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SUSCEPTIBILITY OF CABBAGE APHID, BREVICORYNE BRASSICAE TO NEW CHEMISTRY INSECTICIDES FROM PUNJAB, PAKISTAN

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Several insect pests attack on canola crop but cabbage aphid, Brevicoryne brassicae is the most devastating pest affecting its quality and quantity. B. brassicae has ability to develop resistance against several conventional insecticides which are being used against this pest. The aim of present study was check the toxicity and resistance levels of selected new chemistry insecticides against different field populations of B. brassicae. Four populations collected from Rahim Yar Khan, Bahawalpur, Lodhran, and Multan were tested against selected new chemistry insecticides. Nymphs were used for bioassay through leaf-dip method. Resistance ratios were in the range of 1.00 to 2.11 folds for fipronil, 1.00 to 1.26 folds for spirotetramat, 1.00 to 2.36 folds for emamectin benzoate, 1.00 to 2.83 folds for spiromesifen, 1.00 to 1.49 for chlorantraniliprole, and 1.00 to 1.71 folds for flonicamid, compared to a reference susceptible strain. Information obtained from our results will prove helpful to delay insecticide resistance development in B. brassicae..
Keywords: Brevicoryne brassicae, new chemical insecticides, susceptibility.

INTRODUCTION

Among all varieties of mustard, Brassica napus L. commonly known as canola is an important eatable oil seed crop in the world. Globally, about twenty-one insect pest species attack on the canola crop (Lamb 1989) which include three aphid species i.e. cabbage aphid Brevicoryne brassicae (L.), turnip aphid Myzus persicae (Sulzer) and green peach aphid Lipaphis erysimi (Kaltenbach) (*Aphididae: Homoptera*) causing 30-35% losses to canola crop (Buntin and Raymer 1994). In Southern Punjab, Pakistan, cabbage and turnip aphids are major insect pests of canola (Aslam *et al.*, 2011). Mustard aphid remains active in the field throughout the year while peak population is observed during December-February, main growing period of mustard.
Both adults and nymphs of *B. brassicae* cause damage by sucking the cell sap from leaves, shoots, flower buds and pods (Ahmad and Akhtar 2013). Chlorophyll contents of plants are reduced and changes in color of leaves also occur due to attack of B. brassicae. Plant growth, development of flowers and pods are also badly affected which may sometime cause the death of the effected plants (Aslam and Ahmad 2001). Furthermore, aphids also secrete honeydew which persists for a long time on leaves and acts as a source for sooty mold to grow, which in the long run influences the process of photosynthesis. Short life cycle and very high fecundity rate of B. brassicae causes an extreme amount of damage and yield losses (Lane 1991; Razaq *et al.*, 2011).
Different management strategies have been used to keep the B. brassicae population below the economic threshold level but chemical control is still considered as the best method due to quick knockdown effect and lower cost. Due to the high reproductive potential of B. brassicae and widespread application of insecticides to control its population, resistance has been observed and reported in aphids from different parts of the world (Ahmad *et al.,* 2003; Margaritopoulos *et al.,* 2007; Herron and Wilson 2011; Ahmad and Akhtar 2013). In Pakistan, insecticide resistance has been well documented in many insect pests comprising of Aphis gossypii Glover (Ahmad *et al.,* 2003), Phenacoccus solenopsis Tinsley (Saddiq *et al.,* 2014; Saddiq *et al.,* 2015), Dysdercus koenigii (Fabricius) (Saeed *et al.,* 2018; Saeed and Abbas 2020), Oxycarenus hyalinipennis (Costa) (Ullah *et al.*, 2016), Musca domestica L. (Abbas *et al.*, 2015; Abbas *et al.*, 2015), Bemisia tabaci (Gennadius) (Ahmad and Akhtar 2018) and Amrasca devastans (Distant) (Saeed *et al.,* 2017).
Monitoring insecticides resistance in insect pests is an essential component of the insecticide resistance management programs (Abbas *et al.,* 2015; Jan *et al.,* 2015; Saeed *et al.,* 2017). Level of resistance varies with time and space and depends on selection history of insecticides, insect physiology and environment factors to some extent (Rosenheim and Hoy 1986). Slap dash spraying of a particular insecticide against an insect species leads to extended levels of resistance and control failures (Saeed *et al.,* 2020). It is evident that the development of resistance is inevitable but could be delayed or managed by adopting proper strategy. Different

management strategies like rotation of insecticides with dissimilar mode of action, mixtures of insecticides, and dose refuge strategy (Abbas *et al.,* 2015). Foundation of all these strategies lies mainly on the information regarding level of susceptibility of a specific insect species to chosen insecticides. Determination of the susceptibility level provides baseline data that is helpful in selection of an effective insecticide to obtain optimum level of control. It not only reduced the cost of protection but also retains the efficacy of an insecticide for longer duration (Saddiq *et al.,* 2015; Saeed *et al.,* 2017; Saeed *et al.,* 2018).

Previously, resistance to organophosphates and pyrethroids has been reported in B. brassicae (Ahmad and Aslam 2005; Ahmad and Akhtar 2013) but the information to novel mode of action insecticides is lacking.

Due to lack of baseline susceptibility data of *B. brassicae* against reduced risk new chemical insecticides such as fipronil, spiromesifen, spirotetramat, chlorantraniliprole and flonicamid, the present study was carried out screen the most effective insecticides against *B. brassicae*.

MATERIALS AND METHODS

The population of *B. brassicae* was collected from the canola plants, from different areas of Punjab Pakistan including Lodhran, Rahim Yar Khan, Multan and Bahawalpur and shifted to separate plastic jars covered with the fine mesh cloth for aeration purpose. After collection, *B. brassicae* were exposed to the insecticides in the laboratory without further rearing. Nymphs were used for examination and strain with low LC50 value was considered as a reference strain. Commercially formulated insecticides used for bioassays were: flonicamid (Ulala 50WG; ICI, Pakistan), spiromesifen (Oberon 24SE; Bayer Crop Sciences, Pakistan), chlorantraniliprole (Coragen 20SC, FMC, Pakistan), fipronil (Fipronil 5%SC; Jaffer Agro Services, Pakistan), spirotetramat (Movento 100SC; Bayer Crop Sciences, Pakistan) and emamectin benzoate (Proclaim 1.9EC; Syngenta, Pakistan). Leaf-dip bioassays on the second nymphal instar of all populations of B. brassicae were carried out (Ahmad *et al.,* 2003). Each bioassay was comprised of five concentrations having five replicates each of them and five serial dilutions were made for each insecticide tested. Fresh and cleaned leaves of cauliflower were dipped in insecticide solution for 10s and then kept for 1-1.5 hours at room temperature for air drying. After drying treated leaves were placed in Petri dishes having filter paper treated with water. One Petri dish was considered as one replicate and five nymphs were exposed in each replicate. Hence, 25 nymphs were utilized for one concentration and total of 150 individuals inclusive of control were exposed per bioassay. For control groups, fresh cauliflower leaves immersed in tap water were offered to nymphal instars in the Petri dish. The mortality rate was evaluated after 72 h of exposure. Nymphs were recorded as dead if they were unable to move after a lighter touch with a smooth brush. Mortality data were corrected with the help of Abbott's formula (Abbott 1925) and analyzed with Probit analysis with EPA Probit Analysis Program (version 1.5) (EPA 1999) to determine LC50 values and their 95% confidence intervals (CI). Resistance ratios (RR) were obtained by dividing the LC50s of experimental strains by the LC50 of a strain with the lowest LC50 for each tested insecticide .Resulting RR vales were based on (RR) = 1 indicates no resistance; RR = 2-10, tolerance; RR = 11-20, low resistance; RR = 21-50, moderate resistance; RR = 51-100, high resistance, and RR > 100, very high resistance (Ahmad *et al.,* 2007).

RESULTS

Population collected from Bahawalpur showed tolerance with a 2.11-fold resistance against fipronil, whereas the populations from Multan (1.64-fold), Rahim Yar Khan (1.54-fold), and Lodhran (1.00-fold) showed no resistance to fipronil compared with the susceptible strain (Table 1). The populations collected from Rahim Yar Khan, Lodhran, Multan and Bahawalpur showed no resistance with a RR of (1.26), (1.20), (1.11) and (1.00), respectively, against spirotetramat (Table 1).The population collected from Lodhran and Multan showed tolerance with (2.36-fold and 2.25-fold, respectively. While populations collected from Rahim Yar Khan and Bahawalpur showed no resistance against emamectin benzoate with RR of 1.86 and 1.00 (Table 1). The population collected from Bahawalpur showed tolerance against spiromesifen with the RR of (2.83). While the populations collected from Lodhran, Rahim Yar Khan and Multan showed no resistance with RR of (1.94), (1.69) and (1.00), respectively (Table 1).The populations collected from Lodhran, Multan, Rahim Yar Khan and Bahawalpur showed no resistance with (1.49), (1.13), (1.06) and (1.00) resistance ratios, respectively, to chlorantraniliprole (Table 1).The populations collected from Lodhran, Multan, Rahim Yar Khan and Bahawalpur showed no resistance to flonicamid (Table 1).

DISCUSSION

Different new chemical insecticides were used to assess the level of resistance on B. brassicae collected from four locations of southern Punjab such as Multan, Lodhran, Rahim Yar Khan, and Bahawalpur. The result of our study showed that there was no to very low resistance (tolerance) in B. brassicae populations collected from different localities. However, insects should not be assumed resistant until ten-fold of resistance is observed (Valles *et al.,* 1997). Our results revealed less than 10-fold resistance to all tested insecticides in all tested populations. Yet, the susceptibility to new

Table 1 Toxicity and susceptibility to selected insecticides in *Brevicoryne brassicae* populations

Insecticide	Location	N	Slope ± SE	X2	Df	P	LC_{50} [95% CI] (ppm)	RR
Fipronil	Lodhran	25	1.41 ± 0.30	0.74	4	0.94	3.55 (1.883-5.291)	1.00
	RYK	25	1.58 ± 0.30	0.30	4	0.99	5.45 (3.575-7.755)	1.54
	Multan	25	1.25 ± 0.28	0.34	4	0.98	5.84 (3.408-9.092)	1.64
	Bahawalpur	25	0.95 ± 0.27	0.63	4	0.95	7.50 (0.421-1.477)	2.11
Emamectin benzoate	Bahawalpur	25	1.16 ± 0.29	0.64	4	0.96	2.77 (0.958-4.576)	1.00
	RYK	25	1.20 ± 0.29	0.47	4	0.97	5.15 (2.771-8.070)	1.86
	Multan	25	1.32 ± 0.29	2.06	4	0.72	6.22 (3.815-9.492)	2.25
	Lodhran	25	1.36 ± 0.29	1.02	4	0.90	6.53 (4.110-9.894)	2.36
Spirotetramat	Bahawalpur	25	1.61 ± 0.31	1.27	4	0.86	4.13 (2.57-5.857)	1.00
	Multan	25	1.05 ± 0.27	0.48	4	0.97	4.57 (2.068-7.582)	1.11
	Lodhran	25	1.46 ± 0.30	0.78	4	0.94	4.95 (3.044-7.201)	1.20
	RYK	25	1.10 ± 0.28	0.64	4	0.96	5.19 (2.617-8.462)	1.26
Spiromesifen	Multan	25	1.25 ± 0.46	0.68	4	0.95	3.25 (0.01-8.99)	1.00
	RYK	25	0.85 ± 0.33	0.13	4	1.00	5.52 (0.01-15.40)	1.70
	Lodhran	25	1.09 ± 0.35	0.69	4	0.95	6.32 (0.27-14.45)	1.94
	Bahawalpur	25	1.31 ± 0.36	1.50	4	0.82	9.24 (1.60-17.20)	2.83
Chlorantraniliprole	Bahawalpur	25	1.46 ± 0.67	0.65	4	0.95	1.85 (0.00-6.06)	1.00
	RYK	25	1.03 ± 0.41	1.36	4	0.85	1.96 (0.00-6.91)	1.06
	Multan	25	1.02 ± 0.40	0.29	4	0.99	2.09 (0.00-7.20)	1.13
	Lodhran	25	1.09 ± 0.40	1.23	4	0.87	2.75 (0.01-8.03)	1.49
Flonicamid	Bahawalpur	25	1.08 ± 0.47	0.67	4	0.95	4.10 (0.00-15.90)	1.00
	RYK	25	1.46 ± 0.67	0.65	4	0.95	4.63 (0.00-13.13)	1.13
	Multan	25	1.25 ± 0.46	0.68	4	0.95	6.75 (0.024-18.70)	1.65
	Lodhran	25	1.18 ± 0.44	1.21	4	0.88	7.02 (0.01-20.06)	1.71

RR, resistance ratio calculated as LC_{50} of field population/LC_{50} of susceptible population.

chemistry pesticides in B. brassicae is described here for the first time from the selected areas of southern Punjab.

Fipronil is a member of phenylparazole group with GABA-gated chloride channel blocker action and emamectin benzoate is a glutamate-gated chloride channel allosteric modulator (IRAC 2020). These insecticides have safe biological profile and are being used widely for the management of various insect pests. In this study, susceptibility to tolerance to fipronil and emamectin benzoate was observed in B. brassicae populations. Varying level of fipronil resistance has previously been documented in different pests such as susceptibility to very low resistance in A. devastans (Abbas *et al.,* 2018), M. domestica (Abbas *et al.,* 2015b), very high resistance in P. xylostella (Wang *et al.,* 2016), high resistance in Sogatella furcifera (Horváth) (Tang *et al.,* 2010), and low to very high resistance in Spodoptera litura (Fabricius) (Ahmad *et al.,* 2008)

Susceptibility to very high resistance to emamectin benzoate has been documented in T. tabaci (Lebedev *et al.,* 2013), *B. brassicae* (Ahmad and Akhtar 2013), *S. litura* (Ahmad and Gull 2017), M. domestica (Abbas *et al.*, 2015b) and S. exigua

Spirotetramat and spiromesifen are acetyl CoA carboxylase inhibitor insecticides (IRAC 2020) and recent widely applied insecticides for the control of aphids, cotton bugs, and whiteflies. These insecticides have shown safe biological profile against natural enemies, which revealed that these insecticides are better option for the control of different phloem feeding insect pests (Nauen and Schnorbach 2005). In this study, no resistance to spirotetramat was observed in the tested B. brassicae populations. Low to high level of resistance against spirotetramat has recently been reported in A. devastans (Abbas *et al.,* 2018), B. tabaci (Peng *et al.,* 2017), A. gossypii (Pan *et al.,* 2017) and P. solenopsis (Ejaz and Shad 2017). Resistance to spiromesifen has been documented in B. tabaci (Prabhaker *et al.,* 2008), Trialeurodes vaporariorum Westwood (Karatolos *et al.,* 2012), Neoseiulus californicus, Tetranychus urticae Koch (Sato *et al.,* 2016).

Flonicamid is a chordotonal organ modulator (IRAC 2020) and widely usedinsecticide for sucking pests such as B. tabaci, A. devastans, T. tabaci, B. brassicae, A. gossypii and D. koenigii in Pakistan since 2014. In current study, no resistance to flonicamid was observed in all the tested B. brassicae populations. Similarly, susceptibility/no resistance to flonicamid has been reported in A. devastans (Abbas *et al.,* 2018), B. tabaci (Roditakis *et al.,* 2014), and A. gossypii. Chlorantraniliprole is a member of diamide group with ryanodine receptor modulators (IRAC 2020) and widely used for lepidopteran pests. In current study, no resistance to chlorantrniliprole was observed in all the tested B. brassicae populations. Susceptibility to high resistance to chlorantraniliprole has been detected in M. domestica (Shah and Shad 2020), Helicoverpa punctigera (Wallengren) (Bird and Walker 2019), Choristoneura rosaceana (Harris) (Sial and Brunner 2012), and S. exigua (Lai *et al.,* 2011; Lai and Su 2011).

In the current research, no resistance found against spiromesifen, flonicamid and chlorantraniliprole. While no resistance to a very low level of resistance found against spiromesifen, fipronil and emamectin benzoate in B. brassicae collected from four different localities. In Pakistan, new chemicals are very effective insecticides for encountering sucking insect pests such as cotton mealy-bug, thrips, aphids, plant hoppers and whiteflies (Abbas *et al.,* 2018). Consequently, the cause for no resistance against spiromesifen, flonicamid and chlorantraniliprole may be due to lesser application of these insecticides at these localities. However, present results revealed that the RR values of these insecticides have proved their effectiveness against this pest.

It is necessary to develop efficient management plans as soon as possible for delaying further resistance development resulting in failure of products. Resistance to different insecticides with different modes of action revealed that the phenomenon of cross-resistance or multiple-resistance may also be present in the tested populations of B. brassicae However, further studies are needed to confirm these findings and to design effective management plans. Developing countries, such as Pakistan, have issues like insecticide resistance due to the indiscriminate use of insecticides for control of different insect pests of agricultural or medical importance (Abbas *et al.*, 2015b; Saeed *et al.*, 2018). There are many problems including improper use of pesticides with incorrect dosage, the use of low-quality pesticide formulations and the use of inappropriate application methods. The appropriate use of insecticides may decrease the severity of resistance and control failures of B. brassicae management on canola crops in Punjab, Pakistan. Restricted use of insecticides to which resistance has developed, insecticide mixtures and rotation of insecticides with unrelated modes of action could be helpful for resistance management in sucking insects (Basit *et al.*, 2013; Abbas *et al.,* 2015a). In addition, standard resistance monitoring activities should be planned that would help to identify the effectiveness of insecticides for sucking insect management. IPM also includes the use of cultural practices, economic thresholds, and preservation of natural enemies**.**

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