



Research Article

Assessment of Toxicity of New Chemistry Insecticides against *Coccinella septempunctata* under Laboratory Conditions

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Abstract | Ladybird beetle (*Coccinella septempunctata*) is present in various crops like cotton, sunflower, fruit plants, and vegetables. *C. septempunctata* is the free living predatory specie of sucking insect pests such as aphids, mealybugs, and whiteflies and consume many preys during its lifetime. *C. septempunctata* larvae and adults are both active predators. The present study regarding toxicity of new chemistry insecticides against *C. septempunctata* was conducted under laboratory conditions. The toxic potential of six synthetic insecticides Emamectin benzoate (200ml/acre), Indoxacarb (175ml/acre), Lufenuron (200ml/acre), Spinetoram (80ml/acre), Chlorantraniliprole (50ml/acre), and Flubendiamide (50ml/acre) were tested on *C. septempunctata*. The leaf dip bioassay method was performed by collecting sunflower leaves and a 2cm leaf disc with larvae was dipped in insecticides. Data regarding mortality was recorded after 24, 48, 72, 96, and 120 hours. Mortality rates were as follows: 28% for emamectin benzoate, 26% for indoxacarb, 22% for lufenuron, 22% for Spinetoram, 16% for chlorantraniliprole, 14% for chlorantraniliprole, and 10% for Flubendiamide. However, Lufenuron, Spinetoram, and Chlorantraniliprole exhibited moderate toxicity, Indoxacarb and Emamectin benzoate showed severe toxicity, and Flubendiamide showed low toxicity. Our findings offer insights for integrating chemical and biological control strategies to manage sucking insect pests comprehensively.

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Keywords | Ladybird beetle, Natural predator, Leaf dip bioassay, Insecticides



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Introduction

The ladybird beetle (*Coccinella septempunctata* L.) is a predatory specie that ingest a large amount of prey over its lifetime. As a result, it helps farmers to keep insect pests under control, which benefits their crops (Solangi *et al.*, 2007). *C. septempunctata* attains an important attention as biotic regulator. In Pakistan, *C. septempunctata* is an important regular competitor of various pest species that impair a variety of crops, since it controls a large number of soft-bodied insect pests, mainly aphids, at both the juvenile and adult stages (Solangi and Lanjar, 2007). The voraciousness of this predator's larvae increases with each subsequent instar, with the fourth-instar larvae being the most dangerous. It is commonly believed that the *C. septempunctata*, can be found all over the world in a variety of habitats apart from those that have been cultivated or naturally occurring (Stark *et al.*, 2007). Both *C. septempunctata* larvae and adults are incredibly omnivorous, nurturing on a wide range of insect pests common in greenhouse and field crops, such as Aphidoidea, Tetranychidae, and Psylloidea (Hodek and Michaud, 2013).

Due to its great colonization capacity, high fertility, and extended life duration, this beetle is used to work against all types of aphids in fields and greenhouse (Youn *et al.*, 2003; Landis *et al.*, 2004; Bianchi and Werf, 2004). The Coccinellidae family is well-known due to the extraordinary appearance of its members and the convex body shape they possess. They measure from 4 and 9 millimeters in length, and as little as 1 millimeter (Saleem *et al.*, 2019; Koren *et al.*, 2012). They inhabit all terrestrial habitat types, including forests, meadows, plains, and mountains (Zahoori *et al.*, 2003).

Insecticides are widely used to control the insects pests while they have harmful effects on the environment and the human health as well as they lead to insecticides resistance in insect pests. Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, and Flubendiamide are some of the most common insecticides used to control insect pests. This study was carried out to evaluate the toxicity of new chemistry insecticides against the *Coccinella septempunctata* under laboratory conditions

Materials and Methods

The experiment was conducted at the Insecticides

resistance laboratory, Entomological Research Institute (31°24'18.1"N 73°03'01.8"E), Ayub Agricultural Research Institute (AARI), Faisalabad, during the year 2021-2022. The experiment was set up with a complete randomization design (CRD), which had six treatments of each insecticide and five replications of each treatment, plus a control. Sipthoran 120SE, Chlorfenapyr 36%, Spinetoram 120SC, Lufenuron 50% Coragen 200 ml, and Steward 150EC, at the rates of 0.4ml, 0.8ml, 0.4ml, 0.86ml, 1.25ml, and 1 ml were taken with the aid of a micropipette and placed in a beaker, to which 500 ml of water was added. Distilled water served as control. The adults and larvae of ladybird beetles were collected from the wheat crop at Ayub Agricultural Research Institute Faisalabad, Punjab, Pakistan fields and brought into the laboratory, where they were maintained at a temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of $60 \pm 5\%$.

Leaf dip bioassay

The wheat leaves used in this bioassay were collected from the field, washed with distilled water, and air dried. To accommodate the size of Petri dishes, a leaf cutter was used to trim the leaves to the appropriate lengths. The 25 leaves for each dosage of insecticides ($5 \times 5 = 25$) were dipped in the solution. After drying up, the leaves were placed into petri dishes already having filter paper and field collected wheat aphids were placed on each leaf at the rate of 6 aphids/ leaf. The petri dishes were maintained at $27 \pm 1^\circ\text{C}$, $60 \pm 5\text{RH}$. The same procedure was adopted for the bioassay study of field collected lady bird beetle larvae for each (first, second, third larvae, separately) at the rate of 50 larvae/leaf. After 24, 48, 72, 96, and 120 hours, the data regarding mortality was recorded.

Statistical analysis

Analysis of variance (ANOVA) was done on the data, and then Fisher's least significant difference (LSD) test at 5% P-value was used to isolate the means. To calculate the percentage of mortality in *Coccinella septempunctata* at each stage of development (adult, first, second, and third instar larvae) mortality percentage formula was applied to each insecticide.

$$\text{Percent Mortality} = \frac{\text{Number of insect died}}{\text{Total number of insect present}} \times 100$$

Results and Discussion

Percentage mortality of adult ladybird beetle

The results, summarized in the table, demonstrated

that each insecticides (Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, Flubendiamide) was significantly different from one another concerning the mortality of adult ladybird beetle. The application of Flubendiamide was significantly less harmful. After 24 hours, the adult mortality rate was 20%, 16%, 14%, 10%, 8%, 4% respectively. After 48 hours, mortality rate was 36%, 30%, 28%, 20%, 14%, 8%. After 72 hours, mortality rate was 50%, 44%, 40%, 28%, 20%, 12%. After 96 hours, mortality rate was 64%, 56%, 54%, 34%, 22%, 18%. After 120 hours, mortality rate was 78%, 70%, 64%, 40%, 30%, 22%, was observed in Table 2 and Figure 1.

Table 1: *Insecticides with their recommended field dose/acre, trade name, common name and concentration in ppm.*

Insecticides	Trade Name	Recommended field Dose
Emamectin Benzoate	Proclaim 1.9EC	200ml/acre
Indoxacarb	Encore 150SC	175mi/acre
Lufenuron	Match 150EC	200ml/acre
Spinetoram	Tracer 120SC	80ml/acre
Chlorantraniliprole	Coragen 200SC	50ml/acre
Flubendiamide	Belt 480SC	50ml/acre

Table 2: *A comparison of means for the data regarding the bioassay of new chemistry insecticides against adult of seven spotted ladybird beetle under laboratory condition.*

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
Emamectin benzoate	2.00 ± 2.17 ^a	3.60 ± 2.07 ^a	5.00 ± 1.58 ^a	6.40 ± 1.14 ^a	7.80 ± 1.10 ^a
Indoxacarb	1.60 ± 1.14 ^{ab}	3.00 ± 1.22 ^{ab}	4.40 ± 1.14 ^a	5.60 ± 1.14 ^a	7.00 ± 1.0 ^a
Lufenuron	2.00 ± 1.00 ^a	2.80 ± 1.30 ^{abc}	4.00 ± 1 ^a	5.40 ± 1.14 ^a	6.40 ± 1.14 ^a
Spinetoram	1.00 ± 1.00 ^{ab}	2.00 ± 1.00 ^{bcd}	2.80 ± 1.30 ^{bc}	3.40 ± 1.14 ^b	4.00 ± 1.00 ^b
Chlorantraniliprole	0.80 ± 0.84 ^{bc}	1.40 ± 1.14 ^{cde}	2.00 ± 1.00 ^{cd}	1.80 ± 1.30 ^c	3.00 ± 1.22 ^{bc}
Flubendiamide	0.40 ± 0.55 ^{bc}	0.80 ± 0.84 ^{bc}	1.20 ± 1.10 ^d	1.80 ± 1.30 ^c	2.20 ± 1.30 ^c

Means sharing similar alphabets are not significantly different to each other

Percentage mortality of ladybird beetle of 1st instar larvae

Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, and Flubendiamide were all considerably different from one another in terms

of their ability to eradicate first-instar larvae of the ladybird beetle, as described in the table 3. The application of Flubendiamide was significantly less harmful. After 24 hours, the first instar mortality rate was 30%, 28%, 22%, 16%, 14%, 10%. After 48 hours, mortality rate was 38%, 32%, 24%, 18%, 18%, 16%. After 72 hours, mortality rate was 58%, 50%, 46%, 36%, 28%, 26%. After 96 hours, mortality rate was 76%, 66%, 58%, 46%, 34%, 28%. After 120 hours, mortality rate was 92%, 84%, 66%, 50%, 40%, 34%, mortality was observed in Table 3 and Figure 2.

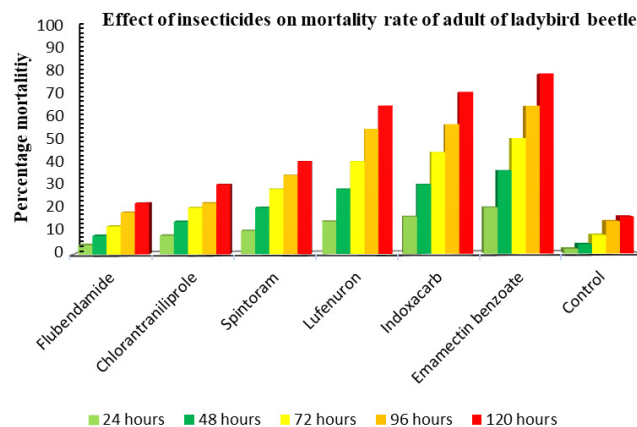


Figure 1: *Percentage mortality of adult ladybird beetle*

Table 3: *A comparison of means for the data regarding the efficacy of different treatments against 1st instar of larvae of seven spotted ladybird beetle.*

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
Emamectin benzoate	2.80 ± 1.25 ^a	4.40 ± 1.3 ^a	5.80 ± 1.55 ^a	7.60 ± 1.25 ^a	9.20 ± 1.14 ^a
Indoxacarb	2.60 ± 1.22 ^{ab}	3.80 ± 1.43 ^{ab}	5.00 ± 1.12 ^{ab}	6.60 ± 1.30 ^{ab}	8.40 ± 2.2 ^{ab}
Lufenuron	2.20 ± 1.30 ^{abc}	3.20 ± 1.12 ^{abc}	4.60 ± 1.21 ^{bc}	5.80 ± 1.30 ^{bc}	6.60 ± 1.25 ^{bc}
Spinetoram	1.60 ± 1.00 ^{abc}	2.40 ± 1.15 ^{bc}	3.60 ± 1.54 ^{bc}	4.60 ± 1.00 ^{cd}	5.00 ± 1.85 ^{de}
Chlorantraniliprole	1.40 ± 0.87 ^{abc}	1.80 ± 1.00 ^c	2.80 ± 1.00 ^c	3.40 ± 1.66 ^{de}	4.00 ± 1.96 ^{de}
Flubendiamide	1.00 ± 1.21 ^{bc}	1.80 ± 0.67 ^c	2.60 ± 1.00 ^c	2.80 ± 1.25 ^e	3.40 ± 1.45 ^{de}

Means sharing similar alphabets are not significantly different to each other

Percentage mortality of ladybird beetle of 2nd instar larvae

The results, summarized in the table, demonstrated that every insecticides (Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, Flubendiamide) was significantly different from one

Table 4: A comparisons of means for the data regarding on efficacy of different treatments against 2nd instar of larvae of seven spotted ladybird beetle

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
Emamectin benzoate	2.00 ± 1.17 ^a	3.80 ± 2.17 ^a	5.00 ± 2.25 ^a	6.00 ± 2.17 ^a	8.80 ± 2.17 ^a
Indoxacarb	2.00 ± 1.5 ^a	3.40 ± 1.56 ^{ab}	5.40 ± 1.52 ^a	6.20 ± 1.55 ^a	7.20 ± 2.2 ^{ab}
Lufenuron	1.200 ± 0.5 ^{ab}	3.00 ± 2.1 ^{ab}	4.00 ± 2.2 ^{ab}	5.40 ± 1.24 ^{ab}	6.40 ± 1.54 ^{bc}
Spinetoram	1.00 ± 0.4 ^{ab}	2.20 ± 1.24 ^{bc}	3.20 ± 1.54 ^{bc}	3.80 ± 1.24 ^{bc}	4.80 ± 1.00 ^{cd}
Chlorantraniliprole	0.600 ± 0.5 ^{ab}	1.60 ± 1.12 ^{cd}	2.20 ± 1.5 ^{cd}	3.00 ± 1.56 ^{cd}	3.60 ± 1.56 ^{de}
Flubendiamide	0.40 ± 1.00 ^b	1.40 ± 1.00 ^{cd}	1.40 ± 0.5 ^d	2.40 ± 1.56 ^{cd}	2.80 ± 1.56 ^e

Means sharing similar alphabets are not significantly different to each other

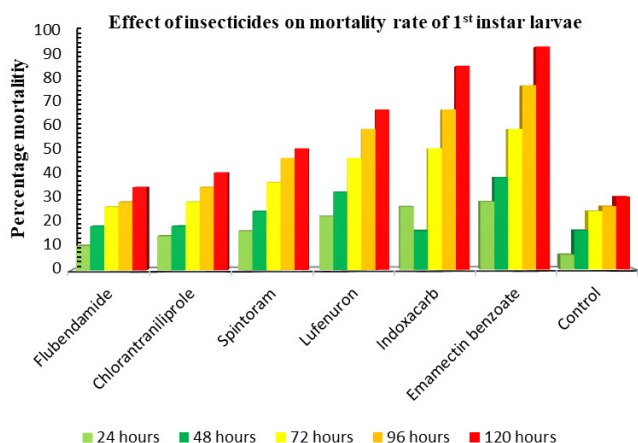


Figure 2: Percentage mortality of 1st instar larvae

another concerning the mortality of ladybird beetle second instar larvae. The application of Flubendiamide was significantly less harmful. After 24 hours, the second instar mortality rate was 20%, 12%, 12%, 10%, 6%, 4%. After 48 hours, mortality rate was 38%, 34%, 30%, 22%, 16%, 14%. After 72 hours, mortality rate was 54%, 50%, 40%, 32%, 22%, 14%. After 96 hours, mortality rate was 66%, 62%, 54%, 38%, 28%, 24%. After 120 hours, mortality rate was 88%, 72%, 64%, 48%, 36%, 28%, mortality was observed in Table 4 and Figure 3.

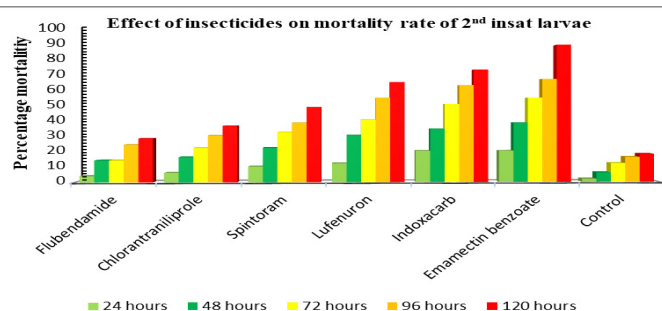


Figure 3: Percentage mortality rate of 2nd instar larvae

Percentage mortality of ladybird beetle of 3rd instar larvae

The results, summarized in the table, demonstrated that every insecticides (Emamectin benzoate, Indox-

carb, Lufenuron, Spinetoram, Chlorantraniliprole, Flubendiamide) was significantly different from one another concerning the mortality of ladybird beetle third instar larvae. The application of Flubendiamide was significantly less harmful. After 24 hours, the third instar mortality rate was 28%, 18%, 12%, 12%, 6%, 4%. After 48 hours, mortality rate was 44%, 38%, 32%, 22%, 16%, 10%. After 72 hours, mortality rate was 60%, 50%, 50%, 36%, 20%, 20%. After 96 hours, mortality rate was 72%, 68%, 62%, 50%, 30%, 28%. After 120 hours, mortality rate was 86%, 84%, 72%, 52%, 32%, 32%, mortality was observed in Table 5 and Figure 4.

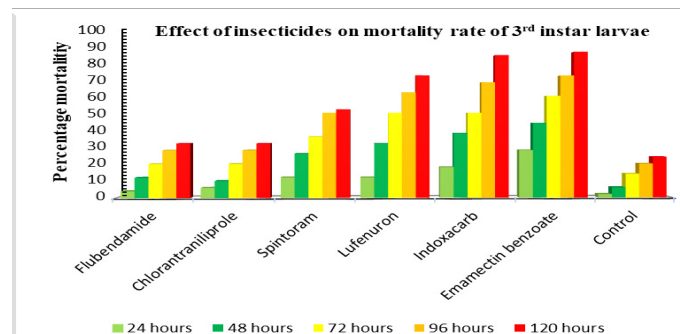


Figure 4: Percentage mortality rate of 3rd instar larvae

Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, and Flubendiamide were employed to determine the effectiveness against the seven-spotted ladybird beetle. Some insecticides proved to be less harmful than others, while others revealed to be more sensitive. Emamectin benzoate, Indoxacarb, Lufenuron, Spinetoram, Chlorantraniliprole, and Flubendiamide are the six insecticides tested for toxicity against the seven-spotted ladybird beetle.

In our results, on adult stage Emamectin benzoate showed the 20 % mortality, Indoxacarb showed 16, Lufenuron showed 14, Spinetoram showed 10,

Table 5: Comparisons of means for the data regarding the efficacy of different treatments against 3rd instar of larvae of seven spotted ladybird beetle

Treatments	24 hours	48 hours	72 hours	96 hours	120 hours
Emamectin benzoate	2.80 ± 1.25 ^a	4.40 ± 1.00 ^a	6.00 ± 2.25 ^a	7.20 ± 1.25 ^a	8.60 ± 2.25 ^a
Indoxacarb	1.80 ± 1.1 ^{ab}	3.80 ± 1.32 ^a	6.00 ± 1.55 ^a	6.80 ± 2.25 ^a	8.40 ± 2.65 ^a
Lufenuron	1.20 ± 1.1 ^{bc}	3.20 ± 2.1 ^{ab}	5.00 ± 1.1 ^{ab}	6.20 ± 2.25 ^a	7.20 ± 2.65 ^a
Spinetoram	1.20 ± 1.00 ^{bc}	2.60 ± 2.1 ^{abc}	3.60 ± 1.24 ^{bc}	5.00 ± 2.1 ^{ab}	5.20 ± 2.4 ^c
Chlorantraniliprole	0.60 ± 1.00 ^{bc}	1.00 ± 0.55 ^c	2.00 ± 1.0 ^{cd}	2.80 ± 1.32 ^{bc}	3.20 ± 2.1 ^c
Flubendiamide	0.40 ± 1.65 ^{bc}	1.20 ± 1.55 ^{bc}	2.00 ± 1.5 ^{ab}	2.80 ± 1.00 ^{bc}	3.20 ± 2.7 ^{ab}

Means sharing similar alphabets are not significantly different to each other

Chlorantraniliprole showed 8, and Flubendiamide showed 4 % mortality after 24 hours. After 48 hours Emamectin benzoate showed the 36 % mortality, Indoxacarb showed 30, Lufenuron showed 28, Spinetoram showed 20, Chlorantraniliprole showed 14, and Flubendiamide showed 8 % mortality. After 72 hours Emamectin benzoate showed the 50 % mortality, Indoxacarb showed 44, Lufenuron showed 40, Spinetoram showed 28, Chlorantraniliprole showed 20, and Flubendiamide showed 12 % mortality. After 96 hours Emamectin benzoate showed the 64 % mortality, Indoxacarb showed 56, Lufenuron showed 54, Spinetoram showed 34, Chlorantraniliprole showed 22, and Flubendiamide showed 18 % mortality and after 120 hours Emamectin benzoate showed the 78 % mortality. Indoxacarb showed 70, Lufenuron showed 64, Spinetoram showed 40, Chlorantraniliprole showed 30, and Flubendiamide showed 22 % mortality. [Fatima et al. \(2016\)](#) conducted the research on same species with some other insecticides, and in the results Emamectin was proved as an insecticide with the highest mortality rate insecticide among the selected insecticides to control adult as well as 1st instar larvae.

On 1st instar larvae Emamectin benzoate showed 28 % mortality, Indoxacarb showed 26, Lufenuron showed 22, Spinetoram showed 16, Chlorantraniliprole showed 14, and Flubendiamide showed 10 % mortality after 24 hours. After 48 hours Emamectin benzoate showed 38 % mortality, Indoxacarb showed 32, Lufenuron showed 24, Spinetoram showed 18, Chlorantraniliprole showed 18, and Flubendiamide showed 16 % mortality. After 72 hours Emamectin benzoate showed 58 % mortality, Indoxacarb showed 50, Lufenuron showed 46, Spinetoram showed 36, Chlorantraniliprole showed 28, and Flubendiamide showed 26 % mortality. After 96 hours Emamectin benzoate showed 76 % mortality, Indoxacarb showed

66, Lufenuron showed 58, Spinetoram showed 46, Chlorantraniliprole showed 34, and Flubendiamide showed 28 % mortality. In addition, 120 hours Emamectin benzoate showed 92, Indoxacarb showed 84, Lufenuron showed 66, Spinetoram showed 50, Chlorantraniliprole showed 40, and Flubendiamide showed 34 % mortality. As mentioned [Fatima et al. \(2016\)](#) conducted the trails on different stages and Emamectin showed higher mortality rate.

On 2nd instar larvae Emamectin benzoate showed 20 % mortality, Indoxacarb showed 12, On third instar larvae Emamectin benzoate showed 28 % mortality, Indoxacarb showed 18, Lufenuron showed 12, Spinetoram showed 12, Chlorantraniliprole showed 6 and Flubendiamide showed 4 % mortality. After 48 hours Emamectin benzoate showed 44 % mortality, Indoxacarb showed 38, Lufenuron showed 32, Spinetoram showed 16, Chlorantraniliprole showed 10, and Flubendiamide showed 9 % mortality. After 72 hours Emamectin benzoate showed 60 % mortality, Indoxacarb showed 50, Lufenuron showed 50, Spinetoram showed 36, Chlorantraniliprole showed 20, and Flubendiamide showed 20 % mortality. After 96 hours Emamectin benzoate showed 72 % mortality, Indoxacarb showed 68, Lufenuron showed 62, Spinetoram showed 50, Chlorantraniliprole showed 28, and Flubendiamide showed 28 % mortality. After 120 hours Emamectin benzoate showed 86 % mortality, Indoxacarb showed 84, Lufenuron showed 72, Spinetoram showed 52, Chlorantraniliprole showed 32, and Flubendiamide showed 32 % mortality. [Tank et al. \(2007\)](#) conducted the research on this species and selected some insecticides and botanicals to compare the efficacy, which proved that Emamectin showed greater controlling potential.

Conclusions and Recommendations

The current study showed that the Flubendiamide recommended as less toxic effect against *C. septempunctata* while Chlorantraniliprole, Lufenuron, and Spinetoram moderately toxic and Emamectin benzoate, Indoxacarb were recorded as highly toxic. Whereas *C. septempunctata* showed destructive response to the insecticidal treatments for both beetle mortality and reduce aphid feeding.

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Novelty Statement

Ladybird beetles, the unsung heroes of pest control, have demonstrated to be a resilient species. It appears they have a soft place for chemical concoctions despite their valiant efforts to combat pests. Among the six analysed chemicals, Indoxacarb and Emamectin benzoate were the beetle's kryptonite, resulting in severe toxicity. With its minimal levels of toxicity, however, Flubendiamide emerges as the white saviour. If you're in need of a dependable insecticide for your garden, Flubendiamide is here to save the moment due to its commendable insecticide efficacy and low impact on beneficial beetles.

Author's Contribution

Aasma Rasheed: Wrote original draft.

Dilbar Hussain and Muhammad Asrar: Supervised the study.

Usama Saleem: Wrote methodology and reviewed.

Saddam Hussain: Wrote abstract.

Zeeshan Javed and Abdul Ghaffar: Proofreading.

Mashal Shahzadi: Draw the graphs.

Muhammad Sohail Qadir: Provide technical input.

Muhammad Saleem: Statistical analysis.

Mawra Rafique: Formal analysis.

Ayesha Ikram: Methodology.

Saad Rasheed: Conceptualization.

Conflict of interest

All the authors declare that they have no any conflict of interest.

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