 96 hr exposure to different treatments is represented in Table 7. The corrected mortalities of adults *C. septempunctata* were evaluated by IOBC toxicity rating scale for pesticide under laboratory conditions. All the treatments were found slightly harmful to the adults of *C. septempunctata* at 96 hr according to IOBC rating for laboratory assays.



Figures 5-6. Mean percentage mortality of *Coccinella septempunctata* after 24 hr and 48 hr residual exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.



Figures 7-8. Mean percentage mortality of *Coccinella septempunctata* after 72 hr and 96 hr residual exposure to pesticides. Percentage error is added on bars and different letters on bars indicate statistically significant differences at p≤0.05, in one-way ANOVA followed by the Tukey's multiple comparison test.

Table 7. Mean and Corrected Mortality of *Coccinella septempunctata* after 96 hr exposure to Pesticides under residual effect and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)	Corrected Mortality (%)	IOBC* Rating
Insecticides	Alphamethrin	4.33 ±1.15	34.61	2
	Quinalphos	4.66 ±0.57	38.45	2
	Thiamethoxam	7.33 ±0.57	69.22	2
	Dimethoate	6.33 ±1.15	57.69	2
	Chlorpyrifos +Cypermethrin	4.33 ±1.52	43.33	2
	Dichlorvos	4.00 ±1.00	30.77	2
Fungicides	Myclobutanil	5.66 ±0.57	49.99	2
	Flusilazole	5.33 ±0.52	46.15	2

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Residual effect of pesticides on immature stages of *C. septempunctata*

Like in above experiment newly hatched first instar grubs and fourth instar grubs were used for this experiment. We observed 96.66% and 100% mortality of first instar grubs in the treatment alphamethrin after 24 and 72 hr respectively. However, treatments with thiamethoxam and quinalphos were found to cause 100% mortality in first instar grubs after 24 hr. Whereas, other treatments were found to cause 100%

mortality after 48 hr. Myclobutanil and flusilazole were also observed to cause 100% mortality in first instar grubs after 72 hr. Besides this, control showed 3.33% and 10% mortality after 72 and 96 hr respectively. All the eight pesticides were found harmful to first instar grubs according to IOBC rating scale for pesticides under laboratory conditions (Table 8).

Table 8. Mean Mortality of first instar grubs of *Coccinella septempunctata* after residual treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC*Rating
		24hr	48hr →	72hr	96hr	
Insecticides	Alphamethrin	96.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Quinalphos	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	93.33±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	86.66 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dichlorvos	93.33 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	4
Fungicides	Myclobutanil	90.00 ±0.00	96.66±0.00	100 ±0.00	100 ±0.00	4
	Flusilazole	86.66 ±0.00	93.33 ±0.00	100 ±0.00	100 ±0.00	4
Control	Distilled water	0.00±0.00	0.00±0.00	3.33±0.00	10.00±0.00	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

In the fourth instar grubs of *C. septempunctata* treatment alphamethrin showed 93.33% mortality at 24 hr and 100% mortality was perceived after 72 hr. However, in treatments like quinalphos and thiamethoxam 100% mortality was observed after 48 hr. 100% mortality was also observed in fourth instar grubs after 96 hr in treatments dichlorvos and chlorpyrifos +cypermethrin. Fungicides, myclobutanil and flusilazole were found to cause 96.66% and 86.66% mortality respectively. According to IOBC rating scale for pesticides under laboratory conditions, all the insecticides were found to be harmful to the fourth instar grubs except fungicide flusilazole and myclobutanil that are moderately harmful. Moreover, in control 6.66% mortality of fourth instar grubs was observed (Table 9).

Table 9. Mean Mortality of Fourth instar grubs of *Coccinella septempunctata* after residual treatments of Pesticides and toxicity rating.

Pesticide Group	Treatment	Mean Mortality (±SD)				IOBC* Rating
		24 hr	48 hr →	72 hr	96 hr	
Insecticides	Alphamethrin	93.33±0.57	96.66 ±0.57	100 ±0.00	100 ±0.00	4
	Quinalphos	96.66 ±0.57	100 ±0.00	100 ±0.00	100 ±0.00	4
	Thiamethoxam	96.66 ±0.57	100 ±0.00	100 ±0.00	100 ±0.00	4
	Dimethoate	90.00±0.1	96.66 ±0.57	100 ±0.00	100 ±0.00	4
	Chlorpyrifos +Cypermethrin	83.33 ±0.57	93.33 ±0.57	96.66 ±0.57	100 ±0.00	4
	Dichlorvos	86.66 ±0.57	90.00 ±0.1	96.66 ±0.57	100 ±0.00	4
Fungicides	Mycobutanil	73.33 ±0.57	76.66 ±0.57	86.66 ±0.00	96.66±0.57	3
	Flusilazole	66.66 ±0.57	73.33 ±0.57	80.00 ±0.57	86.66±0.57	3
Control	Distilled water	0.00±0.00	0.00±0.00	0.00±0.00	6.66±0.57	1

*1, Harmless (<30% Mortality), 2, Slightly Harmful (30-79 % Mortality), 3, Moderately Harmful (80-99% Mortality) and 4, Harmful (>99% Mortality).

Behavioral effect of pesticides on *C. septempunctata*

Behavioral effect of pesticides on predator *C. septempunctata* was carried out under laboratory conditions (28.55 ± 2.83° C and 75.15 ± 6.06% relative humidity).

Young kale plant sprayed with two insecticides, alphamethrin and thiamethoxam that caused highest mortality and one fungicide (myclobutanil) with lowest mortality in the previous experiment were used for recording behavioral activities. Distilled water was used as control. Four main behavioral activities; resting, walking, preening and feeding were observed. The present observations showed that *C. septempunctata* spent more time in resting when subjected to insecticide alphamethrin and thiamethoxam as compared to myclobutanil and control (Fig. 9a). Similarly, *C. septempunctata* on control plants spent lower amount of time in preening compared to plants sprayed with alphamethrin (Fig. 9b). Regarding the behavioral activity of feeding, *C. septempunctata* on control plants spent significantly longer time in feeding. In case of the plants sprayed with alphamethrin, *C. septempunctata* spent less time in feeding. Moreover, *C. septempunctata* also spent longer time in feeding on plants sprayed with myclobutanil as compared to thiamethoxam (Fig. 9c). Total walking time of *C. septempunctata* did not differ much among the plants subjected to pesticides and Control (Fig. 9d).

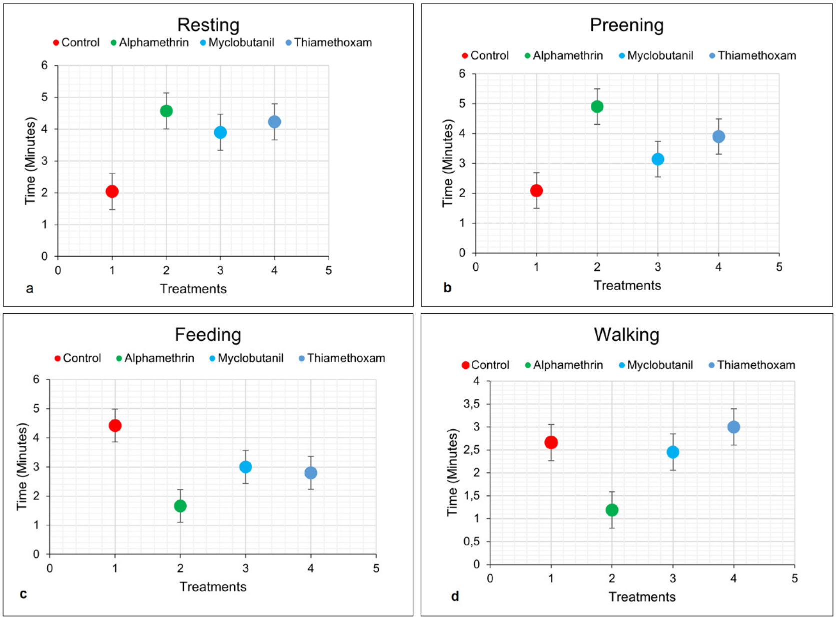


Figure 9. Time in minutes allocated to (a) resting (b) preening (c) feeding and (d) walking by adults of *Coccinella septempunctata* on plants treated with different pesticides and distilled water (control). Duration of the observation period was 11 minutes. Different points with standard error bars showing behavior among different treatments.

DISCUSSION

The laboratory contact toxicity assay conducted on immature stages of *coccinella septempunctata* with different insecticides showed highest mortality rates. Results obtained in the present study revealed that in particular alphamethrin, quinalphos and thiamethoxam caused highest mortality on all the tested ladybird beetles. The results attained with different insecticides is in agreement with others reported in the literature, according to which the compounds belonging to chemical group organophosphate, organochlorides, neonicotinoids pyrethroids and thiazole insecticides are highly harmful to natural enemies (Hassan et al, 1994; Hirai,1993; Gorri et al, 2015; Martinou et al, 2014; He, Zhao, Zheng, Desneux, & Wu, 2012; Lanka, Ottea, Davis, Hernandez, & Stout, 2013 and Liu & Zhang, 2012). Similar study was observed by Mollah, Rahman, & Alam (2013) in which they evaluate the effect of some insecticides on the abundance and mortality of predacious ladybird beetles in bean ecosystem. Their results showed the highest number of dead ladybird beetles after treatment with insecticide Curtap followed by Esfenvalerate, Deltrametrin, Cypermethrin, Fenitrothion, Fenvelarate and Emamectin benzoate. Also, Shah & Khan (2014) studied the coccinellid biodiversity under pesticide pressure in horticulture ecosystem of Kashmir valley. The observations are in agreement with the present results that pesticide treated ecosystem support less number of ladybird beetle species as compared to untreated horticulture ecosystem. Ujjan et al, (2017) worked on toxicity of insecticides and biopesticides against predatory beetle, *Menochilus sexmeculatus* Fabricius. The insecticides Curacron and Novastar showed severe toxicity to the egg and adults of *M. sexmaculatus*. On Contrary, the influence of 7 pesticides (6 insecticides & 1 acaricide) on different stages (adults, larvae, eggs) of *C. septempunctata* and adults of *Adalia bipunctata* were evaluated under laboratory conditions by Olszak, Ceryngier, & Warabieda (2004). Their results showed that aphids contaminated with chemicals such as pirimicarb, novaluron, pyriproxyfen and fenpyroximate did not cause serious effects on the *C. septempunctata* and *A. bipunctata*.

In second assay under residual effect of pesticides higher percentage of mortalities were shown by immature grubs of *C. septempunctata*. Similarly, all the pesticides when tested on adults of *C. septempunctata* showed reduced survival rate. Serious effect of thiamethoxam and dimethoate on the adults of *C. septempunctata* were observed. Indirect effect of pesticides is related to the residues remaining after foliar application that indirectly affect natural enemies by inhibiting adult emergence (Teodoro, Pallini, & Oliveira, 2009; Rill, Grafton-Cardwell, & Morse, 2008). Also some natural enemies are indirectly affected by consuming contaminated food or prey. Besides toxic effects, the behavioral effect of pesticides on adults of *C. septempunctata* was observed. Some behavioral activities of adults of *C. septempunctata* were carefully observed on pesticide treated plants. The present results showed that the adults of *C. septempunctata* changed behavior on pesticide treated plants like less feeding and walking. They show lower activities and learning response. The sub lethal dose does not cause adults death but inference in biological traits that may reduce the population. Earlier studies indicated that behavioral effect of insecticides may result in a rapid disruption of predatory behavior and a potential decrease in the ability of coccinellids to trace and capture their prey (Singh, Walters, & Port, 2001; Singh, Walters, Port, & Northing, 2004; Stark, Banks, & Acheampongl, 2004; Stark, Vargas, & Bank, 2007). The behavioural responses may also alter the predator's hunt pattern (Thornham, 2005; Thornham, Blackwell, Evans, Wakefield, & Walters, 2008). Dent (2000) documented that non-lethal effects of pesticides include weakening of the predatory insects, changing their behaviour and lengthening the development period of the immature stages which will lead to the reduced prey consumption and reproductive ability.

It is evident from the present results, that all the pesticides tested were observed slightly harmful against adults of *C. septempunctata*. This proves helpful in deciding the most suitable pesticide to be used according to target predator. However, the major concern is about immature stages of *C. septempunctata*, as all the pesticides tested proved harmful against them. Thus, we suggest that the use of pesticides against serious pests should be in accordance with the compatibility of predators and pesticides. Different aspects such as class of pesticide applied, type of natural enemy, pesticide preparation, concentration to which natural enemies are exposed to, contact time, timing of application and developmental life stage exposed to pesticide should be considered. The outcome of the laboratory assay highlights the importance of recommending the selective pesticides for different pest management.

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