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EVALUATION OF NEWER INSECTICIDES FOR THE MANAGEMENT OF *MYZUS PERSICAE* (SULZER)

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ABSTRACT: Green peach aphid, *Myzus persicae* (Sulzer) (Aphididae: Hemiptera) is one of important pest which causes direct and indirect losses to many horticultural crops. To deal with this pest insecticide application remains the principal method to avoid its losses. Therefore, the present study was aimed to evaluate two insecticides with novel mode of action as an alternative to the existing insecticides used for management of *M. persicae*. Further leaf dip method was followed to evaluate the test chemicals against *M. persicae*. Probit analysis and LC_{50} data suggest that flonicamid is as effective as imidacloprid. The flonicamid also has different mode of action and can be used as an alternate chemical to manage *M. persicae* in potato and other crop ecosystems. This is the first study that indicates insecticidal resistance in *M. persicae* population in India.

KEYWORDS: *Myzus persicae, mtCOI,* Imidacloprid, Flonicamid, Pymetrozine, Probit analysis, LC50.

INTRODUCTION

Green peach aphid, *Myzus persicae* (Sulzer) (Aphididae: Hemiptera) is an extremely polyphagous aphid species which has been reported to feed on more than five hundred species of host plants belongs to at least forty different families including several important agricultural crops (Van Emden and Harrington, 2007). The *M. persicae* causes direct yield losses to many vegetable crops by sucking the plant sap as well as indirect losses by transmitting plant viruses (Naga et al., 2020). Virus transmission abilities of *M. persicae* are generally considered to be more economically important as it is known to transmit more than 100 plant viruses mainly in non-persistence manner (Blackman and Eastop, 2000). The *M. persicae* is also known to be a major pest in potato and which cause significant yield losses indirectly by transmitting many plant viruses that leads to degeneration of the seed tubers (Kreuze et al. 2020). The potato crop is mainly grown using seed plot technique in Indo-gangetic plains during winter season. However, crops which are grown beyond the seed plot technique require insecticide protection. Imidacloprid is widely used to manage many sucking pests including *M. persicae* and this intensive use may lead various problems like insecticide resistance, resurgence and toxicity to non-target. The resistance problem has emerged in many countries to insecticides from four different modes of action subgroups: carbamates (1A), organophosphates (1B), synthetic pyrethroids (3A), and neonicotinoids (4A) (Bass et al. 2014). The resistance to insecticides has also been recorded with various intensities in the Indian population of *M. persicae* (Halder et al. 2007; Gavkare et al. 2013; Halder and Rai 2018).

In the present study we have evaluated the two newer insecticides flonicamid and pymetrozine against *M. persicae* populations collected from potato. These chemicals have a unique mode of actions where flonicamid

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acts as modulator of chordotonal organ and pymetrozine acts as chordotonal organ TRPV channel modulators (IRAC, mode of action classification scheme, Version 10.1). Due to this unique mode of action these insecticides were chosen in the present study where the efficacy of these chemicals was compared with the recommended chemical imidacloprid.

MATERIALS AND METHODS

Materials used for bioassay

Bioassay of insecticides was carried out against two *M. persicae* populations and both the populations were collected from Jalandhar, (Punjab). Out of these populations one was collected during 2013 and since then was being maintained on Chinese cabbage, *Brassica pekinensis* since 2013 at ICAR-CPRI, Shimla under glasshouse. This population since its collection has not been exposed to insecticides and called as laboratory population.

The second population was collected during the January, 2021 form the same location and called it as Jalanadhar population in this set of experiment. The *M. persicae* population collected from Jalandhar, Punjab (India) was identified morphologically using taxonomic keys. The identification was further confirmed by sequencing the mitochondrial cytochrome oxidase 1 gene (*mtCOI*) using method previously established by Folmer *et al.* (1994). The Polymerase Chain Reaction (PCR) reaction was performed with slight modification as described below, the reaction consisted of 2x EmeraldAmp® GT PCR Master Mix 10 µl, 1 µl each forward and reverse primers, DNA template (30 ng) and nuclease free water was added to make the total reaction volume of 20 µl. PCR was carried out with an initial denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 1 min, and a final extension at 72°C for 10 min. Mitochondrial sequence (*mtCOI*) of *M. persicae* and sequences retrieved from GenBank, NCBI were subjected to multiple sequence alignment with using clustalW followed by construction of phylogenetic tree using Neighbor-Joining method (Saitou and Nei, 1987).

Insecticides evaluated against the *M. persicae* have been mentioned in table 1. The Jalandhar population was only evaluated against imidacloprid and flonicamid.

Insecticidal bioassay

Leaf dip method was followed to evaluate the test chemicals against *M. persicae* as described by IRAC Test method (https://irac-online.org/content/uploads/2009/09/Method_001_v3_june09.pdf).

Appropriate test dilutions of each formulations was prepared in 0.01% Triton x-100 with a staring from dose 10 fold higher from the field recommended dose (mentioned in table 1) (De Little and Umina 2017) and five serial dilutions were prepared. Leaf discs of 4 cm diameter were excised using the surgical blades from fresh uninfected Chinese cabbage leaves. These leaves discs were

Table 1. Insecticides details used for bioassay against *M. persicae*

Test chemical	Field recommended dose (gms of a.i/ ha)	MoA (IRAC, mode of action classification scheme, Version 10.1)
Imidacloprid 17.8% SL	25	Nicotinic acetylcholine receptor (nAChR) competitive modulators
Flonicamid 50% WG	75	Chordotonal organ Modulators undefined target site
Pymetrozine 50% WG	150	Chordotonal organ TRPV channel modulators

allowed to dip in the insecticide dilutions of test chemicals for about 10 sec and dried in incubator at 25°C for about 45 minutes. The control leaf discs were only dipped into 0.01% Triton x-100.

Further these leaf discs were placed over a agar base in about 4,2 cm diameter vials and covered with muslin cloth after releasing the test insect. A drop of water was also poured over the agar base in each vial to keep the leaf discs hydrated. About 30 aphids (from synchronized populations) were released over the leaf discs and incubated at 21°C in incubator.

The aphid response was assessed after 24h in case of imidacloprid (IRAC test method 1) and after 120h in case of flonicamid and pymterozine (IRAC test method 19). The aphid responses were observed as live, morbid and dead. Adults were considered live if they moved and held themselves normally, and morbid if their movement or posture was abnormal. Results were expressed as percentage mortality and for untreated mortality corrected using Abbott's formula (Abbott, 1925). The mortality data were subjected to probit analysis using Polo plus and after Finney, (1971). A resistance ratio was also calculated by dividing the LC_{50} of field population by LC_{50} of the lab population.

The resistance for the population was scaled as follows: < twofold, no resistance; 2- to 10-fold, very low resistance; 11- to 20-fold, low resistance; 21- to 50-fold, moderate resistance; 51- to 100-fold, high resistance; >100-fold, very high resistance (TorreseVila *et al.,* 2002; Saddiq *et al.,* 2014).

RESULTS AND DISCUSSION

Molecular identification of aphid species

The aphids identified as *M. persicae* based on the molecular analysis. Multiple sequence alignment with reference sequence revealed the aphid species as *M. persicae*. The *mtCOI* sequence of aphid had shown 100 percent similarity with reference sequences (Accessions no. KM577343.1, MK396780.1) at NCBI database and the representative *mtCOI* sequence of *M. persicae* has been submitted at NCBI (Accession no. MK814190.1).

Insecticidal bioassay

A signification differences in the responses of *M. persicae* populations to test chemicals, indicating a change of sensitivity against insecticides in the Jalandhar population.

The flonicamid had shown highest toxicity with LC_{50} as 1.195 ppm which was however on par with the LC_{50} of imidacloprid (4.214 ppm) (Table 2). Pymetrozine was found to be the least effective chemical against *M. persicae* with LC_{50} 142.77 ppm (fiducial limit 31.214- 1511.8 ppm) (Table 2).

Bioassay of the flonicamid and imidacloprid were also revealed similar responses in Jalandhar population as observed with the lab population (Table 3). The flonicamid had shown higher toxicity with LC_{50} of 28.420 ppm which was however at par with the LC_{50} of imidacloprid (53.345 ppm) (Table 3). Previous reports also

Table 2. Laboratory evaluation of Insecticides against *M. persicae* adults laboratory population

Test chemical	n	LC_{50} (95% CI) (ppm)	Chi-Square	df*	Heterogeneity	SE	Slop
Imidacloprid	180	4.214 (1.087-12.451)	0.369	4	0.092	0.094	0.552
Flonicamid	180	1.195 (0.163-4.407)	2.022	4	0.506	0.082	0.401
Pymetrozine	180	142.77 (23.849-3101.7)	1.051	4	0.263	0.059	0.249

n = numbers of insects used for bioassay; CI, confidence intervals; *df = degrees of freedom; SE= Standard Error

Table 3. Laboratory evaluation of Insecticides against *M. persicae* adults, Jalandhar population.

Test chemical	n	LC_{50} (95% CI) (ppm)	Chi-Square	df*	Heterogeneity	SE	Slop	RR
Imidacloprid	357	53.345 (21.328 -145.631)	0.467	4	0.131	0.057	0.432	12.6
Flonicamid	400	28.420 (9.280 -106.384)	0.327	4	0.082	0.053	0.337	23.7

n = numbers of insects used for bioassay; CI, confidence intervals; *df = degrees of freedom; SE= standard error, RR= resistance ratio.

suggested similar level of response (LC_{50}) to laboratory population *M. persicae* (Gavkare et al. 2013). The bioassay of pymetrozine against Jalandhar population could not be performed as the collapsed due to parasitoids infestation arrived along with field samples. Our finding of flonicamid efficacy against *M. persicae* is supported by previous workers where it has found more effective then imidacloprid, thiamethoxam and acetamiprid (Chandi and Gill, 2019; Sretenovic et al., 2019). The flonicamid has been also found highly effective against aphid and whitefly in cotton ecosystem (Ghelani *et al*., 2014).

The resistance ration of imidacloprid and flonicamid were 12.6 and 23.7 respectively, revels change in response of *M. persicae* against these chemicals in the field population (table 3). The resistance ratio indicates a low level of resistance against imidacloprid and moderate resistance prevalent against flonicamid in the Jalandhar population (TorreseVila *et al.*, 2002; Saddiq et al., 2014). Altered response of *M. persicae* against imidacloprid has been previously recorded by Halder and Rai (2018). The slight change in the sensitivity of *M. pericae* has been recorded in the Greece by (Ntantos 2020).

Intensive use of insecticides over many years has led to the development of widespread and multiple forms of resistance. The first report of resistance *M. persicae* species dates back to 1955 (Anthon, 1955). Resistance in *M. persicae* now reported to most classes of insecticide, including the organophosphates, carbamates, pyrethroids, cyclodienes, and neonicotinoids.

The *M. Persicae* employs seven independent mechanisms of resistance against the insecticides (Puinean *et al.*, 2010; Bass *et al.*, 2014). Hence there is a need to continuously evaluate the effective chemicals having different mode of action against *M. persicae* to manage the insecticide resistance populations.

CONCLUSION

Overall this study highlights that the flonicamid is as effective as imidacloprid which has different mode of action and can be used as an alternate chemical to manage *M. persicae* in potato and other crops ecosystems. Apart from the effectiveness of the chemical the results also highlights the change in the sensitivity of the imidacloprid and flonicamid in the field population (Jalandhar, Punjab) so there is a need to continuous monitoring of the filed population for resistance monitoring.

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