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Evaluation of Resistance to Different Insecticides Against Field Populations of *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) in Multan, Pakistan*

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Abstract.- *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious pest of fruits and vegetables in Pakistan. The cover sprays against control of fruit flies are being extensively used in Pakistan. For evaluation of resistance to insecticides LC_{50} of six commonly used insecticide trichlorfon, bifenthrin, malathion, methomyl, λ-cyhalothrin and spinosad was determined in fifteen field populations of *B. zonata* in Multan, Pakistan. Susceptible to high resistance was recorded for trichlorfon (1.00- fold to 41.82-fold). Susceptible to moderate resistance level was observed for bifenthrin (1.39 to 13.59-fold), cyhalothrin (1.07 to 18.24 fold), spinosad (1.15 to 14.08-fold) and for malathion (1.07 to 19.36-fold) while the fly was susceptible to methomyl. The results suggest that *B. zonata* has developed resistance to trichlorfon, malathion, bifenthrin, λ-cyhalothrin and spinosad and there is need of resistance management programs for restoring the efficacy of insecticides based control measures.

Key words: *Bactrocera zonata,* insecticides resistance.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are important insect pest of vegetables and fruits in Pakistan. Besides annual estimated loss of $200 million, rejection of export commodity especially mangoes has further devastated the conditions due to quarantine issues of this pest from developed countries. Aesthetic value of guava, bitter gourd and other vegetables restrict their purchase due to presence of maggots and hence this monitary deficit is usually not added to post harvest losses (Stonehouse *et al.,* 1997; Abdullah *et al.,* 2002; Latif, 2004). Eleven species of fruit flies have been traced from Pakistan, among the most frequently present in field are *Bactrocera zonata, B. cucurbitae* and *B. dorsalis* (Abdullah and Latif, 2001; Abdullah *et al.,* 2002; Stonehouse *et al.,* 2002) present on mango, guava, ber, apple, bitter gourd, snak gourd and musk melon (Khan and Musakhel, 1999; Sultan *et al*., 2000; Khan *et al.,* 2005). *B. zonata* has proved a serious pest of guava, mango and citrus orchards with an estimated 50-55% infestation only in guava fruits in Pakistan (Syed, 1970). The presences of male adults of *B. zonata* in traps in orchards of mango and guava observed as 74.66 and 46.62% respectively, in Pakistan (Khan *et al*., 2005).

Farmers mostly rely on cover sprays for the management of fruit fly with least success in its control. The cover sprays against fruit flies are being extensively used in Pakistan and this application of insecticide is increasing day by day (Stonehouse *et al.,* 1997). Endrin, dialordin, dipterex (trichlorfon), dimecron, diazinon and malathion has been applied on mango orchards as a cover spray in Pakistan (Panhwar, 2005). Resistance to insecticides has been attributed to selection pressure, fruit flies experience during life time. Owing to number of generation and plenty of host plants, fruit flies are not exception to this pressure. The development of insecticide resistance has been reported in different Tephritid insect pests all over the world. It has been observed that laboratory and field strains of *B. dorsalis* and *B. cucurbitae* has developed resistance to DDT and methoxychlor in Hawaii but remained susceptible to malathion during 1950-1960, while *Ceratitis capitata* (Wiedemann) also remained as susceptible to the tested insecticides (Keiser, 1989). In Taiwan

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toxicity of seven insecticides (OPs, methomyl and pyrethroids) was assayed in laboratory with population of *B. dorsalis* (Hendel) and resistance were observed among all the tested insecticides with different ratios (Hsu and Feng, 2000). A survey of susceptibility level of insecticides in two strains of *B. zonata* from Multan and Faisalabad in Punjab, Pakistan revealed that strains of *B. zonata* were found to be resistant against trichlorfon, λ-cyhalothrin, bifenthrin and malathion (3-19 fold) while susceptible to spinosad by fruit dip assay. Trichlorfon showed the highest resistance level (10-19 fold) followed by bifenthrin (8-11 fold), λ-cyhalothrin (4-9 fold) and malathion (3-6 fold) (Ahmad *et al.*, 2010). Keeping in the view the development of resistance in fruit fly, present study was conducted to evaluate the level of resistance against insecticides in the adult field populations of *B. zonata* from Multan, Punjab Pakistan.

MATERIALS AND METHODS

Specimens collection of fruit fly

The specimens of full grown larvae of fruit fly, *B. zonata*, from infested mangoes were collected from Alam Pur, Bosan, Nawab Pur, Mohammad Pur, Sher Shah, Tara Garh, Khokhran, 5-Faiz, Lar, 11-MR, 12-MR, 10-T, Multani Wala, Kotla Mahran, Qadir Pur Ran, Multan, Punjab during the year 2008. The collected specimens with infested fruits were put into plastic jar provided with sand in the bottom and marked the specific population name for pupal emergence.

Rearing of fruit fly

Fruit fly was reared under laboratory conditions at Department of Agricultural Entomology, University of Agriculture, Faisalabad under controlled temperature (26±2^{o}C) and relative humidity (60±5%) with a photoperiod of 12:12 (L:D). Healthy guava, mango and peach fruits were offered to the adult flies confined in the cages for their egg laying. Infested fruits were kept in a plastic jars having sterilized sand until pupation. The pupae were taken out from the sand and shifted into separate adult rearing cages of 30 ×30 ×30cm dimensions. The different sides of cages were furnished with iron wire covered with mesh cloth. The adult flies were offered artificial adult food containing sugar mixed with hydrolysate (3:1 w/w), source of water was provided in the form of wet cotton.

Susceptible strain

Culture of susceptible strain of *B. zonata* was obtained from fruit fly rearing labs. of Nuclear Institute of Agriculture (NIA), Tandojam for comparative study which was established for last 16 years without exposing any insecticides. The culture of this susceptible strain was maintained in same laboratory under conditions as maintained for rearing of field collected strains.

Selection of insecticides

Six insecticides were selected for present research trial includes trichlorfon (98% purity, Jiangsu Anpon, China) malathion (95%, Jiangsu Huangma Agrochemicals, China), bifenthrin (95%, Agro-Care Chemical, China), λ-cyhalothrin (95%, Jiangsu Huangma Agrochemicals, China), methomyl (98%, Agro-Care Chemical, China) and spinosad (93% Shenzhen Crop Star Chemical, China) technical grade was used in different concentration dissolved in acetone (98%, Riedel -de Haën®, Germany). Trichlorfon and malathion were selected being commonly using insecticides for the control of fruit flies and remaining were exclusively in use to other crops in areas of specimen collection.

Bioassay of insecticides

The resistance of fruit flies against different insecticides was determined by topical application (Anonymous 1979). A total of 50 adult flies (3-5 days old) were treated with different concentrations of insecticides in acetone. The adult flies were anaesthetized with CO_2 for 10-15 seconds before application of insecticides. For each treatment, 1 µl of insecticide solution was applied on the pro-notum of equal numbers of male and female with help of Burkard micro-applicator. Tested flies were shifted into paper cups of 250 ml capacity, top covered with muslin cloth and fed diet sugar, hydrolysate and water (4:1:5 w/w) soaked in a small piece of cotton. All treated flies were maintained under standard laboratory conditions. Control flies were treated with acetone only.

Statistical analysis of data

Mortality data against each insecticide with respective concentration treatment was recorded after 24 hours and analyzed by Probit analysis (Finney, 1971) using the software POLO-PC (LeOra Software, 1987). LC_{50} value of field populations and susceptible strain was compared to determine the ratio of resistance. The resistance factors were evaluated by dividing LC_{50} values of field strains to LC_{50} value of susceptible strain. Resistance factor (RFs) was followed as described by Torres-Vila *et al.* (2002): Susceptibility (RF=1), low resistance (RF= 2-10), moderate resistance (RF= 11-30), high resistance (RF= 31-100) and very high resistance (RF >100).

RESULTS AND DISCUSSION

The present study carried out to evaluate the resistance ratio of six insecticides *viz.*, trichlorfon, bifenthrin, malathion, methomyl, lamda-cyhalothrin and spinosad against fifteen field populations of *B. zonata*.

Fig. 1. Baseline LC_{50} values of insecticides for susceptible strain.

LC_{50} of insecticides for susceptible strain

Baseline LC_{50} values of trichrofon, bifenthrin, malathion, methomyl, λ-cyhalothrin and spinosad against susceptible strain of *B. zonata* are shown in Figure 1. Malathion showed the lowest activity (LC_{50}, 4.96μgmL^{-1}) followed by in methomyl (3.88 μgmL^{-1}), spinosad (3.69 μgmL^{-1}), bifenthrin (2.58μgmL^{-1}), λ-cyhalothrin (2.42μgmL^{-1}) and trichlorfon (2.38μgmL^{-1}) against susceptible strain of *B. zonata*.

Resistance to insecticides

Resistance ratios of *B. zonata* collected from 15 different locations of Multan are shown in Table I.

Trichlorfon

For trichlorfon high resistance ratio LC_{50} ranged from 99.55 to 83.30 μgmL^{-1} was observed in the order Qadir Pur Ran (41.82-fold) > Mohammad Pur > 10-T > Kotla Mahran > Bosan > Alam Pur > Multani wala > Nawab Pur. The moderate resistance ratio with LC_{50} ranging from 44.87 to 41.49μgmL^{-1} followed the order: Sher Shah > Khokhran, 5-Faiz > Tara Garh strain. Populations from 12-MR, 11-MR and Lar were recorded as susceptible to trichlorfon.

The range of resistance (1-41.82 fold) in present study are at par with the findings of Zhang *et al.* (2007) who reported moderate resistance ratio (16.42- 27.42-fold) in 5 strains and low resistance ratio (4.33-8.04-fold) in 4 strains of *B. dorsalis* to trichlorfon in South China. Ahmad *et al.* (2010) reported low to moderate resistance ratios to trichlorfon. Jin *et al.* (2010) reported 70.4-fold resistance ratio to trichlorfon against *B. dorsalis* in China.

Bifenthrin

Moderate resistance ratio as shown by LC_{50} ranged from 35.08 to 26.07μgmL^{-1} in the order Qadir Pur Ran > 10-T > Multani wala > Mohammad Pur > Alam Pur > Kotla Mahran > Bosan > Nawab Pur. Low resistance ratio with LC_{50} from 14.66 to 13.37μgmL^{-1} was recorded in Sher Shah (5.68-fold) followed by Khokhran > Tara Garh > 5-Faiz. Populations from 12-MR, 11-MR and Lar were found susceptible to bifenthrin. Our results partially agreed to Oke (2002).

Malathion

Malathion has also shown variation in resistance factors (1.07-fold to 19.36-fold) when treated with different populations of *B. zonata* (Table I). Moderate resistance ratio with LC_{50} ranged from 96.06-76.66μgmL^{-1} in the order Qadir Pur Ran > 10-T > Bosan > Mohammad Pur > Kotla Mahran > Multani wala > Alam Pur > Nawab Pur. Strains of Sher Shah > Khokhran > 5-Faiz > Tara Garh had low resistance ratio with LC_{50} ranging from 44.58 to 38.16μgmL^{-1}, while populations from

Table I.- **Toxicity of different insecticides on the adult field populations of *B. zonata*.**

Population	LC_{50} (μgmL^{-1}) FL (95%)	Fit of Probit Line		RR*
		Slope±SE	χ^2	
	Trichlorfon			
Alam Pur	85.75 (54.84-128.56)	1.96± 0.24	3.98	36.02
Bosan	86.19 (67.51-108.64)	1.69± 0.23	1.27	36.21
Nawab Pur	83.30 (67.57-101.59)	2.03± 0.25	0.72	35.00
Mohammad Pur	90.86 (72.52-113.14)	1.82± 0.23	2.38	38.17
Sher Shah	44.87 (36.07-54.03)	2.07± 0.30	1.72	18.85
Tara Garh	41.49 (31.85-51.07)	1.84± 0.29	2.46	17.43
Khokhran	43.10 (34.32-52.02)	2.05± 0.30	1.76	18.10
5-Faiz	42.22 (32.46-51.98)	1.83± 0.29	0.71	17.73
Lar	2.40 (1.51-3.47)	2.65± 0.28	5.31	1.00
11-MR	2.47 (1.92-3.08)	2.82± 0.29	4.07	1.03
12-MR	2.60 (1.91-3.41)	2.74± 0.29	3.13	1.09
10-T	90.79 (59.38-136.23)	1.97± 0.24	3.89	38.14
Multani wala	84.42 (56.44-122.03)	2.07± 0.24	3.68	35.47
Kotla Maharan	89.65 (55.34-140.64)	1.95± 0.24	4.58	37.66
Qadir Pur Ran	99.55 (67.92-147.00)	1.98± 0.24	3.47	41.82
	Bifenthrin			
Alam Pur	28.84 (23.02-35.43)	1.98± 0.23	1.31	11.17
Bosan	26.76 (21.02-33.14)	1.89± 0.23	1.62	10.37
Nawab Pur	26.07 (19.92-32.81)	1.73± 0.22	0.39	10.10
Mohammad Pur	29.15 (23.63-35.41)	2.13± 0.24	0.69	11.29
Sher Shah	14.66 (11.48-18.10)	1.94± 0.24	0.70	5.68
Tara Garh	13.89 (10.77-17.23)	1.91± 0.24	0.38	5.38
Khokhran	14.41 (11.41-17.64)	2.06± 0.24	0.09	5.58
5-Faiz	13.37 (10.25-16.66)	1.88± 0.23	0.41	5.18
Lar	3.61 (2.75-4.41)	3.59± 0.41	5.65	1.39
11-MR	4.02 (2.02-5.88)	3.61± 0.40	10.65	1.55
12-MR	4.31 (2.63-5.96)	3.39± 0.39	7.47	1.67
10-T	32.21 (26.68-38.61)	2.35± 0.25	1.00	12.48
Multani wala	29.87 (24.54-35.93)	2.28± 0.25	0.44	11.57
Kotla Maharan	26.56 (21.48-32.19)	2.17±0.25	1.50	10.41
Qadir Pur Ran	35.08 (27.94-43.60)	1.85±0.22	0.61	13.59
	Malathion			
Alam Pur	79.40 (61.75-98.51)	1.89±0.23	0.10	16.00
Bosan	92.83 (73.11-115.21)	1.85±0.21	2.40	18.71
Nawab Pur	76.66± (61.37-93.13)	2.17±0.25	0.06	15.51
Mohammad Pur	89.32 (71.39-109.28)	2.03±0.23	0.65	18.00
Sher Shah	44.58 (35.22-54.47)	2.12±0.25	1.01	8.98
Tara Garh	38.16 (29.00-47.47)	1.98±0.25	1.13	7.69
Khokhran	42.42 (33.98-51.29)	2.29±0.27	1.69	8.55
5-Faiz	41.66 (26.70-57.77)	2.15±0.26	3.09	8.39
Lar	5.32 (4.40-6.34)	2.47±0.27	2.33	1.07
11-MR	5.49 (4.45-6.65)	2.18±0.25	1.51	1.10
12-MR	5.66 (4.58-6.86)	2.15±0.24	0.73	1.14
10-T	93.61 (76.91-112.41)	0.91±0.30	0.91	18.87
Multani wala	82.66 (66.06-100.82)	2.09±0.24	0.29	16.66
Kotla Maharan	89.15 (70.11-110.43)	1.88±0.23	0.13	17.97
Qadir Pur Ran	96.05 (75.29-119.82)	1.80±0.22	0.26	19.36

Continued

Population	LC_{50} (µgmL^{-1}) FL (95%)	Fit of Probit Line		RR*
		Slope±SE	χ²	
	Methomyl			
Alam Pur	7.76 (6.47-9.07)	2.78±0.31	2.86	2.00
Bosan	7.79 (6.51-9.04)	2.78±0.35	1.26	2.01
Nawab Pur	8.53 (7.31-9.78)	3.05±0.36	2.44	2.19
Mohammad Pur	39.77 (31.26-50.65)	1.67±0.21	1.67	16.43
Sher Shah	4.37 (3.38-5.42)	1.96±0.24	0.49	1.12
Tara Garh	4.60 (3.52-5.75)	1.84±0.23	0.39	1.18
Khokhran	4.49 (3.39-5.65)	1.78±0.23	0.59	1.15
5-Faiz	3.98 (3.07-4.93)	2.02±0.25	0.25	1.02
Lar	4.24 (3.22-5.30)	1.86±0.23	0.25	1.09
11-MR	4.27 (3.11-5.47)	1.64±0.22	0.80	1.10
12-MR	4.74 (3.61-5.96)	1.78±0.23	1.87	1.22
10-T	5.41 (4.28-6.78)	1.87±0.23	0.53	1.39
Multani wala	7.50 (6.29-8.67)	2.92±0.36	0.87	1.93
Kotla Maharan	37.04 (28.62-47.61)	1.58±0.21	1.72	15.31
Qadir Pur Ran	44.15 (35.14-55.96)	1.75±0.22	0.71	18.24
	λ-cyhalothrin			
Alam Pur	31.95 (25.95-38.92)	2.09±0.24	1.37	13.20
Bosan	32.16 (25.35-40.12)	1.80±0.22	0.03	13.28
Nawab Pur	36.80 (28.77-46.66)	1.67±0.21	0.45	15.20
Mohammad Pur	39.77 (31.26-50.65)	1.67±0.21	1.67	16.43
Sher Shah	16.16 (9.91-22.42)	2.68±0.31	4.79	6.67
Tara Garh	15.40 (9.32-22.31)	2.41±0.29	5.25	6.36
Khokhran	17.70 (11.62-25.25)	2.31±0.28	4.36	7.31
5-Faiz	14.13 (11.86-16.46)	2.78±0.30	2.05	5.83
Lar	2.59 (2.13-3.09)	2.39±0.26	2.18	1.07
11-MR	2.71 (2.24-3.23)	2.39±0.26	2.74	1.11
12-MR	2.55 (2.07-3.06)	2.30±0.26	0.77	1.05
10-T	33.71 (26.23-42.62	1.68±0.21	0.30	13.92
Multani wala	32.92 (25.22-42.17)	1.58±0.21	0.62	13.60
Kotla Maharan	37.04 (28.62-47.61)	1.58±0.21	1.72	15.30
Qadir Pur Ran	44.15 (35.14-55.96)	1.75±0.22	0.71	18.24
	Spinosad			
Alam Pur	48.78 (39.06-59.14)	2.15±0.25	0.56	13.21
Bosan	49.23 (40.34-58.75)	2.41±0.27	1.47	13.34
Nawab Pur	50.70 (39.06-63.21)	1.80±0.23	0.46	13.73
Mohammad Pur	50.84 (40.74-61.73)	2.11±0.25	0.82	13.77
Sher Shah	26.21 (16.72-37.01)	2.33±0.28	4.38	7.10
Tara Garh	24.18 (19.44-29.07)	2.15±0.28	2.27	6.55
Khokhran	28.28 (23.67-33.27)	2.48±0.29	2.53	7.66
5-Faiz	27.39 (18.09-38.23)	2.39±0.29	4.24	7.42
Lar	4.27 (3.57-5.00)	2.64±0.32	1.00	1.15
11-MR	4.36 (3.62-5.13)	2.53±0.31	0.60	1.18
12-MR	4.26 (3.59-4.96)	2.78±0.33	0.53	1.15
10-T	51.38 (41.18-62.43)	2.11±0.25	0.44	13.92
Multani wala	49.27 (39.46-59.78)	2.15±0.25	0.43	13.35
Kotla Maharan	47.94 (38.74-57.78)	2.25±0.26	2.32	12.99
Qadir Pur Ran	51.97 (41.94-62.82)	2.17±0.25	0.56	14.08

LC_{50}, lethal concentration of field population; FL, fiducial limits; ±SE, standard error; *RR, resistance ratio; (LC_{50} of field population/LC_{50} of susceptible strain).

Lar, 11-MR and 12-MR remained susceptible to malathion. Hsu and Feng (2000) reported resistance of this against malathion. Hsu and Feng (2002) reported 29 fold resistance to fenthion, malathion, methomyl and cyfluthrin) against *B. cucurbitae*. Our results are partially in agreement with Ahmad *et al.* (2010) who evaluated the insecticides resistance in two strains of *B. zonata* in Punjab, Pakistan. Nadeem *et al*. (2010) reported the susceptibility of *B. zonata*. Our results also showed that three populations of *B. zonata* were highly susceptible to malathion.

Methomyl

Methomyl showed very slight variation in resistance factors (1.02 fold to 2.45 fold) when assayed with different populations of *B. zonata* (Table I). Very low (LC_{50} ranging 9.51 to 7.76µgmL^{-1}) resistance ratio was noted in Kotla Maharan, Nawab Pur, Mohammad Pur, Bosan and Alam Pur. Other tested populations of 5-Faiz, Lar, 11-MR, Sher Shah, Khokhran, Tara Garh, 12-MR, 10-T, Multani wala and Qadir Pur Ran were observed as susceptible to methomyl. Virtually there is no comparable study against methomyl in Pakistan, however, our work is contradictory to the results reported by El-Aw *et al.* (2008) who compared the toxicity of different insecticides against peach fruit fly (*B. zonata)* and found that methomyl was more effective than the other three tested insecticides. Hsu and Feng (2002) compared the resistance level in different insecticides against six field population of *B. dorsalis* (Hendel) and *B. cucurbitae* and got the highest resistance ratio (43-fold) observed to methomyl against *B. dorsalis* among the tested strains which also fluctuate in our study.

λ-Cyhalothrin

λ-cyhalothrin showed resistance factors ranging in from 1.05- to 18.24 fold against different populations of *B. zonata* (Table I). The order of resistance is in Qadir Pur Ran > Mohammad Pur > Kotla Maharan > Nawab Pur > 10-T > Multani wala > Bosan > Alam Pur populations of *B. zonata* showed moderate resistance ratio (LC_{50} 44.15 to 31.95µgmL^{-1}) whereas, Khokhran > Sher Shah > Tara Garh > 5-Faiz, low resistance ratio (LC_{50} from 17.70 to 14.13µgmL^{-1}). The populations of 12-MR, 11-MR and Lar were evaluated as susceptible to λ-cyhalothrin. Ahmad *et al.* (2010) have also reported low to moderate resistant ratio for λ-cyhalothrin (4-9 fold) and bifenthrin (8-11 fold) against *B. zonata* strains from Multan and Faisalabad.

Spinosad

The resistance factor for spinosad ranged from 1.15 to 14.08 (Table I) in the order Qadir Pur Ran > 10-T > Mohammad Pur > Nawab Pur > Multani wala > Bosan > Alam Pur > Kotla Mahran. The flies showed moderate resistance with LC_{50} ranging from 51.97 to 47.94µgmL^{-1}. Low resistance ratio was observed in Khokhran, 5-Faiz, Sher Shah, and Tara Garh, Populations from 12-MR, Lar and 11-MR were susceptible to spinosad. Our findings are in the line with the work carried by Steven and McQuate (2000), who reported that malathion was the most effective among the tested insecticides. Findings of Ahmad *et al.* (2010) revealed that *B. zonata* strains from Multan and Faisalabad remained susceptible, whereas in this study, three populations among 15 were found susceptible to spinosad.

CONCLUSIONS

It is concluded that *B. zonata* has developed resistance against trichlorfon, malathion, bifenthrin, λ-cyhalothrin and spinosad, while they still remained susceptible to methomyl.

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