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ASSESSMENT OF RELATIVE TOXICITY OF COMMON INSECTICIDES AGAINST ADULT AND GRUBS OF COCCINELLA SEPTEMPUNCTATA UNDER LABORATORY SETTINGS

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A B S T R A C T

One of the significant aphid-eating predators that are natural is the ladybird beetle *Coccinella septempunctata*. *C. septempunctata* is a biological control agent and effective in integrated pest management strategies (IPM). In the development of IPM, chemical insecticides are routinely utilized, to boost crop protection and support the activity of biological control agents. Research on the relative toxicity of multiple insecticides on seven spotted ladybird beetle *C. septempunctata* L. instars was conducted in a lab environment to gauge the *C. septempunctata*'s reaction to powerful insecticides. Six insecticides were treated as Spinetoram 120SC (0.4%/0.5L), Steward 150EC (0.86/0.5L), Coragen 200ml (0.4%/0.5L), Siphtoram 120SE (0.8/0.5L), Chlorfenapyr 36% (1.25/0.5L), and Lufenuron 50% (1.0/0.5L) to the 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instars of *C. septempunctata*. After 4, 12, 24, 48, and 72 hours of the treatment, the results of the proportional death were determined. Ongoing investigation was done at 60±5% RH and 25±2°C. When administered on adult ladybird beetles, Lufenuron was detrimental in 99.4 % cases, Chlorfenapyr in 88.4% Steward in 85.5% of cases, Coragen in 83.2%, and Siphtoram in 77.6%, Spinetoram was very little hazardous substance based on the death rate, followed by, Coragen, Steward, Chlorfenapyr, and Lufenuron.

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INTRODUCTION

The seven-spotted ladybird beetle (*Coccinella septempunctata*) serves as one of the World's most significant aphid-eating natural predators. In terms of diversity and biological control, the order Coleoptera comes in first and its family 'Coccinellidae' is exclusively predatory. About 71 out of 6000 predatory species are native to Pakistan (Irshad, 2001). *Coccinella septempunctata* (L) is a common and widely recognized species of British beetle. It serves as a general predator of a number of problematic parasitic insects, the ladybird beetle provides a useful biological control vector. Aphids such as green peach aphid (*Myzus pericael*), wheat aphids (*Sitobion avenae*) and cotton aphids (*Aphis gossypii Glover*) are among the insects on which beetle species feed (Yu *et al.*, 2014). It has a wide geographical distribution and in China, the *C. septempunctata* (L) is an abundant and helpful generalized arthropod eater (Zhang *et al.*, 2011; Singh *et al.*, 2004; Lu *et al.*, 2012). The larval and mature stages are polyphagous as they consume Aphidoidea, Psylloidea, and Coccoidea mites, as well as mites from a variety of plants. *C. septempunctara* (L) has received a lot of interest as a biological control agent, due to its efficiency in managing a number of soft-bodied insect pests, like aphids. It heavily eats both the juvenile and mature stages of aphids. It probably feeds on wheat aphids and has a considerable impact on reducing the quantity of aphids in the planting area. The introduction of this biological predator into fields affects the aphid population growth (Youn *et al.*, 2003; Seo and Youn, 2002). The fourth instar larvae of this predator have a greater destructive than the earlier instars (Rizvi *et al.*, 1994). It may consume up to 40 aphids every day as a grown-up group and become more dangerous when it is fully grown and eat 53 aphids per day (Soni *et al.*, 2007). The effective application of this biological agent plays a key role in keeping the population of various pests below acceptable limits (Ghosh *et al.*, 2007).

C. septempunctata has tremendous capacity for reproduction and is also used as an indicator species to study the detrimental effects of predators and pesticides that help to control pests in nature (Wu *et al.*, 2021; Chi *et al.*, 2021). Singh (2015) (Singh, 2015)evaluated how new chemical reactions affected the biocontrol participants of agricultural insect pests. Harmful effects of various insecticides against ladybird beetle larval instars were observed and Spinetoram was found to be the most dangerous pesticide that has been utilized to control the population size. Superficial use of Spinetoram was highly dangerous (74.7% potentially hazardous) as the mortality rate fluctuated between 40-63% (Abbas *et al.*, 2014; Fernandez, 2015). Steward had a significant latent effect on larval instars and mature individuals, pertaining to their numbers by 55% and 85% correspondingly (Khan *et al.*, 2015). When evaluating the toxicological effects caused by different chemical treatments on *Coccinella septempunctata* (L) in a controlled laboratory setting, Solangi, Lanjar and Lohar (2007), noticed the probability of death spiked when insect killers were used at higher doses. The researchers additionally highlighted that all pesticides became potentially hazardous to attackers after a period of 96 hours of exposure as the chemical pattern changed during the course of 96 hours. Additionally, study demonstrated that all insect repellents lowered its predator's densities when used at different dilutions however, their effects culminated within seven days of intensive treatment rather than 96 hours(Solangi *et al.*, 2007). Chlorfenapyr is classified as somewhat dangerous, triggers mild to moderate insect damage and contributes to 25% to 50% death (Elzen, 2001). Lufenuron and Chlorfenapyr have been demonstrated as detrimental to ladybird beetles, however, Lufenuron proved more dangerous, causing 91.66% deaths (Tavares *et al.*, 2010). In light of the aforementioned considerations, the current study was carried out to assess insecticides that are less toxic to the ladybird beetle and effective in eliminating wheat pests so that their population can be preserved to manage aphids in wheat fields.

MATERIALS AND METHOD

A complete randomization design (CRD), with five replicas, and a single control group was conducted at the Ayub Agricultural Institute in Faisalabad. Mature ladybird beetles *C. septempunctata* were collected from field and kept at 25 ± 2^oC and 60-65% relative humidity. The 1st, 2nd, 3rd and 4th instars of *C. septempunctata* were also collected and kept at same environmental conditions (25 ± 2^oC and 60-65% relative humidity). All these grubs and instars were treated with six insecticides of given concentrations. The insecticides were, Spinetoram 120SC (0.4%/0.5L), Steward 150EC (0.86/0.5L), Coragen 200ml (0.4%/0.5L), Siphtoram 120SE (0.8/0.5L), Chlorfenapyr 36% (1.25/0.5L), and Lufenuron 50% (1.0/0.5L). Leaf dip bioassay was employed for treatments. For this purpose, the dried and cleaned sunflower leaves were used. Every leaf was immersed in a solution of each insecticide for 10 seconds, dried out, and placed in a petri dish that measured 100mm in diameter. Taken from the field, on each leaf, 6 adult *C. septempunctata* were applied. The instars of larvae were placed in petri dishes (50 larvae/petri dish). Mortality of adults and instars was observed after 4, 12, 24, 48 and 72 hours of treatments. The data about % mortality was recorded and subjected to Tuckey's post hoc multiple comparison tests for the comparison of means at $p<0.05$.

RESULTS

C. septempunctata were chosen to evaluate the adverse impacts of multiple insecticides and percentage mortality was observed after 4, 12, 24 and 48 hours of application on 1st, 2nd, 3rd, and 4th instar of *C. septempunctata* larvae. The effect of all insecticides was also observed on adult *C. septempunctata* after 72 hours of application. Nearly 94% mortality was observed in larval instars after 48 hours of application of insecticides thus observation could not be made after 72 hours.

Effects of Insecticides on Adult *C. septempunctata*

The analysis of variance revealed a significant difference ($p\leq0.05$) among treatments after 4 hours of application. Maximum % mortality was observed in adult ladybird beetles in T6 when Lufenuron was applied. It was observed that spinetoram was found to be least toxic for adult *C. septempunctata* after 4 hrs. After 12 hours of application, maximum mortality was observed in T5 and T6 ($p\leq0.05$) when chlorfenapyr and lufenuron applied

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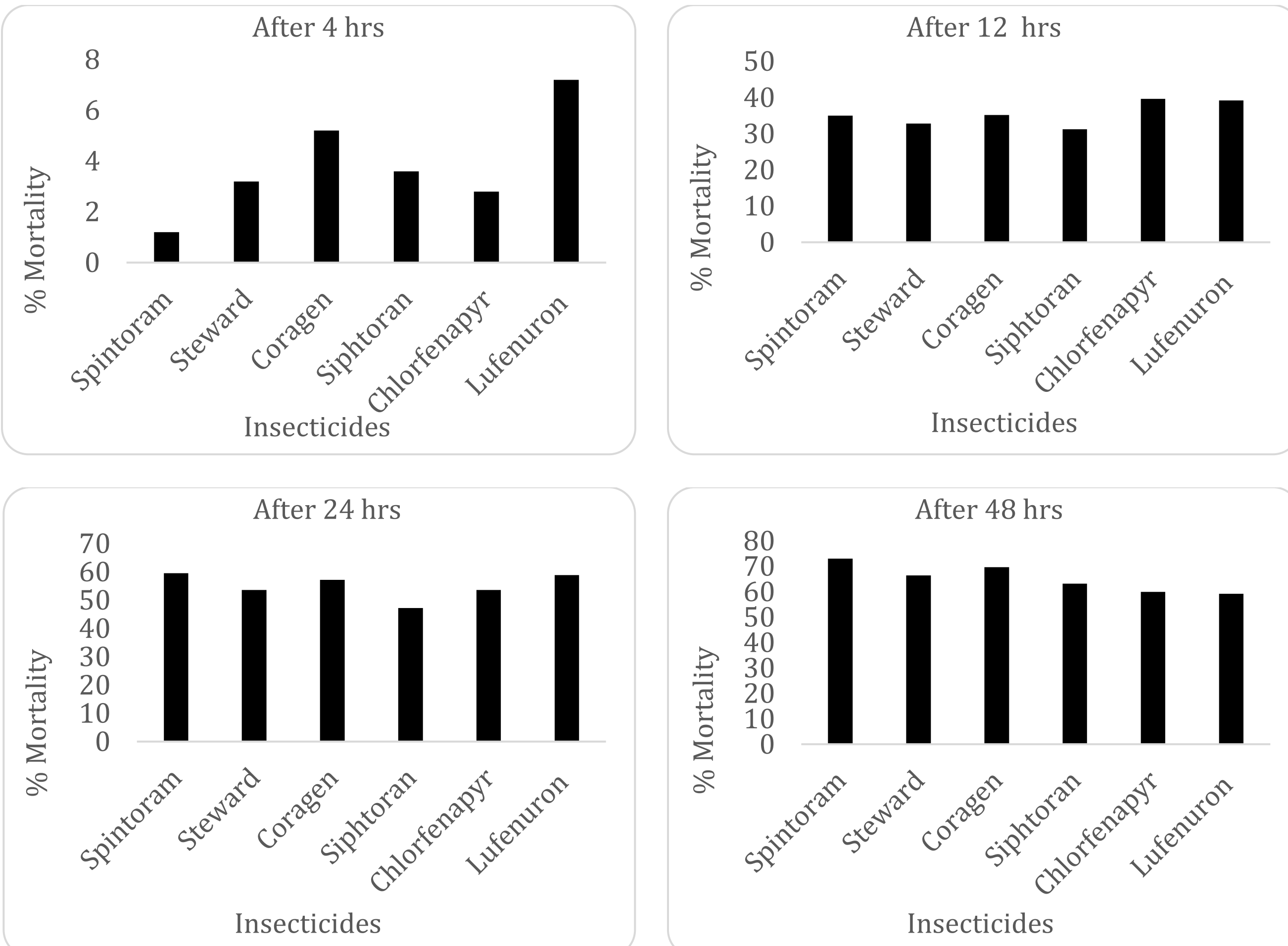
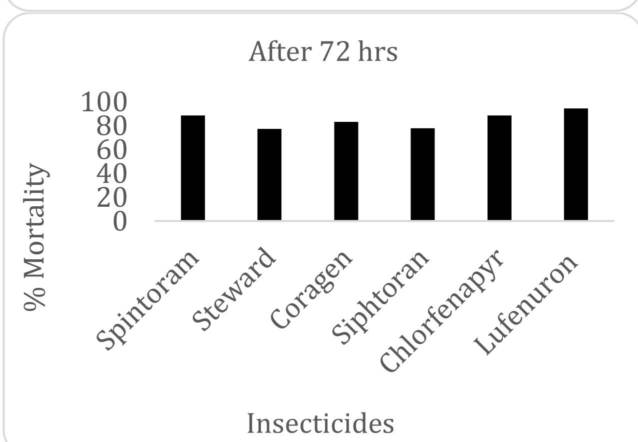
followed by coragen, spinetoram, steward and siphtoran hence steward was proved least toxic for the adult *C. septempunctata* after 12 hrs. Treatment T1, T3 and T6 had maximum mortality among treatments after 24 hours of the application when spinetoram, coragen and lufenuron were applied followed by steward, chlorfenapyr and siphtoran. The least toxic among all treatments was siphtoran on adult *C. septempunctata.* Spinetoram (T1) was the most toxic among all treatments after 48 hours of application followed by coragen, steward, spinetoram, chlorfenapyr and lufenuron. Lufenuron (T6) was the most toxic among all treatments followed by siphtoran, chlorfenapyr, coragen, spinetoram and steward after 72 hours of application (figure 1).

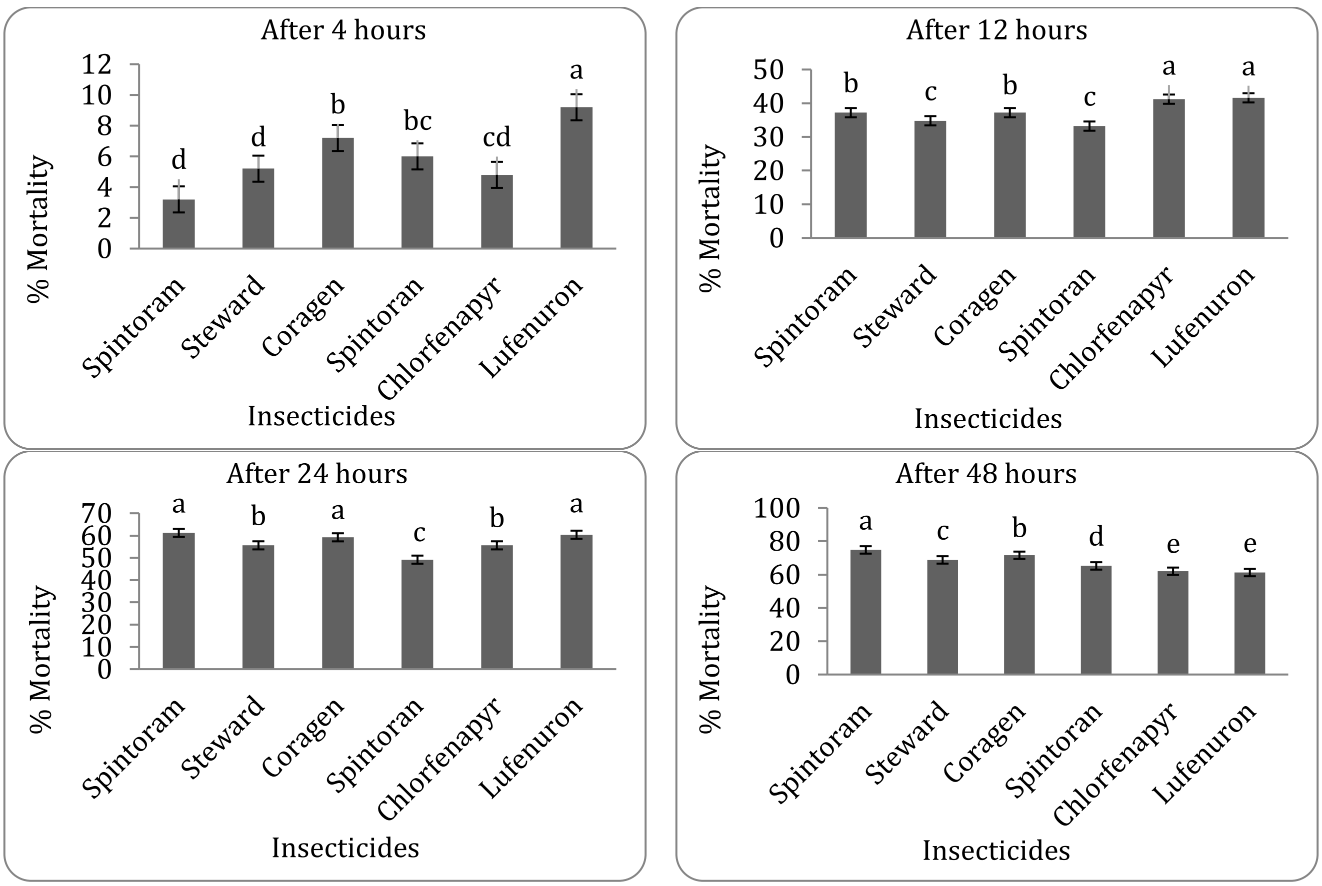




Figures 1. Detrimental impacts of different insecticides on adult *C. septempunctata* and % mortality after 4, 12, 24, and 48 hours of applications.

Effects of Insecticides on 1^{st} Instar of *C. septempunctata*

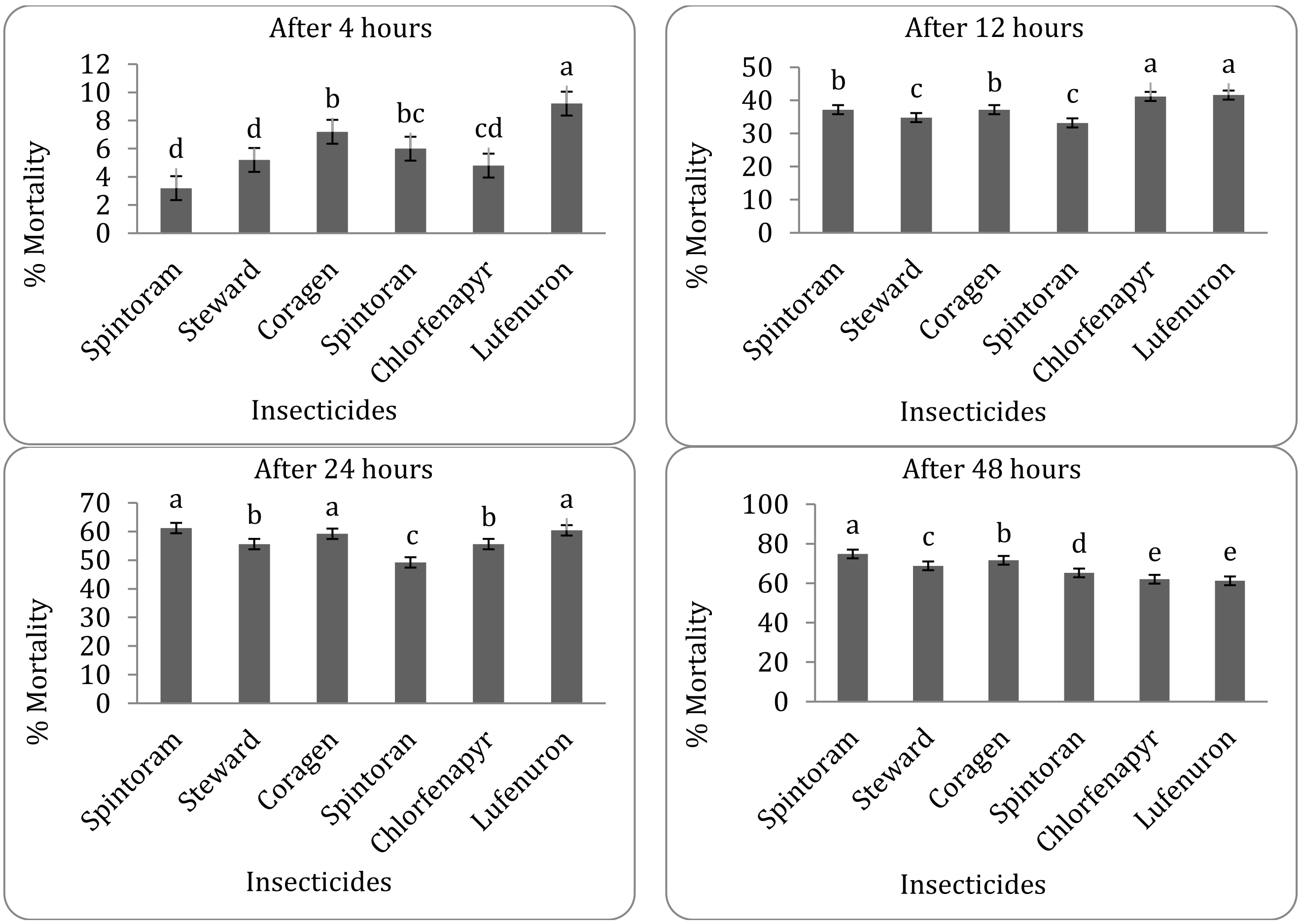
The analysis of variance revealed a significant difference ($p \leq 0.05$) among all treatments after 4 hours. The maximum mortality rate of the first instar of larvae of *C. septempunctata* was observed in T6 when lufenuron was applied followed by coragen, siphtoran, steward, chlorfenapyr and spinetoram. Results showed that spinetoram was proved least toxic for the 1^{st} instar of *C. septempunctata* larvae after 4 hours. The maximum detrimental effect was noticed ($p \leq 0.05$) by T5 and T6 treatment after 12 hours of the application when chlorfenapyr and lufenuron were applied followed by coragen, spinetoram, steward and siphtoran. The least toxic insecticide for 1^{st} instar after 12 hours of application was spinetoram. After 24 hours of application, the maximum mortality was observed ($p \leq 0.05$) by T1, T3 and T6 when spinetoram, coragen and lufenuron were applied followed by steward, chlorfenapyr and siphtoran. The least toxic insecticide for 1^{st} instar of *C. septempunctata* after 24 hours of application was siphtoran. The toxic effect of insecticides after 48 hours of application was observed and maximum mortality was induced by spinetoram (T1) ($p \leq 0.05$) followed by coragen, steward, siphtoran, chlorfenapyr and lufenuron. The least toxic insecticide proved against the 1st instar of *C. septempunctata* was lufenuron (Figure 2).



Figures 2. Detrimental impacts of different insecticides on1st instar of *C. septempunctata* and % mortality after 4, 12, 24, and 48 hours of applications.

Effects of Insecticides on 2^{nd} Instar of *C. septempunctata*

Treatments of insecticides against 2^{nd} instar of *C. septempunctata* revealed significant differences ($p \leq 0.05$) at 4, 12, 28 and 48 hours of application. The maximum % mortality of 2^{nd} instar larvae of *C. septempunctata* was observed in T6 when lufenuron was applied followed by coragen, siphtoran, steward, chlorfenapyr and spinetoram. It was observed that spinetoram was proved least toxic for the 2^{nd} instar larvae of *C. septempunctata* after 4 hours of application. The analysis of variance showed a significant difference ($p \leq 0.05$) among treatments after 12 hours of applications against 2nd instar of larvae. The maximum mortality of 2nd instar larvae was observed in T5 and T6 when chlorfenapyr and lufenuron were applied followed by spinetoram, coragen, steward, and siphtoran. It was noticed that spinetoram was proved least toxic for the 2nd instar of larvae after 12 hours of application. After 24 hours of application, T1 and T6 (spinetoram and lufenuron) were observed as the maximum toxic with the highest rate of mortality ($p \leq 0.05$) followed by coragen, steward, chlorfenapyr and spinetoram. The least toxic insecticide against 2nd instar was siphtoran. Treatment T1 (spinetoram) had a more lethal effect after 48 hours of application followed by coragen, steward, siphtoran, chlorfenapyr and lufenuron. Lufenuron was least toxic for 2nd instar of *C. septempunctata* (Figure 3).



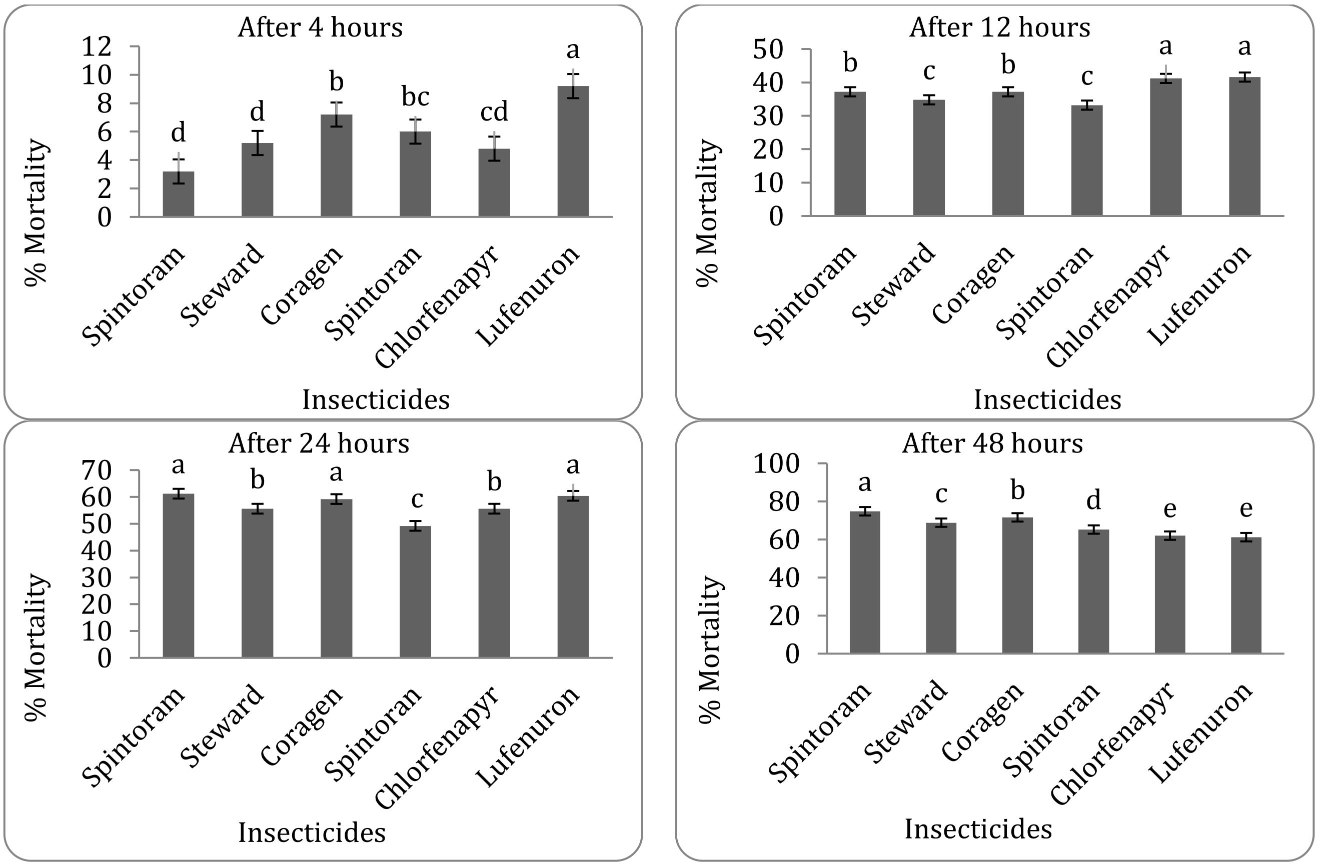
Figures 3. Detrimental impacts of different insecticides on2nd instar of *C. septempunctata* and % mortality after 4, 12, 24, and 48 hours of applications.

Effects of Insecticides on 3rd Instar of *C. septempunctata*

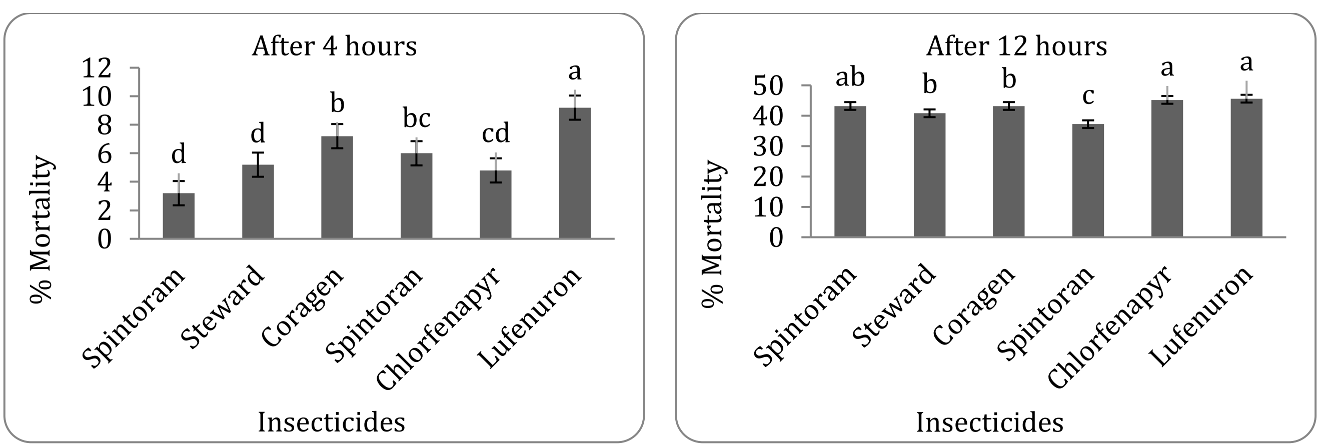
The analysis of variance depicted a significant difference ($p \leq 0.05$) among treatments after 4 hours of application, maximum mortality was observed in lufenuron (T6) followed by coragen, siphtoran, steward, chlorfenapyr and spinetoram. After 12 hours of application, maximum mortality was observed by chlorfenapyr and lufenuron (T5 and T6) followed by coragen, spinetoram, steward and siphtoran thus proven siphtoran as least toxic for 3rd instar of larvae of *C. septempunctata*. After 24 hours of application, treatment T1 (spinetoram) was found to be more lethal followed by lufenuron, coragen steward, chlorfenapyr and siphtoran. Again, siphtoran was proven as the least toxic for 3rd instar of larvae after 24 hours. Spinetoram had the maximum lethal effect (96% mortality rate) after 48 hours of application followed by coragen, steward, siphtoran, chlorfenapyr and lufenuron (Figure 4).

Effects of Insecticides on 4th Instar of *C. septempunctata*

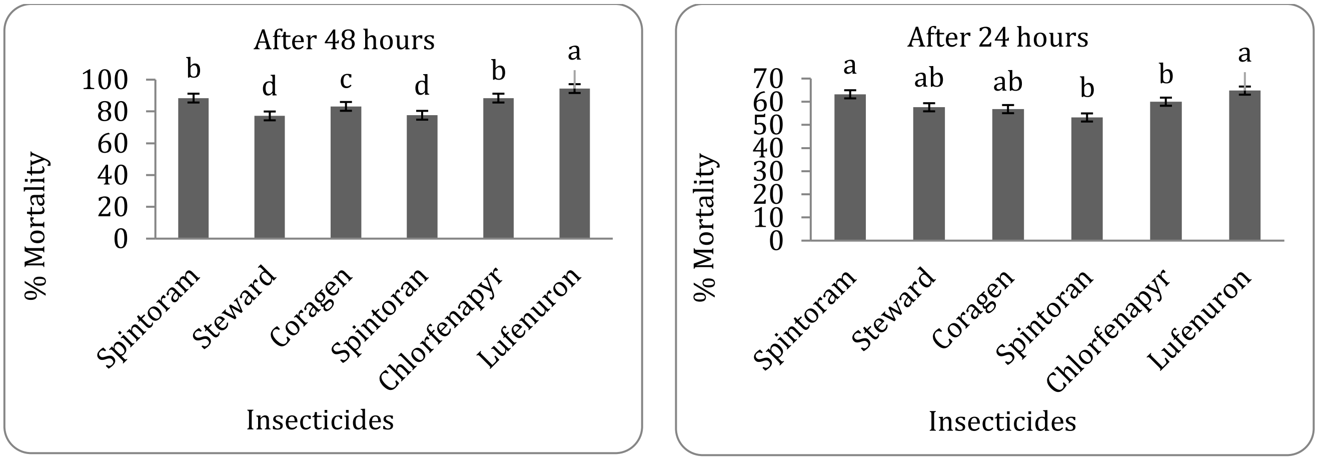
The analysis of variance revealed significant differences (p≤0.05) among treatments after 4 hours of application and maximum mortality (13%) was observed by lufenuron (T6) followed by coragen, siphtoran, steward, chlorfenapyr and spinetoram. After 12 hours of application, Treatment T6 (lufenuron) was found to be more lethal (45.6%) followed by coragen, spinetoram, steward and siphtoran. Lufenuron (T6) and spinetoram (T1) were more toxic at the 4th instar of larvae followed by chlorfenapyr, steward, coragen, and siphtoran after 24 hours of application. Lufenuron (T6) was found to be the most lethal (94.4%) after 48 hours of application followed by chlorfenapyr, spinetoram, coragen, siphtoran and steward. The steward was found to be least toxic against 4th instar of *C. septempunctata* (figure 5).



Figures 4. Detrimental impacts of different insecticides on3rd instar of *C. septempunctata* and % mortality after 4, 12, 24, and 48 hours of applications.



Figures 5 (a). Detrimental impacts of different insecticides on 4th instar of *C. septempunctata* and % mortality after 4 and 12 hours of applications.



Figures 5 (b). Detrimental impacts of different insecticides on 4th instar of *C. septempunctata* and % mortality after 24 and 48 hours of applications.

DISCUSSION

In an integrated pest management program, coccinellids are widely used as biocontrol agents against a variety of insect pests. In the family of coccinellids, there are both generalists and specialists of various prey types. Aphidophagous generalist predators can feed on a variety of aphid species with essentially no preference. As a biocontrol agent for mealybugs, thrips, and whiteflies, the cultivation of specific specialized ladybirds is also encouraged. The best way to mass rear these coccinellids is to optimize the abiotic parameters like temperature and light as well as the food in the form of prey and mating tactics in terms of the ideal age and mating partners (Pervez *et al.*, 2020). We examined the mortality of *Coccinella septempunctata* with certain common insecticides including spinetoram, steward, chlorfenapyr, lufenuron, siphtoran, and coragen and found that, spinetoram was the safest and least dangerous insecticide against adult *Coccinella septempunctata*. Based on multiple evaluations, spinetoram appeared to be safe for *C. septempunctata* adults. Compared to the other chemicals employed in this experiment, chlorfenapyr was more harmful to all stages of the coccinellids. These findings are in accordance with Ozawa and Uchiyama (2016) who confirmed that spinetoram is moderately to seriously harmful to adult lady bird beetle (Ozawa and Uchiyama, 2016). The findings are also supported by another study (Sabry *et al.*, 2014) who determined that Spinetoram can be used to reduce ladybird populations because it is most poisonous to them during their larval stages. The most sensitive endpoints for survival and development of ladybird beetle are 2nd-instar larval stage (Yu *et al.*, 2014). Mughal et al. (2017) (Mughal *et al.*, 2017) found that chlorpyrifos was more toxic among all other seven insecticide and imidachloprid was the safest on adult lady bird beetle after 72 hours (Chlorpyrifos > Indoxacarb > Spinosad > Acetamaprid > Ememectin Benzoate > Leufenoron > Imidachloprid). Xiao et al (2016) draw attention to the significance of evaluating the negative impacts of low imidacloprid concentrations on predator species, even at the transgenerational level (Xiao *et al.*, 2016). All of the insecticides were found to be unsafe, and they all had negative impacts on the coccinellid beetle *C. septempunctata's* life characteristics (Afza *et al.*, 2021). Spirotetramat is toxic to several growth stages but it has a very short residual activity (Lefebvre *et al.*, 2012). Bostanian *et al*. (2001) found that chlorpyrifos was very harmful to adult beetles as compared to other pesticides(Bostanian *et al.*, 2001). Solangi *et al*. (2007) utilized a variety of insecticides and observed that all the insecticides caused higher mortality of the 4th instar grubs when applied at high concentration but, denitol was shown to be much more harmful with 72% and 90% mortality at intervals of 96 hours and one week, respectively. Tracer, on the other hand, had a lower toxin level, with mortality rates of 30% and 38%, respectively, after 96 hours and one week (Solangi *et al.*, 2007).The verdicts of the study were also supported by Tavares et al.(2010) who determined that lufenuron induced 91.66 % mortality in adult and 95.86% in larval stages after 48-72 hours of treatment (Tavares *et al.*, 2010).

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COMPETING INTERESTS

No competing interest of the study exist.

CONFLICT OF INTEREST

Authors claim no conflict of interest exists.

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