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INSECTICIDES RESISTANCE IN THE FIELD SELECTED STRAINS OF *BACTROCERA ZONATA* (SAUNDERS) (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Seven insecticides *i.e*., trichlorfon, malathion bifenthrin, lambda-cyhalothrin, spinosad, cypermethrin and chlorpyrifos were studied for resistance against three field strains *viz*., M_1, M_2 and SWL derived from twenty nine field populations of *B. zonata.* Results showed that high resistance ratios were found towards trichlorfon (80.81-, 35.91- and 69.92-fold), while moderate resistance ratios were recorded to malathion and chlorpyrifos in three selected strains (28.15-, 20.96-, 27.22 and 25.52-, 17.79-, 24.81-fold). The selected strains (M_1, M_2 and SWL) exhibited low to moderate resistance ratios against pyrethroids (Bifenthrin, lambda-cyhalothrin, cypermethrin) and microbial insecticide (Spinosad). It is concluded that the three field selected strains had developed more resistance against organophospahtes (Trichlorfon, malathion and chlorpyrifos) as compared to pyrethroids (Bifenthrin, lambda-cyhalothrin and cypermethrin) and microbial insecticide (Spinosad).

Key words: *Bactrocera zonata*, organophosphates, pyrethroids, microbial, resistance.

INTRODUCTION

Fruit fly is a serious pest of fruit and vegetables in Pakistan. The losses reported about seven billion Pakistani rupees at the farmer level (Stonehouse *et al*., 1997). There are about 44 species belong to genus *Bactrocera* (Syed, 1969; Kapoor *et al*., 1980) among 250 species of family Tephritidae, which are distributed in temperate, tropical and sub-tropical regions of the world (Robinson and Hopper, 1989). About 11 species of fruit flies have been recorded from Pakistan and the most prominent among them are *Bactrocera zonata, B. cucrbitae* and *B. dorsalis* (Abdullah and Latif, 2001; Abdullah *et al.*, 2002; Stonehouse *et al.*, 2002). *B. zonata* has been reported as most prevailing species in mango, guava, apricot, peach, plum, melon, pear, jujube, persimmon and cucurbit in Pakistan (Anonymous, 2008).

The cover spray of different insecticides against fruit flies is extensively used in Pakistan and its applications are increasing day by day owing to quick controlling effect against this pest (Stonehouse *et al.*, 1997). The extensive use of insecticides, number of generation and plenty of host plants of this pest leads to develop insecticides resistance towards commonly used insecticides against them all over the world. Hsu and Feng (2000) carried out laboratory tests against *Bactrocera dorsalis* to determine the toxicity of five insecticides *i.e.*, fenitrothion, malathion, fenthion, naled and trichlorfon. The results showed the significant increase in the level of LD_{50} (1.9-4.3 folds) for all the tested insecticides except trichlorfon. Zhang *et al*. (2007) worked to monitor the resistance ratio in 11 strains of *B. dorsalis* in South China against three insecticides trichlorfon, abamectin and alphamethrin. Results revealed that moderate resistance ratio was developed in five strains and low resistance ratio in four strains, while two strains were remained susceptible against trichlorfon. The alphamethrin bioassay was recorded as moderate resistant level in three strains and low level of resistance in four strains, while four strains were remained susceptible. Ahmad *et al.* (2010) conducted a study to evaluate the insecticides resistance level in two strains of *B. zonata* in Punjab, Pakistan. They found that *B. zonata* strains from Multan and Faisalabad were observed as low to moderate resistant ratios towards trichlorfon, lambda-cyhalothrin, malathion and bifenthrin. In 2011, Haider *et al*. studied the resistance level to 10 insecticides *viz*., Talstar, Confidor, Curacron, deltamethrin, Dipterex, Proclaim, Karate, malathion, Tracer and Steward against field population of *B. zonata* collected from Multan, Pakistan. They reported that high level of resistance was observed in Dipterex (65.32-fold), while moderate resistance level to Curacron (13.20-fold) against population of *B. zonata*. Low resistance level was observed to Confidor, Talstar, Karate, malathion and deltamethrin, whereas, Tracer and Steward was remained susceptible against *B. zonata* populations.

The development of different insecticides resistance against *B. zonata* field populations in Pakistan was reported from above narrated discussion. To understand the mechanism of insecticides resistance and its

management against *B. zonata* populations, it is necessary to study the level of resistance to different commonly used insecticides against the field selected strains of *B. zonata*.

MATERIALS AND METHODS

Susceptible strain

Susceptible strain of *B. zonata* was used in the study as mentioned by Nadeem *et al.*, (2012).

Specimen collection of *B. zonata*

The specimens of full grown larvae of fruit fly, *Bactrocera zonata*, were collected from infested mangoes and guava fruits from fifteen sites of mango orchards of tehsil Multan and fourteen locations of guava orchards areas of tehsil Chichawatni, District Sahiwal (Nadeem *et al.*, 2012 and 2014). After insecticides bioassay among the twenty nine field populations of Multan and Sahiwal areas, the populations which showed resistance to different insecticides with similar trend of LC_{50} and over lapping fiducial limits were pooled together and permitted them to interbreed freely in rearing cages. Three categories were made from the twenty nine field populations and selected two from Multan area and one from Sahiwal area and named as M_1, M_2 and SWL strains, respectively, reared, bioassay of insecticides, their configuration of LC_{50} and over lapping fiducial limits (95%) were taken according to standard procedures as described by Nadeem *et al.*, (2012.

M_1 strain

Populations of the sites of Alam Pur, Bosan, Nawab Pur, Mohammad Pur, Multani wala, Kotla Maharan and Qadir Pur Ran that showed resistance from moderate to high category towards insecticides *viz*., bifenthrin, lambda-cyhalothrin, spinosad, malathion and trichlorfon with overlapping fiducial limits and almost similar trend in LC_{50} were selected. These selected populations were pooled together in a separate rearing cage and allowed them to breed freely further and named as M_1 strain and reared as referred above.

M_2 strain

Out of fifteen populations, the four populations of sites *i.e*., Sher Shah, Tara Garh, Khokhran and 5-Faiz who had showed resistant to five insecticides (Bifenthrin, lambda-cyhalothrin, spinosad, malathion and trichlorfon) with low to moderate resistance. Then, these similar behavioral populations were pooled together and permitted them to breed into a separate rearing cage and named as M_2 strain and reared as referred above.

SWL strain

The populations of 105/7R, 111/7R, 113/12L, 6/14L, 168/9L, 31/11L, 174/9L, 15/11L and 184/9L from Sahiwal area which showed moderate to high resistance after assayed to bifenthrin, lambda-cyhalothrin, spinosad, malathion and trichlorfon. These populations were then pooled together in a separate cage in rearing laboratory and named as SWL strain and reared as referred above.

Insecticides

The technical grade of five insecticides were selected for present study which included trichlorfon, bifenthrin lambda-cyhalothrin, malathion and spinosad the purity specification and source as described in Nadeem *et al.*, (2012) to estimate the LC_{50} of selected resistant strains (M_1, M_2 and SWL). To check the stability of resistance in selected strains, two more technical grade insecticides organophosphate and pyrethorid *i.e.*, chlorpyrifos (97% Purity, Jiangsu Huangma, CHINA) and cypermethrin (94% Purity, Jiangsu Huangma, CHINA) were also included in the bioassay process. The technical grade of above mentioned insecticides were used in different concentration dissolved in acetone (98%, Riedel -de Haën®, Germany).

Bioassay of insecticides

The procedure of bioassay of insecticides against selected resistant strains of *B. zonata i.e*., M_1, M_2 and SWL carried to determine the level of resistance as referred above.

Statistical analysis

The data was analyzed statistically. LC_{50} was determined through probit analysis (Finney, 1971) and resistance ratios (RRs) were fallowed as described by Torres-Vila *et al.* (2002).

RESULTS

Susceptible baseline values:

LC_{50} values of trichlorfon, bifenthrin, malathion, methomyl, lambda-cyhalothrin and spinosad in the susceptible strain of were used to determine the resistance level in field selected strains of *B. zonata* (Table 1).

Table 1. Baseline LC_{50} for the susceptible strain of *B. zonata*.

Insecticides	LC_{50} (μgmL^{-1})	Reference
Trichlorfon	2.38	Nadeem *et al*. (2012)
Malathion	4.96	
Bifenthrin	2.58	
Lambda-cyhalothrin	2.42	
Spinosad	3.69	
Cypermethrin	2.35	Determined by bioassay against susceptible strain in laboratory which were obtained from NIA, Tandojam.
Chlorpyrifos	3.86	

Insecticides resistance in the selected strains of *B. zonata*

Selected resistant strains (M_1, M_2 and SWL) of *B. zonata* showed variation in their resistance ratios when treated with seven insecticides *i.e.*, trichlorfon, malathion, bifenthrin, lambda-cyhalothrin, spinosad, cypermethrin and chlorpyrifos (Table 2).

M_1, M_2 and SWL strains showed high level of resistance to trichlorfon (80.81-, 35.91- and 69.92-fold), while other tested organophosphates insecticides *i.e.*, malathion and chlorpyrifos were observed as moderate level of resistance (28.15-, 20.96-, 27.22-fold and 25.52-, 17.79- and 24.81-fold).

In case of pyrethorids (Bifenthrin, lambda-cyhalothrin and cypermethrin) assay to M_1 and SWL strains showed no variation in their resistance ratios. Bifenthrin, lambda-cyhalothrin and cypermethrin were proved moderately resistant having resistance ratios as 19.67-, 17.52- and 19.33-fold against M_1 strain, while 17.84-, 13.45- and 18.07-fold in SWL strain, respectively. In M_2 strain, bifenthrin, lambda-cyhalothrin and cypermethrin showed low resistance level (8.73-, 9.01- and 9.79-fold). Effect of spinosad was observed in low resistance category with resistance ratios of 14.59-, 12.12- and 10.57-fold in M_1, M_2 and SWL strains correspondingly.

DISCUSSION

Results obtained from selected field resistant strains (M_1, M_2 and SWL strains) of *B. zonata* showed high resistance level toward trichlorfon to 80.81-, 35.91- and 69.92-fold. These findings are consistent with the work of Jin *et al*. (2010), who investigated insecticide resistance to three insecticides *viz*., trichlorfon, *β*-cypermethrin and avermectin against 25 strains of *B. dorsalis* and found high resistance level (70.4-fold) to trichlorfon. Results of present study are in the line to the results reported by Hsu *et al.* (2004) who found Malathion as moderate resistance (14.7-fold) against *B. dorsalis* to Malathion. Our results are at par with the findings of Haider *et al*. (2011), who reported that trichlorfon gave high resistance ratio (65.32-fold) against field populations of *B. zonata* tested at Multan, Pakistan.

Results about malathion against M_1, M_2 and SWL strains were contradictory to the findings of Haider *et al*. (2011) who reported low level of resistant (5.54-fold) to malathion, whereas, we recorded moderate level of resistance (28.15-, 20.96-, 27.22-fold) towards malathion against *B. zonata.* This difference may be due to high exposure of malathion against *B. zonata* in the specimen collection area.

Virtually there is no comparable reference about chlorpyrifos resistance in the literature about fruit flies in Pakistan. Present findings revealed that M_1, M_2 and SWL strains had moderate resistance level (25.52-, 17.79- and 24.81-fold) to chlorpyrifos. These results may be attained due to the behavior of adult fruit flies who visited to the other sprayed crops for nectars as their food and that crop might be sprayed with chlorpyrifos. Dong *et al*. (2002) assayed the toxicity of ten different insecticides against melon fruit fly and reported that malathion, dimethoate,

diazinon, chlorpyrifos, fenitrothion, fenthion and deltamethrin gave 100% mortality of fruit flies four hours post treatment and findings about chlorpyrifos are contradictory to our results, which may be due to area difference.

Bifenthrin, lambda-cyhalothrin and cypermethrin showed low to moderate level of resistance with resistance ratio ranged from 8.73- to 19.87-fold in M_1 M_2 and SWL strains. In case of bifenthrin and lambda-cyhalothrin, our results are partially agreed to the work reported by Ahmad *et al*. (2010), who observed that bifenthrin and lambda-cyhalothrin showed low resistance level against *B. zonata* but we found that resistance ratios extended low to moderate in M_1, M_2 and SWL strains of *B. zonata.* In case of cypermethrin, there is no direct study available in literature to compare resistance in fruit flies in Pakistan, whereas, results of cypermethrin showed similar trend as that of bifenthrin in M_1, M_2 and SWL strains. Results about cypermethrin are conflicting to the results reported by Kalia (1995) who found tested four insecticides *viz*., endosulfan, cypermethrin, methyl parathion and monocrotophos against *B. dorsalis* and found cypermethrin and methyl parathion as the most effective followed by monocrotophos and endosulfan on the basis of mortality after 24 hours treatments.

Findings about assay of spinosad against M_1, M_2 and SWL strains showed moderate level of resistant with 14.59-, 12.12- and 10.57-fold resistance ratios, respectively, are agreed to the work reported by Moustafa *et al*. (2009) who studied the efficacy of malathion, lefenuron and spinosad against *B. oleae* under field condition were found that lufenuron was the most effective followed by spinosad and malathion against *B. oleae.* Ahmad *et al.* (2010) reported that insecticides resistance level in two strains of *B. zonata* from Multan and Faisalabad remained susceptible to spinosad are inconsistent to our results.

Table 2. Effect of insecticides on the adult populations of selected strains of *B. zonata*.

Strain	Insecticides	LC_{50} (μgmL^{-1})	FL (95%)	Fit of Probit Line			RR*
				Slope ± SE	χ^2	H	
M_1	Trichlorfon	192.34	162.20-221.26	2.78 ± 0.36	3.39	1.13	80.81
	Malathion	139.67	119.84-158.53	3.41 ± 0.39	2.32	0.77	28.15
	Bifenthrin	51.29	31.88-72.80	2.06 ± 0.25	3.50	1.16	19.67
	Lambda-cyhalothrin	42.40	24.94-60.10	2.26 ± 0.28	4.24	1.41	17.52
	Spinosad	53.84	42.78-65.65	2.06 ± 0.25	1.90	0.63	14.59
	Cypermethrin	45.43	37.40-54.45	2.28 ± 0.25	2.46	0.82	19.33
	Chlorpyrifos	98.54	73.49-125.28	1.63 ± 0.23	0.86	0.28	25.52
M_2	Trichlorfon	85.48	72.34-98.42	2.79 ± 0.37	2.86	0.95	35.91
	Malathion	104.01	88.06-119.51	2..86 ± 0.37	2.98	0.99	20.96
	Bifenthrin	22.54	17.60-28.08	1.82 ± 0.22	0.13	0.04	8.73
	Lambda-cyhalothrin	24.81	18.32-31.85	1.57 ± 0.22	2.40	0.80	9.01
	Spinosad	44.75	25.96-64.93	2.02 ± 0.26	3.92	1.30	12.12
	Cypermethrin	23.10	17.96-28.59	1.93 ± 0.24	2.30	0.76	9.79
	Chlorpyrifos	68.68	58.85-78.67	2.89 ± 0.37	1.88	0.62	17.79
SWL	Trichlorfon	166.41	140.89-191.22	2.86 ± 0.37	2.98	0.99	69.92
	Malathion	135.02	77.77-185.02	2.87 ± 0.37	5.72	1.90	27.22
	Bifenthrin	46.05	34.32-58.23	2.06 ± 0.38	2.58	1.19	17.84
	Lambda-cyhalothrin	32.57	27.71-37.38	2.86 ± 0.37	2.03	0.67	13.45
	Spinosad	39.03	33.14-44.85	2.83 ± 0.37	2.07	0.69	10.57
	Cypermethrin	42.47	36.04-49.20	2.76 ± 0.37	2.47	0.82	18.07
	Chlorpyrifos	95.79	66.41-124.15	2.71 ± 0.37	5.77	1.92	24.81

LC_{50} (Lethal concentration); FL (Fiducial limit); ± SE (Standard Error); H: Heterogeneity, RR* (Resistance Ratio) LC_{50} of field selected strain/LC_{50} of susceptible strain.

CONCLUSIONS

From the above results, it is concluded that trichlorfon had adopted high resistance in M_1, M_2 and SWL strains, while malathion and chlorpyrifos showed moderate resistance. The selected strains M_1, M_2 and SWL were found low to moderate resistance when treated to bifenthrin, lambda-cyhalothrin, cypermethrin and spinosad.

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