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Evaluation of Toxicity of Few Novel Insecticides Against Different Aphid Species (*Rhopalosiphum maidis*, *Myzus persicae*, *Liphaphis erysimi*)

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ABSTRACT

Aphids are important insect pests and are considered a major threat to various crops. In the laboratory experiment, our objective is to assess the toxicity level of some newer synthetic insecticides, *viz.* Imidacloprid, Flonicamid, and Spirotetramat against different species of aphids *viz.* maize leaf aphids (*Rhopalosiphum maidis*), green peach aphids (*Myzus persicae*), and mustard aphids (*Liphaphis erysimi*). The leaf dip bioassay was conducted to evaluate the LC_{50} and LT_{50} values. Among these novel molecules, Spirotetramat was the most toxic insecticide against *R. maidis* and *M. persicae,* with median lethal concentrations (LC_{50}) of 0.68 and 3.99 ppm, and Flonicamid was the most toxic against *L. erysimi* with an LC_{50} value of 5.79 ppm. The median lethal concentrations for the Imidacloprid, Flonicamid, and Spirotetramat are different for each species of aphids. The LT_{50} values of the given insecticides revealed that the Imidacloprid has the potential for giving effective control of *R. maidis, M. persicae,* and *L. erysimi* species, as evidenced by the shorter time required for 50% mortality with LT_{50} values of 44.53, 49.19 and 44.90 hrs respectively with median lethal concentrations of 4.20, 5.14 and 10.86 ppm. The results indicated variations in toxicity among these different chemicals against different insect species.

INTRODUCTION

Aphids are one of the most known herbivorous pests worldwide and have effectively taken advantage of the agricultural environment (Srigiriraju et al. 2010). Aphids are the common insect pests of crops that cause major economic loss (Dewhirset et al. 2010).

Maize aphid is also known as corn leaf aphid (*Rhopalosiphum maidis*, Hemiptera: Aphididae). *R. maidis* has a wide range of hosts of the Graminae, which includes 30 genera of cereal crops, specifically corn, barley, sorghum, and *Miscanthus sinensis* grass (Kieckhefer & Kantack 1980, Pointeau et al. 2014). *R. maidis* is a polyphagous, sap-sucking insect pest that may cause a 40% yield loss (Table 1) (Alam et al. 2019). It is distributed all over India, and the maize dwarf mosaic virus and maize leaf fleck virus are transmitted by *R. maidis* (So et al. 2010). The infestation of *R. maidis* is found in the whorl stage of plants. The infestation increases during the tassel emergence stage. Infestation of aphids in maize tassel reduces pollination and transmits bacteria, viruses, and fungi in cob, leaf, and other parts of the plant (Alam et al. 2020). Adults of the *R. maidis* are bluish-green. The wingless females are parthenogenetic (reproducing without mating) and of green or whitish green. Most of the time, *R. maidis* reproduces parthenogenetically (Blackman & Eastop 2000, Kumar et al. 2018).

The green peach aphid, *Myzus persicae* (Sulzer) (Aphididae: Hemiptera), is an extremely polyphagous aphid species that has been reported to feed on over 500 species of host plants from at least forty different families, involving several important crops (Van Emden 2017). *M. persicae* is the major pest in potato crops, causing major losses (Table 1) in yield indirectly by transmitting many plant viruses (Table 2) that lead to the degeneration of the seed tubers (Kreuze et al. 2020). The wingless adult aphids are greenish or yellowish and have lateral stripes on the body.

Lipaphis erysimi (Kalt.) is the primary pest in all mustard-growing regions of the country. It mainly affects the different cruciferous crops such as cabbage, radish, and rape. But mainly harms the crops of the Brassicaceae family *e.g.*, mustard and rapeseed (Koramutla et al. 2016). The mustard aphid's nymphs and adults suck the cell sap from the leaves, inflorescences, and immature pods, resulting in poor yield. They have also been discovered to prefer flowers over leaves for feeding (Dewhirst et al. 2010). This species transmits over 13 different viruses in the Brassicaceae family (Adhab & Schoelz 2015). The heavy infestation of *L. erysimi* shows many symptoms in mustard plants, which results in stunted growth, crinkling of leaves, yellowing, and dried-up plants. The wingless or apterous aphids of *L. erysimi* are yellowish-green or grey-green, small in size, faded, waxy white coating on the body (Gautam et al. 2019).

Due to the high capacity of virus transmission of those above-mentioned aphid species, heavy economic loss has been recorded in the crop yield by various scientists (Harris & Maramorosch 2014). Adverse effects of insecticides, especially due to organophosphate and carbamates, insecticides,

Table 1: Yield losses(%) caused by aphid vectors in different crops.

S.No.	Aphid species	Crop	Yield loss	Reference
1.	*Rhopalosiphum maidis*	Barley	30%	(Maanju et al. 2023)
		Barley	17.1–30%	(Murti et al. 1968, Bhatia et al. 1973)
		Corn	28.14%	(Al-Eryan & Al-Tabbakh 2004)
		Maize	40%	(Alam et al. 2019)
		Maize	10–20%	(Subedi 2015)
2.	*Myzus persicae*	Sweet pepper	31.49-100%	(Sharma et al. 2021)
		Potato	90%	(Sidauruk & Sipayung 2018)
3.	*Lipaphis erysimi*	Mustard	35.4 to 91.3%	(Brar et al. 1987)
		Mustard	100%	(Singh & Sachan 1999)
		Mustard	91%	(Bunker et al. 2006, Kular & Kumar 2011)
		Brassica spp.	65 to 96%	(Dhillon et al. 2022)

Table 2: List of aphid vectors and their disease transmission.

S.No.	Aphid species	Virus	Reference
1.	*Myzus persicae*	Turnip Yellow Virus (TuYV)	(Nancarrow et al. 2022)
		Cucumber mosaic virus	(Ali et al. 2021, Blackman & Eastop 2000, Naga et al. 2020, Capinera 2001, Eigenbrode et al. 2002, Qi et al. 2021)
		Potato leafroll virus (PLV)	
		Lettuce mosaic virus	
		Watermelon mosaic viruses	
		Potato virus Y (PVY)	
		Beet Yellow Virus (BYV)	(Hossain et al. 2021)
		Pea Enation Mosaic Virus(PEMV)	(Doumayrou et al. 2016)
		Potato Leafroll Virus (PLRV)	(Kumar et al. 2020)
		Tobacco necrotic dwarf virus (TNDV)	(Tolin 2008)
2.	*Rhopalosiphum maidis*	Sunflower Mosaic Virus(SMV)	(Gulya et al. 2002)
		Johnsongrass mosaic virus (JGMV), Maize dwarf mosaic virus (MDMV), Sugarcane mosaic virus (SCMV), and Sorghum mosaic virus (SRMV)	(Klein & Smith 2020)
3.	*Lipaphis erysimi*	Turnip mosaic virus	(Devi et al. 2004)
		Cauliflower mosaic virus Caulimovirus	(Adhab & Schoelz 2015)

neurotoxic impact, and fatality, have been observed in beneficial insects, birds, amphibians, and mammals. Inappropriate use of those toxicants and their persistence have become a significant threat to the environment (Singh et al. 2023). Because of these reasons, our focus was to find the efficacy of some novel insecticides on the aphid population, which has less hazardous consequences on the environment and other beneficial organisms of the crop ecosystem. We had planned laboratory assays for evaluating the lethal concentration. Three insecticides which are mainly novel and used by the farmers for sucking pests, especially against these aphid species, have been considered for our trials, like Flonicamid, Spirotetramat, and Imidacloprid. These chemical insecticides have a specific mode of action. Flonicamid disrupts insect chordotonal organs, and Spirotetramat acts as a lipid biosynthesis inhibitor which decreases the reproductivity and fertility of sucking insect pests (Salazar et al. 2016). Imidacloprid blocks the nicotinic acetylcholine receptor in the central nervous system. Because of this mode of action, we selected these insecticides in the present study to assess their bio-efficacy.

MATERIALS AND METHODS

Experiments of the relative toxicity of insecticides were carried out in the laboratory of the Department of Entomology, Lovely Professional University, Phagwara (Punjab) (31.2560°N, 75.7051°E). *R. maidis, L. erysimi, and M. persicae* populations were collected from their host plant, *viz*., maize, mustard, and potato, respectively. The Species of the aphids, *R. maidis, L. erysimi,* and *M. persicae,* were collected in different seasons. *R. maidis* was collected in the *Kharif* season (June-October), and *M. persicae* and *L. erysimi* were collected in the *Rabi* season (November-April). These species of aphids were collected from the field of the university and its nearby places. *R. maidis* species were collected from the maize, and *M. persicae* was collected from the field of the Potato. *L.erysimi* species were collected from the mustard crop of LPU fields. These aphid species were identified morphologically by using taxonomic keys. We reared *R. maidis* on the leaves of maize or corn, and *M. persicae* on the flower and leaves of the potato, *L. erysimi* on the floral part of the mustard plant in laboratory conditions, where the incubator temperature was atat ± 25°. Required insecticides for the evaluation of toxicity are enlisted in Table 3.

Table 3: Different insecticides were used for the evaluation.

Trade Name	Active ingredient	Company
Shandor	Imidacloprid 17.8% SL	Shanro Agritech
Ulala	Flonicamid 50 WG	UPL
Movento	Spirotetramat 15.31% OD	Bayer

Insecticidal Bioassay

The bioassay process was conducted according to the leaf dip method as given by the IRAC test method with some modifications (https://irac-online.org/methods/aphids-adultnymphs/). The healthy leaves of the different hosts of aphids were collected from the field and washed thoroughly. Later, different concentration levels of each formulation were made by diluting it in distilled water. After that, we make the leaf disc or stripes as per the suitability of the host's leaf. One end of these stripes was covered with a wet cotton plug to avoid dryness. Later on, each bunch of stripes or discs was dipped or treated with insecticide concentrations for 15-20 seconds. The treated leaves were dried up for 15 to 20 minutes. After the drying of treated leaf stripes or discs, those were placed onto the paper cups. On each bunch, 20 apterous aphids of the 3rd nymphal instar were transferred on the treated leaves with the help of a fine pointed brush. The paper cups were covered with a muslin cloth. The cups were further kept in the incubator at ± 25°C and 65 ± 10% relative humidity (RH), with a 16:8 h (L:D) photoperiod. The observation was recorded periodically after 24 hours. The response of aphids was categorized as the live, morbid, and dead insects. Aphids were considered alive if they showed movements or held themselves normally. If their movements or posture are abnormal then those were considered morbid.

Statistical Analysis

The result was interpreted by using percentage mortality and control treatment mortality by using corrected mortality by using Abott's formula (Abbott 1925).

$$\text{Corrected mortality \%} = \frac{\text{Test mortality (\%) - Control mortality (\%)}}{\text{100 - Control mortality (\%)}}$$

The mortality data was calculated by Probit analysis by using Finney's table for the transformation of the percentage of mortality. The statistical analysis was done by using the Poloplus software (2.0 version).

RESULTS

There are differences between the responses of the *R.maidis, M. persicae,* and *L. erysimi* populations against tested chemicals, showing a change in the sensitivity to insecticides within the different species.

Relative Toxicity of Insecticides Against *R. maidis*

Laboratory bioassay revealed that Spirotetramat is the most toxic insecticide against *R. maidis,* with an LC_{50} value of

Table 4: Relative toxicity of the tested insecticides against R. maidis, M. persicae, and L. erysimi with values of LC_{50} and LC_{90}.

Sr. No.	Insecticide	LC_{50} ppm	Fiducial limit 95% Ppm	LC_{90} ppm	Chi-square value	*df*	Heterogeneity
R. maidis							
1	Flonicamid	4.20	2.93-5.31	12.00	3.21	3	1.07
2	Spirotetramat	0.68	0.55-0.832	1.38	3.08	3	1.03
3	Imidacloprid	6.33	5.55-7.11	14.38	2.94	3	0.98
M. persicae							
1	Flonicamid	5.12	2.60-7.61	18.43	4.82	3	1.61
2	Spirotetramat	3.99	2.70-5.30	11.49	3.98	3	1.33
3	Imidacloprid	5.14	4.40- 5.90	14.69	1.54	3	0.51
L. erysimi							
1	Flonicamid	5.79	3.01- 7.89	11.53	5.26	3	1.76
2	Spirotetramat	15.19	14.40- 15.90	21.49	1.76	3	0.59
3	Imidacloprid	10.86	5.27-14.39	30.26	5.92	3	1.97

0.68 ppm and an LC_{90} value of 1.38 ppm when compared with imidacloprid and Flonicamid (Table 4). Whereas the toxicity level of Imidacloprid and Flonicamid was comparatively less, with an LC_{50} value of 6.33 and 4.20 ppm, respectively. LC_{90} values were found at 13.61 and 13.46 ppm, respectively. However, there is a minute difference in the toxicity level of Imidacloprid and Flonicamid. Dose dose-specific response of the aphids against each level of concentration of insecticides was graphically presented in (Fig.1).

Relative Toxicity of Insecticides against *M. persicae*

Relative toxicity of the insecticides against *M. persicae* was analyzed, and the outcomes showed that Spirotetramat was the most toxic insecticide with a value of LC_{50} at 3.99 ppm (fiducial limit 1.55-7.44), and the insecticidal response was

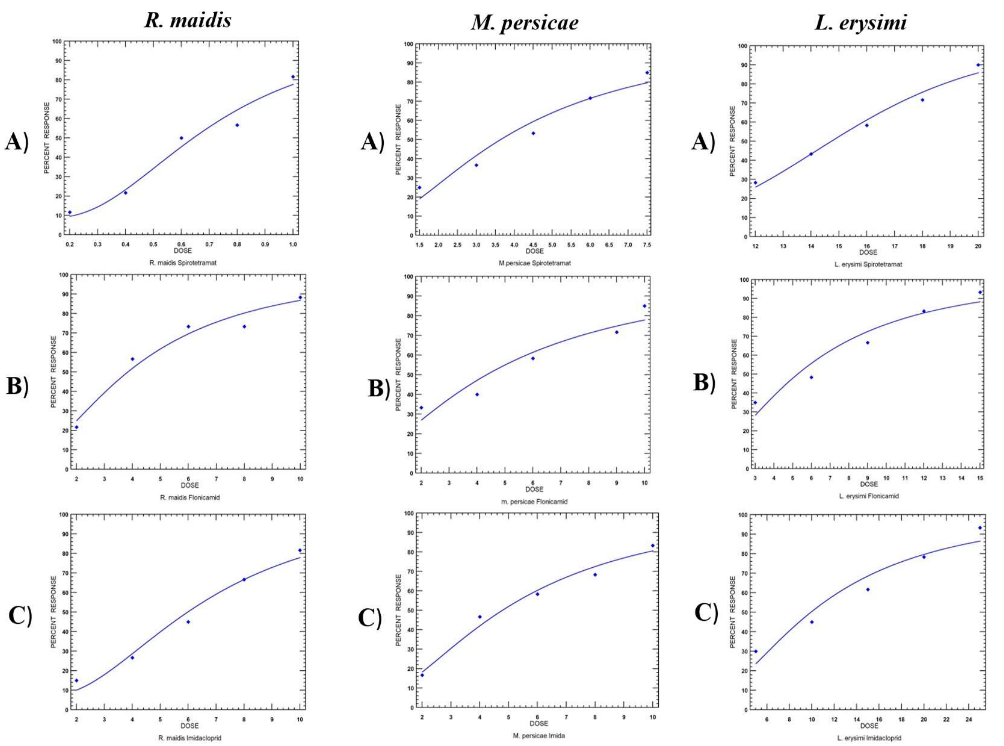

Fig. 1: Graphical representation of the insecticidal responses of the R. maidis, M. persicae, and L. erysimi against insecticides A) Spirotetramat B) Flonicamid C) Imidacloprid. Where response showed the mortality percentage of aphids against each insecticide with their particular level of concentration.

also analyzed in (Fig.1). Bioassay findings of Imidacloprid was evaluated, and the resulted toxicity level was found with an LC_{50} value of 5.14 ppm (Table 4). Flonicamid was found to the toxic as compared to Spirotetramat, with an LC_{50} value of 5.12 ppm. The LC_{90} values of Spirotetramat, Flonicamid, and Imidacloprid were found as 11.49, 18.43, and 14.69 ppm, respectively.

Relative Toxicity of Insecticides against *L. erysimi*

The results of the laboratory bioassay indicate that Flonicamid was found to be the most effective insecticide against *L. erysimi* with an LC_{50} value of 5.79 ppm and LC_{90} value of 11.53 ppm (fiducial limit of 3.01-7.89 ppm) (Table 4). The toxicity level of Imidacloprid showed an LC_{50} value of 10.86 ppm and an LC_{90} value of 30.26 ppm. The effect of the Spirotetramat was seen as less toxic, with LC_{50} value at 15.19 ppm and LC_{90} at 21.49 ppm. At the same time, the toxicity level of Flonicamid was more than the toxicity level of Imidacloprid and Spirotetramat. The insecticidal response of *L. erysimi* against tested formulations is represented in (Fig. 1).

Among all the tested insecticides against different aphid species, our results indicated that Spirotetramat was the most toxic insecticide against *R. maidis* and *M. persicae* with an LC_{50} value of 0.68 and 3.99 ppm except in the case of *L. erysimi*. However, Flonicamide was found to be the most toxic against *L. erysimi*. As per the estimated LC_{50} values, Flonicamid was found to be the second most toxic insecticide against *R. maidis* and *M. persicae,* with an LC_{50} Value of 4.20 and 5.12 ppm, respectively, where Imidacloprid was found to be as least toxic as compared to the other tested insecticides.

Median Lethal Time (LT_{50} and LT_{90}) for Aphid Species Treated with Different Insecticides

In this study, we evaluate the median lethal time LT_{50} and LT_{90} for aphid species *R. maidis, M. persicae,* and *L. erysimi* were treated with the insecticides *viz*. Spirotetramat, Flonicamid, and Imidacloprid. We checked the LT_{50} values of the mentioned insecticide based on the LC_{50} value of each insecticide.

In the case of *R.maidis,* for the bioassay, we prepared levels of concentrations of insecticides as Imidacloprid (6.33 ppm), Floniamid (4.20 ppm), and Spirotetramat (0.68 ppm). Here, laboratory bioassay results indicated that Imidacloprid is the most effective insecticide with an LT_{50} value of 44.53 h. (fiducial limit 33.89-57.88 h) and LT_{90} value at 112.16 h. followed by Flonicamide and Spirotetramat with an LT_{50} value of 58.30 and 64.30 h, respectively, and the LT_{90} values of the insecticides are 144.58 and 162.48 h, respectively (Table 5).

In the case of *M. persicae,* the concentration levels of Imidacloprid (5.14 ppm), Flonicamid (5.12 ppm), and Spirotetramat (3.99 ppm) were used for the assessment of LT_{50} values. After the treatment of insecticides, we observed the duration-wise mortality. The findings of the bioassay showed that the lethal time (LT_{50}) for Imidacloprid was determined to be 49.19 h with a fiducial limit ranging from 39.50 to 61.31 h and LT_{90} value at 173.06 h (Table 5). The LT_{50} values of the Flonicamid and Spirotetramat are 57.45 and 61.99 h, respectively, whereas LT_{90} values are 162.19 and 172.15 h, respectively. However, there is a slight difference between the LT_{50} values of Flonicamid and Imidacloprid.

In the case of *L. erysimi,* the prepared concentrations of Imidacloprid (10.86 ppm), Flonicamid (5.79 ppm), and Spiroteramat (15.19 ppm) were used for the laboratory bioassay. The result indicated that imidacloprid has an LT_{50} at 44.90 h, where the fiducial limit ranges from

Table 5: Relative toxicity of insecticides against different aphid species with the value of an LT_{50} (Median lethal time).

Sr. No.	Insecticide	LT_{50} h	Fiducial limit 95% h	LT_{90} h	Chi-square value	*df*	Heterogeneity
R. maidis							
1	Flonicamid	58.30	36.49-83.24	144.58	3.00	3	1.00
2	Spirotetramat	64.30	51.93-85.18	162.48	6.64	6	1.10
3	Imidacloprid	44.53	33.85-57.88	112.16	5.74	5	1.14
M. persicae							
1	Flonicamid	57.45	44.23-71.85	162.19	2.83	3	0.94
2	Spirotetramat	61.99	52.10-76.48	172.15	3.91	6	0.98
3	Imidacloprid	49.19	39.50-61.31	173.06	1.953	6	0.32
L. erysimi							
1	Flonicamid	47.82	33.32-61.23	156.97	2.47	3	0.82
2	Spirotetramat	49.54	32.84-63.25	189.61	2.96	3	0.98
3	Imidacloprid	44.90	25.29-61.24	208.14	2.73	3	0.91

25.29 to 61.24 h, and has an LT_{90} at 208.14 h. The LT_{50} values of the Flonicamid and Spirotetramat were found at 47.82 and 49.54 h, and the LT_{90} values were 156.97 and 189.61 h, respectively.

According to the results of the median lethal time (LT_{50}), Imidacloprid was found to be the most effective insecticide against *R. maidis*, *M. persicae*, and *L. erysimi,* with an LT_{50} value of 44.53, 49.19, 44.90 h., respectively. Next in the line, Flonicamid, found as the second most effective insecticide against the aphid species with LT_{50} values, was 58.30 h. (*R. maidis*), 57.45 h (*M. persicae*), and 47.82 h. (*L. erysimi*). Interestingly, we observed that in the results of relative toxicity, Spirotetramat was found as the most toxic insecticide, but as per the median lethal time, it was observed as the less effective as compared to the other insecticides with LT_{50} values of 64.30, 61.99, and 49.54 against the *R. maidis*, *M. persicae*, and *L. erysimi* respectively.

DISCUSSION

Selected novel insecticides have different modes of action, and they belong to different classes of insecticides. Spirotetramat belongs to a class known as tetronic acid derivative, which acts as a lipid biosynthesis inhibitor (Brück et al. 2009), whereas Flonicamid belongs to pyridine carboxamides, which mainly acts as an antifeedant and has a unique mode of action targeted to the nervous system of insects (Morita et al. 2007) and Imidacloprid belongs to the neonicotinoid class which mainly targets the nervous system of insects by acting as nicotinic acetylcholine receptor (nAChR) agonists (Bass et al. 2015). Due to these unique modes of action, these insecticides exhibit high insecticidal effectiveness as compared to other synthetic insecticides since we are focusing on these insecticides for our experiment.

The studies carried out by Nauen et al. (2008) showed that Spirotetramat is considered a wide-spectrum insecticide with a new mode of action and is effective against different hemipteran insects, *viz.,* aphids, whiteflies, and psyllids. Also, in a similar research performed by Laurent et al. (2023), it was shown that Spirotetramat and Flonicamid insecticides are effective in controlling aphid species up to 85.6% and 79.9%, respectively, in cabbage crops. The result showed the efficacy of Spirotetramat against *R. maidis*, *M. persicae*, and *L. erysimi* with an LC_{50} value of (0.68, 3.99, and 15.19 ppm, respectively). The present findings are supported by the study conducted by Iftikhar et al. (2022), who showed the efficacy of Spirotetramat 240 SC against cabbage aphids (*B. brassicae*) with an LC_{50} value of 1.304 mgL^{-1}. Udtewar et al. (2021) reported that Flonicamid has a high toxicity level against *R. maidis* with an LC_{50} value of 6.682 ppm, and also recorded that Imidacloprid was less toxic as compared to Flonicamid with an LC_{50} value of 9.028 ppm. As per these results, our study showed a similar trend, with an LC_{50} value of Flonicamid (4.20 ppm) and Imidacloprid (6.33 ppm) against *R. maidis*. In the findings of Neupane and Subedi (2022), the evaluation of insecticidal efficacy against *R. maidis* revealed that the field efficacy of Flonicamid was the most effective insecticide against *R. maidis* and also showed low colony percentage (2.85%), aphid score (2.63%), aphid infestation per plant (7.33%) and high yield production 7904 $kg.ha^{-1}$ with a dose of 0.5 $g.ha^{-1}$.

As per the studies of Umina et al. (2022), the toxicity of Spirotetramat 240 SC against collected populations of *Myzus persicae* from different locations was assessed. The LC_{50} values were found to be 0.095, 0.571, 13.121, 1.515, and 2.447 $mg.L^{-1}$. According to the study conducted by Naga et al. (2021), their laboratory experiment revealed that Flonicamid was a toxic insecticide against a laboratory population and Jalandhar population of *M. persicae* with an LC_{50} value of 1.195 and 28.420 ppm, respectively, which in line with present finding. Imidacloprid showed toxicity with an LC_{50} value of 4.214 ppm against a laboratory population, while the LC_{50} against the Jalandhar population was 53.345 ppm, which is also in accordance with the Laboratory population of our study, where the LC_{50} value of the Flonicamid and Imidacloprid was 5.12 and 5.14 ppm respectively against *M. persicae*. Similarly, Halder and Rai (2018) observed an LC_{50} value of 19.17 ppm for Flonicamid against *M. persicae*, and Imidacloprid showed toxicity with an LC_{50} at 5.019 ppm. However, in this study, Imidacloprid was found to be the most toxic insecticide against *M. persicae*.

The studies of Udtewar et al.(2021) reported that Flonicamid was a toxic insecticide against *L. erysimi* with an LC_{50} value of 10.829 ppm and an LC_{50} value of Imidacloprid at 17.968 ppm. Similarly, an experiment conducted by Halder & Rai (2018) indicated that Imidacloprid was more toxic as compared to Flonicamid, with an LC_{50} value of 5.197 ppm and Flonicamide, with LC_{50} at 33.09 ppm. As per our results of toxicity, Floniamid was found as the most toxic insecticide against *L. erysimi,* with an LC_{50} value of 5.79 ppm, and Imidacloprid, with an LC_{50} of 10.86 ppm, respectively.

CONCLUSION

In general, the research highlights the toxicity level of the different insecticides against different species of aphids. The results indicated fluctuation in the effectiveness of the Spirotetramat, Imidacloprid, and Flonicamid when observed in the insect population and species.

We found that Spiroteramat is a highly toxic insecticide against the *R. maidis* and *M. persicae* because of the distinct mode of action. Whereas, the toxicity level of the Flonicamid is higher in the case of *L. erysimi*. These insecticide has different modes of action and can be used as alternatives in the insect pest management of different crops. The use of regular insecticides over the years causes, increases the resistance capacity of many insect pests. Therefore use of novel and efficient insecticides with proper doses is of utmost importance to combat resistance problems. We found that Imidacloprid showed 50% mortality within a short duration of time. Although Flonicamid 50 WG, Spirotetramat 15.31% OD, and Imidacloprid 17.8% SL, these novel insecticides are suitable and efficient insecticides for managing aphid infestations depending on aphid species, their efficiency level may differ. Hence, advocating proper insecticides for chemical management and employing rotation of the insecticides and the use of newer molecules with unique modes of action could be the safer strategy to counteract with development of resistance in the aphid populations.

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