EFFECT OF NEW CHEMISTRY INSECTICIDES DIRECTLY AND THROUGH POISONED MUSTARD APHIDS ON *COCCINELLA SEPTEMPUNCTATA*

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ABSTRACT-Aphids are highly damaging insect pest of mustard crop that causes direct and indirect losses in many growing areas of Pakistan. In addition to insecticides, biocontrol agents, especially *Coccinella septempunctata*, play a significant role in managing aphid population. The consistent use of insecticides may directly and indirectly (through poisoned aphid) have significant effect on the *Coccinella septempunctata.* Keeping in view the importance the effect of new chemistry insecticides directly and through poisoned mustard aphid on development of C*occinella septempunctata* were assessed in lab conditions. Under laboratory conditions, this experiment was conducted by using CRD layout with four replications of each treatment. Four different concentrations of insecticides (Flonicamid and Spirotetramat) were applied by direct application of pesticides on adults of *C. septempunctata* and indirectly through by feeding poisoned aphid to *C. septempunctata*. The mortality and consumption rate of poisoned aphids were counted after 24, 48 and 74 h of study period. Results showed that in case of direct application, the maximum mortality were recorded 58.75±0.25 and 51.50±0.28% after 72 h of treatment of Spirotetramat and Flonicamid @ 1500 and 150 ppm, respectively. In case of indirect application (through poisoned aphids), the maximum mean mortality percentage was noticed 36.00±1.2% with lowest consumption 70.66±2.62% after 72 h of study period by the treatment of Flonicamid @ 150 ppm conc. Similarly, the maximum mean mortality percentage 43.33±0.72% was noticed with lowest consumption rate of 49.33±2.65% after 72 h of study period by the application of Spitotetramat @ 1500 ppm. The Spirotetramat (50 EC) is considered more toxic than Flonicamid (50 wg). Furthermore, results showed that as consumption rate increases mortality decreases and consumption decreases mortality increases. In some cases, the feeding behavior remains asymptomatic. This study provides insight into the ecological consequences of using new chemistry insecticides and their implications for IPM strategies in agriculture.

Key words: aphids, *Coccinella septempunctata*, Flonicamid, insecticides, natural enemies, Spitotetramat

I. INTRODUCTION

The mustard Aphids (Hemiptera: Aphidoidae) are sap-sucking insect pests and cause severe damage to agro-crops in various areas of the world [1]. They are also considered as key pests of mustard plant and mainly present in Pakistan, China, USA, India and Bangladesh [2, 3]. The nymph of mustard aphid as well as its adult heavily damages the plants by sucking the cell sap from leaf, badly affecting the inflorescence and consequently incomplete growth of pods which ultimately decline mustard yield [2]. Moreover, they also produced considerable amounts of honeydew which causes the growth of fungus and affects the photosynthetic activity of plants due to blackish appearance of pods and leaves [4]. It affects the quality of seed products and causes approximately 70-90 percent yield losses [5, 6]. Due to short life span, aphids have approximately forty generations annually mainly in optimal weather conditions, this means that billions of aphids produced annually by a female, if they are not properly managed [7]. Usually, to control aphids' various pesticides are used which not only affect the environment but, also non-targeted species [8]. The excessive use of pesticides to control aphids, the resistance development against insecticides was reported in many parts of the world [9].

Biological control agents belonging to the family Coccinellidae and Chrysopidae are the most active and cosmopolitan predators which feed on a wide range of insect pests including aphids, mealybugs, whiteflies, leafhoppers, and several other soft-bodied insects [10]. They were also found to feed on the eggs and newly hatched larvae of lepidopterans [11]. The ladybird beetle is considered the most important and successful predator of several pests attacking cotton, sunflower, citrus and vegetables crops [12, 13]. The seven spotted ladybird beetle is considered as the best natural enemies among the others as a valuable predatory insect of aphids. Both larvae and adults of seven spotted ladybird beetles not only feed on aphids, but they also feed on various soft body insects like scales, spider mites, borers, spider mites, mealy bug and eggs of various other pests [14]. The use of natural enemies to control pests provides an advantage to reduce the risks associated with insecticides. The ladybird beetle showed a greater performance to control pests by predation than other natural enemies as a biological control agent [15]. Moreover, it is reported that the ladybird beetle is greatly definite to aphid diet [16].

The biological agents and natural predators or parasites of aphids generally present in agro-ecosystem and usually subjected to insecticides directly during the foliar application [17], and indirectly through the interaction with insecticide residue on the

leaves of plant or consuming poisoned prey [18, 19]. Similarly, natural enemies faced hazardous effects of insecticides, as feeding on nectars of flowers contaminated due to insecticide application and effected hosts [20]. In addition to direct mortality, insecticides may have sublethal effects on physiology, behavior, and biological parameters, including development time, longevity, fecundity, and fertility of beneficial arthropods [20-23]. For example, insect growth regulator hexaflumuron had significant effects on development duration, survival, pupation, and hatching of *C. septempunctata* [24]. Sublethal concentrations of imidacloprid reduced the fecundity of *C. septempunctata*, which could result in markedly lowering their populations [24, 25].

However, with the increasing demand for insecticides and their effects on the environment, it is necessary to find effective strategies to control the mustard aphids to minimize the effect of insecticides on non-targeted organisms and their environment. So, keeping in view study was conducted to find out the "effect of new chemistry insecticides directly and through poisoned mustard aphids on development of *Coccinella septempunctata*". This study provides insight into the ecological consequences of using new chemistry insecticides and their implications for IPM strategies in agriculture.

II. MATERIALS AND METHODS

The experiments were conducted under controlled conditions (at 25±2°C temperature and the 60- 70% RH) in Biological Control Lab of Entomology, University of Agriculture, Faisalabad.

Insect material:

The adult ladybird beetle (*Coccinella septempunctata*) was collected from a mustard field from Entomological research area at Young-Wala and were reared in Biological Control laboratory, Department of Entomology, University of Agriculture, Faisalabad. The predator was reared on aphids in small plastic transparent boxes. Honey solutions were also provided in cages with the help of cotton wicks and newly laid eggs were collected and placed in plastic boxes (90 mm) after hatching, the larvae were provided with aphids inside the boxes. The culture was maintained until there were homogenous populations obtained for the experiment [14].

Aphid:

The aphid population was also collected from different fields of Entomological research area, at Young-Wala and were maintained in biological control laboratory, Department of Entomology, University of Agriculture, Faisalabad. The fresh leaves were provided to aphids in plastic boxes (8.5×6.5×3.5 inches) and leaves were replacing after every 24 hours. The culture was maintained until there was enough population for the experiment.

Bioassay

The bioassay was performed using pesticides directly on adults and larvae of *C. septempunctata* and indirectly through by feeding poisoned aphid to *C. septempunctata* [26]. The effects of new chemistry insecticides were tested using contact toxicity (direct exposure of lady bird beetles) and stomach poisoning (by providing poisoned aphids). Under CRD design, there were four treatments with replicated four times. The two new chemistry insecticides Flonicamid at the concentrations (0, 18.5, 37.5, 75 and 150 ppm) and Spirotetramat at the concentrations (0, 187, 375, 750 and 1500 ppm) were applied directly and indirectly through poison aphids under laboratory conditions. At first the direct effect of insecticides was tested by a direct application method. In second, case the aphids pretreated with different concentrations of both insecticides and were feed to *C. septempunctata*. The mortality and survival rate were recorded after 24, 48 and 72 hours of feeding. Percentage pray consumption was counted by counting left over numbers of aphids divided into the given numbers of aphids multiply by hundred. Survival of *C. septempuntata* was considered successful when larvae developed to adults.

Statistical analysis

The data was subjected to complete random design for ANOVA table. The all-pair wise meaning was calculated using Tuckey HSD test. The significant differences were recorded. The percentage mortality was calculated using mortality formula and lethal concentration (LC50) were calculated using Probit analysis.

III. RESULTS:

Direct exposure of *Coccinella septempunctata* to Flonicamid (50 wg)

The results showed that the direct application of Flonicamid different concentrations had significant effect on the survival of seven spotted ladybird beetles (*Coccinella septempunctata*) after 24 h of treatments ($F_{=1104.07}$, df=4,19, $p<0.05$, LC_{50}=14.925). The highest percentage mortality was recorded 26.00±0.40% after direct treatment of Flonicamid at the rate of 150 ppm, which was not significantly different for treatments T3 and T2, respectively. The lowest mortality percentage was recorded 8.75±0.25% after treatment of Flonicamid @ 18.5 ppm (Fig. 1).

The results showed that the direct application of Flonicamid (LC_{50}=18.567) at various concentrations significantly affect the seven spotted ladybird beetle (*C. septempunctata*) after 48 h of treatments ($F=_{2679.63}$, df=4,19, $p<0.05$). The maximum percentage mortality of seven spotted ladybird beetles were recorded (36.50±0.28%) after treatment of Flonicamid @ 150 ppm, followed by 33.75±0.47, 21.50±0.25 and 18.00±0.28% after treatment of Flonicamid at the rate of 75, 37.5 and 18.5 ppm concentrations, respectively (Fig. 1).

The direct application of Flonicamid (LC_{50}=24.209) at different concentrations significantly affect the seven spotted ladybird beetle (*C. septempunctata*) as compared to control after 72h of treatments ($F_{=4580.66}$, df=4,19, $p<0.05$). The maximum percentage mortality was recorded 51.50±0.28% after the treatment of Flonicamid @ 150 ppm, which was not significantly different from treatment T2 (Flonicamid @ 75 ppm). There was 0.25±0.25% percent mortality was recorded in control group; this mortality may be due to change in lab condition or contaminations (Fig. 1).

Direct exposure of *Coccinella septempunctata* to Spirotetramat (50 EC)

The results showed that the direct application of Spirotetramat different concentrations had significant effect on the survival of seven spotted ladybird beetles (*C. septempunctata*) as compared to control after 24 h of treatments ($F_{=819.69}$, df=4,19, p<0.05, LC_{50}=14.177). The highest percentage mortality was recorded 28.00±0.4% after treatment of Spirotetramat at the rate of1500 ppm conc. which was not significantly different from other treatments. The percentage mortality was recorded 25.50±0.25, 18.75±0.40 and 10.00±0.40% after treatment of Spirotetramat @ 750, 375 and 187 ppm concentrations, respectively. There was no percentage of mortality recorded in control (Fig. 2).

The direct application of Spirotetramat various concentrations had significant effect on survival of *C. septempunctata* after 48 h of treatments ($F_{=1549.46}$, df=4,19, p< 0.05, LC_{50}=15.056) (Fig 2). The maximum mean percentage mortality of were recorded (41.0±0.40%) after treatment of Spirotetramat at the rate of 1500 ppm, which was significantly different from other treatments. The lowest mean percentage mortality was recoded 18.25±0.40% after treatment of Spirotetramat @ 187 ppm. No mortality was recorded in control.

The results exhibited that the direct application of Spirotetramat different concentrations had significant effect on survival of *C. septempunctata* after 72 h of treatments ($F_{=8497.20}$, df=4,19, p<0.05, LC_{50}=27.977) (Fig. 2). The highest mean percentage mortality was recorded as 58.75±0.25% and minimum mortality was noticed as 33.00±0.40% after treatment of Spirotetramat at the rate of 1500 and 187 ppm, respectively.

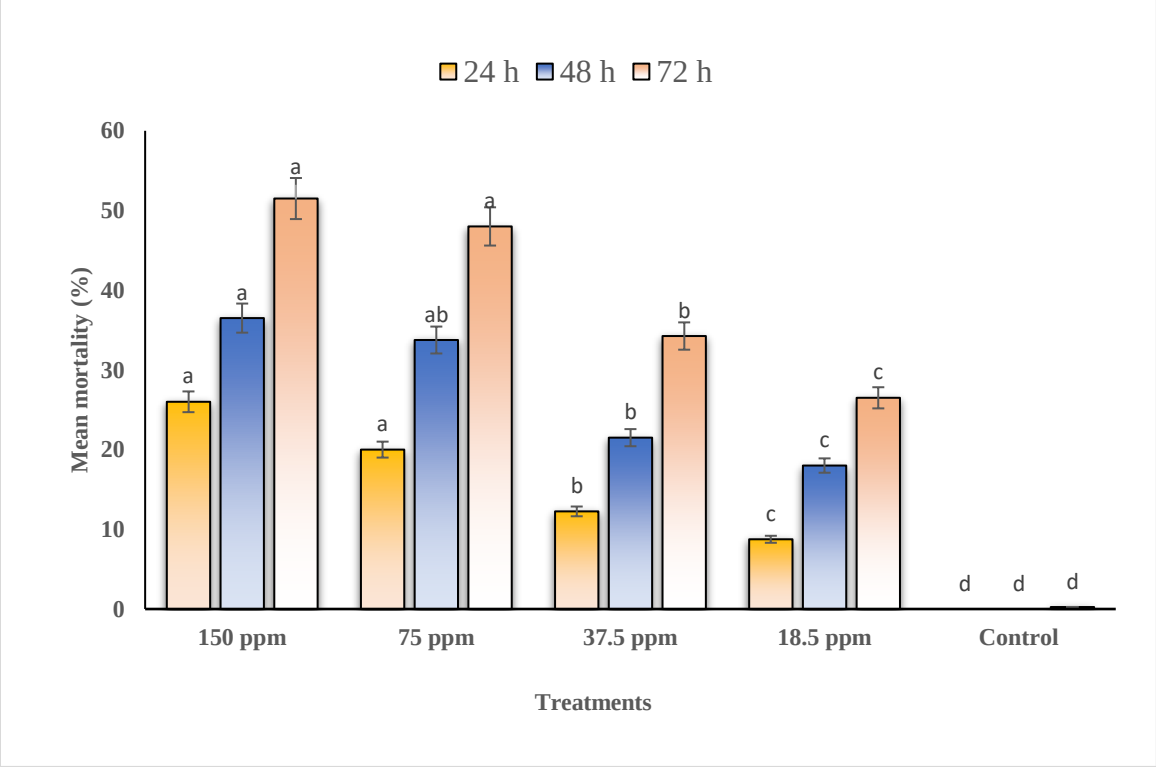

Figure 1: Effect of direct application of different concentrations of Flonicamid (50 wg) on *Coccinella septempunctata* after different intervals of time during the experimental period. Means with standard error and homogeneous group are present, same lowercase letters are not statistically different from each other at Tukey HSD test (p<0.05).

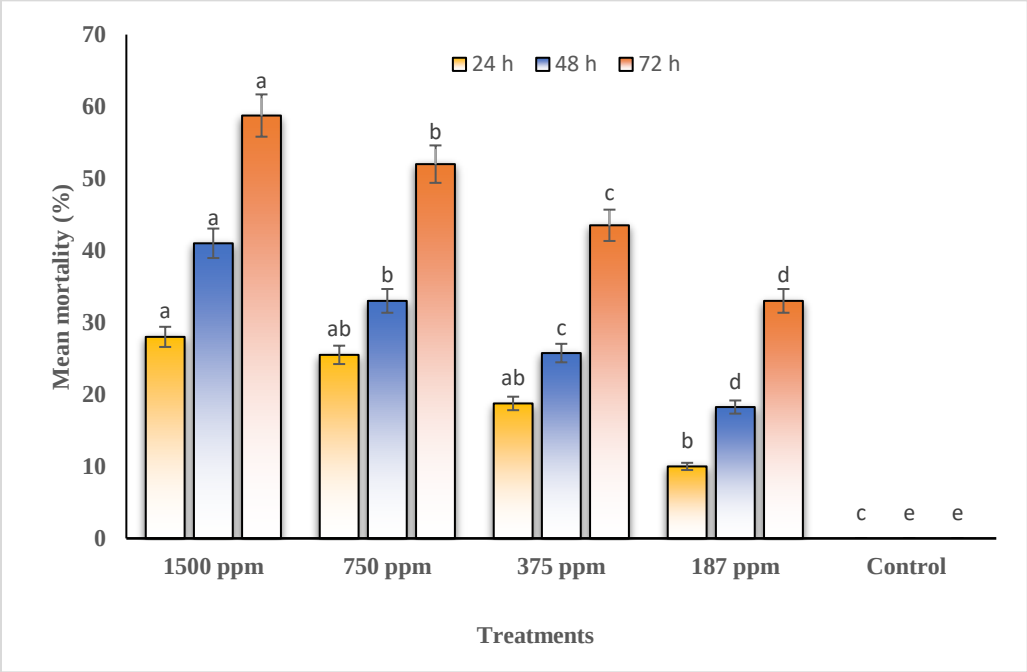

Figure 2: Effect of direct application of different concentrations of Spirotetramat (50 EC) on *Coccinella septempunctata* after different intervals of time during the experimental period. Means with standard error and homogeneous group are present, same lowercase letters are not statistically different from each other at Tukey HSD test (p<0.05).

Indirect effect of Flonicamid on *Coccinella septempunctata* through poison aphids

The results of the experiment indicated the indirect effect of Flonicamid (through poisoned aphids) on *Coccinella septempunctata* after various intervals of feeding period. The consumption rate was not significantly different from each other (Table 1). The consumption rate was not significant different from each other at Tukey HSD test. The mean maximum consumption of poisoned aphids was recorded 73±0.94% with lowest mean percentage mortality 4.00±0.81% after treatment of Flonicamid @ 18.5 ppm (df= 4,19, p=0.2837) (Table 1). Similarly, the lowest mean consumption was recorded 63±1.25% with high mortality rate of 12.00±0.25% after treatment at the rate of 150 ppm of Flonicamid concentration. After 48 h of experimental period the maximum mean consumption was noticed as 75±1.30% with minimum mean mortality rate of 6.00±0.27% after treatment of Flonicamid @ 18.5 ppm (df= 4,19, p=0.7941). The minimum consumption was recorded 59±2.62% with maximum mean mortality 21.67±0.27% (Table 1). Similarly, the highest mean consumption of position aphids was noticed as 85.66±1.30% with lowest mean percentage mortality 12.67±0.91% and lowest consumption was recorded 70.66±2.62% with maximum mean mortality percentage 36.00±1.2 after 72 hours of study period (df= 4,19, p=0.5437, Table 1). The mortality rate of *Coccinella septempunctata* had an indirect relationship with consumption rate, as consumption rate increased mortality decreased vice versa.

Indirect effect of Spirotetramat on *Coccinella septempunctata* through poison aphids

The results of the experiment indicated the indirect effect of Spirotetramat (through poisoned aphids) on *Coccinella septempunctata* after different intervals of feeding period. The consumption rate was not significantly different from each other's Tukey HSD test (Table 2). The mean maximum consumption of poisoned aphids after 24 h of feeding period was recorded 85.25±0.94% with lowest mean percentage mortality 6.67±0.27% after treatment of Spirotetramat @ 187 ppm (df= 4,19, p=0.5437) (Table 1). Similarly, the lowest mean consumption was recorded 59.75±0.85% with high mortality rate of 14.67±0.27% after treatment of Spirotetramat at the rate of 1500 ppm concentration.

After 48 h of experimental period the highest mean consumption was noticed as 77.75±1.89% with minimum mean mortality rate of 7.66±0.27% after treatment of Spirotetramat @ 187 ppm (df= 4,19, p=0.5732). The minimum consumption was recorded 69.25±2.5% with maximum mean mortality 25.67±0.72%, as compared to control (Table 2). Similarly, the highest mean consumption of position aphids was noticed as 87.66±0.85% with lowest mean percentage mortality 11.33±0.27% and lowest consumption was recorded 49.33±2.65% with maximum mean mortality percentage 43.33±0.72% after 72 hours of study period (df= 4,19, p=0.6567, Table 2). The *C. septempunctat* significantly consumed poison aphids mainly treated with lower concentrations of both insecticides. The mortality rate of *Coccinella septempunctata* had an indirect relationship with consumption rate, as consumption rate increases mortality decreases and consumption decreases mortality increases. In some cases, the feeding behavior of seven spotted ladybird beetles remains asymptomatic.

Table 1: Mean consumption and mean mortality percentage of seven *Coccinella septempunctata* treated with different concentrations of Flonicamid after 24, 48 and 72 hours of feeding interval.

Treatments (ppm)	Daily consumption (mean ± S.E)					
	24 h		48 h		72 h	
	Consumption (%)	Mortality (%)	Consumption (%)	Mortality (%)	Consumption (%)	Mortality (%)
150	63±1.25b	12.00±0.25a	59±2.62c	21.67±0.27a	70.66±2.62	36.00±1.2a
75	68±0.91b	9.00±0.51b	62±3.14bc	16.00±0.25ab	78.66±3.14b	26.67±0.54b
37.5	69±0.85b	9.00±0.25b	67±4.17bc	15.33±0.33b	80.33±4.17b	22.33±0.54c
18.5	73±0.94ab	4.00±0.81c	75±1.30ab	6.00±0.27c	85.66±1.30bc	12.67±0.91d
Control	91±0.75a	00±0.00d	93±0.83a	00±0.00d	93.33±0.83a	00±0.00e
p-value	0.2837	0.000	0.7941	0.000	0.5437	0.000

Means with standard error and homogeneous group are present in the table, same lowercase letters are not statistically significant form each other at Tukey HSD test ($p<0.05$).

Table 2: Mean consumption and mean mortality percentage of seven *Coccinella septempunctata* treated with different concentrations of Spirotetramat after 24, 48 and 72 hours of feeding interval.

Treatments (ppm)	Daily consumption (mean ± S.E)					
	24 h		48 h		72 h	
	Consumption (%)	Mortality (%)	Consumption (%)	Mortality (%)	Consumption (%)	Mortality (%)
1500	59.75±0.85b	14.67±0.27a	69.25±2.5b	25.67±0.72a	69.33±2.65d	43.33±0.72a
750	61.25±1.65b	12.33±0.27b	68.00±1.82b	20.33±0.27b	75.00±1.18c	34.00±0.47b
375	64.00±0.91b	8.66±0.25c	64.00±2.16b	17.67±0.72b	82.33±1.49bc	24.67±0.54c
187	85.25±0.94ab	6.67±0.27d	77.75±1.89ab	7.66±0.27c	87.66±0.85ab	11.33±0.27d
Control	88.00±0.91a	00±0.0e	90.00±1.29a	00±0.0d	93.66±0.75a	00±0.0e
p-value	0.5437	0.000	0.5732	0.000	0.6567	0.000

Means with standard error and homogeneous group are present in the table, same lowercase letters are not statistically significant form each other at Tukey HSD test ($p<0.05$).

IV. DISCUSSION

The current study was conducted to check the "effect of new chemistry insecticides directly and through poisoned mustard aphids on development of *Coccinella septempunctata"*. The two new chemistry insecticides Flonicamid at the concentrations (0, 18.5, 37.5, 75 and 150 ppm) and Spirotetramat at the concentrations (0, 187, 375, 750 and 1500 ppm) were applied directly and indirectly through poison aphids under laboratory conditions.

The current study showed that, in case of direct application of insecticides on adults of *Coccinella septempunctata*, the mean maximum mortality was recorded 58.75±0.25 and 51.50±0.28% after the application of Spirotetramat and Flonicamid, respectively. The higher mortality was noticed in the case of Spirotetramat as compared to Flonicamid in all the experimental groups. The present study agreement with Bushsal *et al*. ([26] proposed that Flonicamid had the lowest impact on *C. septempunctat* as compared to Imidacloprid, Nitenpyram, Azadirachtin and Dimethoate, respectively, when ladybird beetles provided with treated Aphis fabae. Similarly, Jalali *et al*. ([27] stated that the Flonicamd are part of bio-rational insecticides did not show harmful impact on two spotted ladybird beetles as treated with 10 times higher concentration from of field recommended dose, this may be due to species difference. However, the current study contradicts with Azod *et al*. [28] who reported that Spirotetramat is considered less lethal than abamectin, but it can alter the pre-oviposition period, percentage of adult emergence and growth of *Cheilomenes sexmaculata*, however, species different from current studies. Similarly, Tengfei *et al*. [29] reported that the Spirotetramat did not affect the *Coccinella septempunctata* when applied through contact, there was no change in physiology and behavior was observed after different treatments. He also stated that Spirotetramat is considered safer for the natural enemies and other beneficial arthropods. Similar findings suggested that there was no hazardous impact noticed in adult and larvae of *Adalia bipunctata* after the treatments of Spirotetramat various concentrations [23]. Likewise, Planes *et al*. [30] proposed that survivorship, hatching potential, egg laying, longevity and fecundity were not changed after the treatment of Spirotetramat. However, the current study disagreed with Pezzini and Koh, [31] who reported that the high mortality was recorded in pirate bugs after the treatment of Flonicamid. Furthermore, he suggested that the Flonicamid insecticide effects insect pests both ingesting and contact with contaminated surface, thus the effect of this pesticide on the true bugs and ladybird beetle could be because of together impact of contact as well as due to ingestion of pesticide treated aphids, as providing green bean aphids and through substrate contact. The Pan *et al*. [32] stated that the different concentrations of Spirotetramat were used to manage the whiteflies and aphids due to its long-lasting impact and consider safest to natural enemies.

The present study proposed that maximum consumption was recorded in control as compared to treatments. The *C. septempunctat* significantly consumed poison aphids mainly treated with lower concentrations of both insecticides. The mortality rate of *Coccinella septempunctata* had an indirect relationship with consumption rate, as consumption rate increases mortality decreases and consumption decreases mortality increases. In some cases, the feeding behavior of seven spotted ladybird beetles remains asymptomatic. The current study was agreement with Afza *et al*. [33] proposed that during the experiment period the seven spotted ladybird beetle was provided with both choice and free choice to feed aphids, the results showed that the *Coccinella septempunctata* mainly chose untreated aphids as compared to treated aphids in lab conditions. He also suggested that the seven spotted ladybird beetle consume more aphids treated with half of the field recommended dose as compared to full dose treatment. Singh *et al*. [34] proposed that half of the field recommended harmful to ladybird beetles while its impact was not detrimental.

The maximum consumption with lowest mortality was noticed when aphids treated with Flonicamid as compared to Spirotetramat. The mortality rate of *Coccinella septempunctata* had an indirect relationship with consumption rate, as consumption rate increases mortality decreases and consumption decreases mortality increases. In some cases, the feeding behavior of seven spotted ladybird beetles remains asymptomatic. The present study agreement with El-Zahi *et al*. [35] suggested that Flonicamid is considered safest insecticide in field condition as compared to imidacloprid and thiamethoxam. Hautier *et al*. [36] also suggested that flonicamid is considered safer than predatory insects that other applied insecticides in filed condition as compared to laboratory conditions. In contrast, Pezzini and Koh [31] proposed that the high mortality in *C. septempunctata* occurred due to ingestion of poison aphids as well as due to contact with surface, because Flonicamid produced toxicity both contact and ingestion method.

The *C. septempunctat* significantly consumed poison aphids mainly treated with lower concentrations of both insecticides. The current study agreement with Tengfei *et al*. [29] suggested that the *Coccinella* septempunctata significantly consumed treated prey such as camellia aphids explore to various concentrations of different insecticides. He further suggested that among the other insecticides treated aphids, the seven spotted ladybird beetle significantly consume camellia aphids explore to Spirotetramat different concentrations as compared to other groups of insecticides. This showed that the Spirotetramat has low lethality as compared to flonicamid. Similarly, Planes *et al*., [30] reported that the Spirotetramat showed sub-lethal impact on mealybug destroyer ladybird beetle. He proposed that the Spirotetramat is considered harmless against the mealy bug destroyer lady beetle when exploring insecticide using prey.

Moreover, the combined direct and indirect impacts of new chemistry insecticide highlight the need for a general approach to pest management. These insecticides may significantly reduce the pest population, however, their impact on natural enemies must be carefully evaluated to avoid ecological balance disruption. Understanding the interactions among aphids, natural enemies and insecticides application can update strategies that promote IPM, like in time applications of insecticides to decrease exposure of natural enemies during critical life stages and application of eco-friendly insecticides could mitigate the adverse effects of insecticides on beneficial insects.

V. CONCLUSION

It was concluded from the current study that the Spirotetramat (50 EC) is considered more toxic than Flonicamid (50 wg). The Spirotetramate (50 EC) showed a high mortality percentage when directly applied to adult seven spotted ladybird beetles as compared to Flonicamid. However, *Coccinella septempuncata* consumed more aphids treated with different concentrations of Spirotetramat as compared to treated aphids with flonicamid different concentrations. Thus, the current study showed that Flonicamid can be recommended for the use in field conditions as it was not harmful for the natural enemies as like that of Spirotetramat. The mortality rate of *C. septempuncata* is not too high to consider lethal, so, both pesticides can be used in IPM strategies.

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