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Determination of Level of Insecticide Resistance in Fruit Fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) by Bait Bioassay

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ABSTRACT

In order to determine the level of resistance in fruit fly (*Bactrocera zonata*) to different insecticides mostly used in the field for fruit fly control, bait bioassays were conducted by using insecticides in protein hydrolysate. Adult fruit flies were collected from Multan area exposed to bait and compared with a laboratory reared population of *B. zonata*. Insecticides included in the bioassays were, Talstar 10 EC, Confidor 70WS, Curacron 50 EC, Deltamethrin 2.5 EC, Diptrex 80 WP, Proclaim 1.9 EC, Karate 2.5 EC, Malathion 57 EC, Tracer 240 SC, Steward 360 SC. Each treatment was repeated three times. The mortality in adult flies was noted at 24 and 48 h after the treatment application and LC_{50} values were estimated using Probit analysis (Polo Plus). These values of field and susceptible populations were compared to determine the level of resistance. The results revealed that the field strain exhibited varying ratios of insecticide resistance; being highest against Diptrex (65.32) followed by Curacron (13.20), Confidor (7.12), Talstar (5.97), Karate (5.73), Malathion (5.54) and Deltamethrin (2.35) at 24 h. The difference in LC_{50} of Proclaim and Tracer between two strains was non-significant at 24 and 48 h after treatment.

Key Words: Fruit fly; *Bactrocera zonata;* Insecticide; Resistance

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are one of the important insect pests of vegetables and fruits in Pakistan. Genus *Bactrocera* (Diptera: Tephritidae: Dacinae) contains 75 described species, largely endemic to Southeast Asia. Among these species, *Bactrocera dorsalis*, *B. papayae*, *B. cucurbitae*, and *B. zonata* are polyphagous pests of international significance (Anthony *et al*., 2005). The hosts of fruit flies recorded in Pakistan are apple, ber, guava, mango, musk melon and bitter gourd (Khan & Musakhel, 1999; Sultan *et al*., 2000; Khan *et al*., 2005). The incidence of fruit fly reduces both yield and quality of fruits and vegetables.

Various control measures such as chemical, biological and cultural are being followed; though latter has not proved vital for the management/control of the fruit flies. Chemical control has been the most important measure. Insecticides for the control of fruit flies have been applied in two ways, viz; baiting and cover spray and have proved successful in a number of cases. The equipment used for applying the bait is simple, so the technique is appropriate for control of fruit flies at either village or commercial levels (Allwood & Drew, 1997). In either case of application method, development of resistance is in offing.

The development of insecticide resistance has been studied in various Tephritid insect pests around the world. It has been reported that populations and laboratory strains of the oriental fruit fly *B. dorsalis* Hendel and the melon fly *B. cucurbitae* Coquillett from Hawaii developed resistance to DDT and methoxychlor but not to malathion, in the late 1950s and 1960s. By contrast, the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was found to be susceptible to these insecticides by topical application method. Four decades later in laboratory selection experiments with a *B. dorsalis* population from Taiwan, resistant lines were produced not only to malathion and other OPs but to insecticides of diverse chemical groups (methomyl & pyrethroid) (Hsu & Feng, 2000). In above study, cases of cross- and multi-resistance were also reported. In Israel, resistance to OPs and to the carbamate (methomyl) has been found in another Tephritid species, *Dacus ciliatus* Loew, treated by topical application method (Maklakov *et al*., 2001).

Regular and indiscriminate use of insecticides can cause resistance in insects. Fruit flies receiving small doses of insecticides over a period of time can attain resistance easily as has been observed in many cases in housefly, *Musca domestica* L. (Scott & Georghiou, 1985). Insecticides in bait sprays or cover sprays can render the

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fruit flies to attain resistance against them. Although recent references from different sources have indicated this possibility (Hsu *et al.*, 2004b; Hsu & Feng, 2006) and a number of insecticides resistant strain developed in the laboratory using topical application method, further reinforce potential of fruit flies for falling prey to insecticide resistance. We have previously demonstrated a resistance ratio up to 19 fold to many insecticides in strains of *B. zonata* icluding one from Multan with fruit dip method (Ahmad *et al.*, 2010). The present paper extends the studies of evaluation of insecticides resistance in *B. zonata* by bait feeding technique.

MATERIALS AND METHODS

The present studies were conducted under laboratory conditions (28±1°C, 55±5% RH) in Department of Agri. Entomology, University of Agriculture, Faisalabad to determine the resistance level in fruit fly, *B. zonata* against ten insecticides, recommended for its control. Field populations of *B. zonata* were collected from the infested and fallen fruits of the mangos and guava from various orchards of Multan. A reference susceptible strain of the test insect (*B. zonata*) was obtained from CABI, Multan in pupal form which has been reared for the last 12 years without insecticide exposure, for comparison. The experiment was laid down according to completely randomized design with three replicates including an untreated control. Rearing of both susceptible and field strains of *B. zonata* were carried in Persplex cages on healthy mango fruits for egg laying. The infested fruits were then placed in a wooden cage having soil at the bottom for pupation. The pupae were isolated from the soil and placed in a separate cage for adult emergence. The adults were fed on banana based artificial diet having ingredients such as egg yolk, sugar, honey, yeast, syrup vitamin B complex blended in the ratio of 2:4:8:2:2:1, respectively in an electric blender to make a paste and was kept in a freezer for subsequent use (Ahmad *et al.*, 2010).

Commercial formulations of tested insecticides were obtained from the respective distributors and were used in baits for the determination of level of resistance against field population of *B. zonata.* Curacron 50 EC, Proclaim 1.9 EC and Karate 2.5 EC were obtained from Syngenta Pakistan Ltd.; Confidor 70 WS and Diptrex 80 WP from Bayer Crop Sciences; Deltamethrin 2.5 EC and Malathion 57 EC from Ali Akbar Group, Pakistan; Tracer 240 SC from Arysta Life Sciences; Steward 360 SC from United Distributors Pvt. Ltd. and Talstar 10 EC from FMC Corporation, Pakistan. Tissue paper dipped in solution (10 mL bait + insecticide solution) in small petri dishes was offered to the fruit flies in plastic jars. Ten fruit flies of known age were transferred to the jar, containing the petri dish. The mouth of plastic jar was covered with a muslin cloth held in place with a rubber band for proper aeration and to prevent escape of fruit flies. Mortality was noted after regular intervals up to 48 h. Irreversible knockdown followed by death of the adult fruit flies was the criterion to determine mortality. The LC_{50} values of susceptible and field strains for each insecticide were estimated using Probit analysis (Polo Plus, LeOra Software 2002-2010) for both the strains (Finney, 1971). These LC_{50} values of susceptible and field strain were compared to determine the level of resistance. The LC_{50}s were considered non-significant if the fiducial limits overlapped with each other (Robertson & Preister, 1992).

RESULTS AND DISCUSSION

The results (Table I & II) revealed that the field strain exhibited varying ratios of insecticide resistance, with highest resistance against Diptrex 80WP was followed by Curacron 50 EC, Confidor 70 WS, Talstar 10 EC, Malathion 57 EC, Karate 2.5 EC and Deltamethrin 2.5 EC in descending order at 48 h.

The highest resistance ratios evaluated for Diptrex were 65.32 and 32.71 after 24 and 48 h, respectively. Hsu and Feng (2000) assayed *B. dorsalis* adults and found triclorofon to be the least effective among the five insecticides tested. The present results are also in line with that of the Kashyap and Hameed (1982) and Zhi-Ping *et al.* (2005) in China, who found low level of resistance in two populations of oriental fruit flies from Chenghai and Minnan however, Diptrex has been found effective against C. *capitata* (Raga & Sato, 2006).

The resistance ratio calculated for Malathion was 5.54 and 4.90 after 24 and 48 h, respectively. Similar results were found by Magana *et al.* (2007) in the field populations of *C. capitata* in spain due to increased frequency of insecticide treatment. Hsu *et al.* (2004b) also found resistance in *Bactrocera dorsalis* against Malathion in Taiwan with RR 14.7, which also strengthens the results.

Moderate level of resistance was computed for Curacron (13.20 & 15.34) after 24 and 48 h, respectively. There have been no comparable results for Curacron resistance against fruit fly but Gogi *et al.* (2007) found Curacron incompatible in attract and kill method for fruit fly. While with same method, resistance against Curacron has been found in *Culex pipiens* in France by Wood *et al.* (1984).

The fruit flies showed low level of resistance against Confidor with 7.12 and 15.20 resistance ratio after 24 and 48 h, respectively. There are no previously documented results of resistance against Confidor in the fruit fly. The present results are in contrary to the results found by Yee and Alston (2006) who reported that Confidor was satisfactorily effective against western cherry fruit fly *Rhagoletis indifferens.* The low level of resistance was also found against Talstar (5.97 & 4.79) after 24 h and 48 h, respectively.

The fruit flies showed low level of resistance against Karate (5.73 & 3.93 RR) after 24 and 48 h, respectively.

Table I: LC_{50} (ppm) and Fit of Probit lines in various strains of *B. zonata* against different insecticides after 24 h

Insecticides	Lab. strain				Multan strain				
	LC_{50} (ppm)	Fit of Probit Line		C.I (95%)	LC_{50} (ppm)	Fit of Probit Line		C.I (95%)	R.R
		Slope±S.E	X^2			Slope±S.E	X^2		
Talstar 10 EC	33.98	1.187±0.266	0.218	17.4945-1.538	202.99	1.096±0.258	0.231	115.626-319.696	5.97
Confidor 70 WS	211.36	1.075±0.272	0.55	124.509-699.429	1504.7	1.077±0.294	0.038	770.04-989.01	7.12
Curacron 50 EC	131.92	1.645±0.296	0.291	95.619-208.945	1741.28	1.662±0.331	0.201	1192.78-3418.37	13.20
Deltamethrin 2.5 EC	1.07	1.496±0.275	2.076	0.751-1.529	2.50	1.504±0.275	1.636	1.785-3.664	2.35
Diptrex 80 WP	5.35	2.693±0.38	3.213	3.459-7.844	349.34	2.155±0.318	1.497	271.949-461.253	65.32
Proclaim 1.9 EC	49.19	1.312±0.283	1.395	23.608-74.296	67.19	1.065±0.26	0.432	30.593-106.287	1.37
Karate 2.5 EC	125.06	1.823±0.307	2.902	93.155-186.603	716.69	1.12±0.271	0.361	442.406-1916.697	5.73
Malathion 57 EC	2.23	0.697±0.25	2.076	0.397-4.428	12.39	0.811±0.251	2.523	5.055-23.322	5.54
Tracer 240 SC	479.48	1.105±0.262	0.092	307.868-1000.018	574.45	1.145±0.267	0.109	368.469-1278.515	1.20
Indoxacarb 360 SC	1341.78	1.537±0.35	0.850	817.117-3993.851	2792.3	1.167±0.347	0.376	1201-4995.1	2.08

Table II: LC_{50} (ppm) and Fit of Probit lines in various strains of *B. zonata* against different insecticides after 48 h

Insecticides	Lab. Strain				Multan Strain				
	LC_{50} (ppm)	Fit of Probit Line		C.I (95%)	LC_{50} (ppm)	Fit of Probit Line		C.I (95%)	R.R
		Slope±S.E	X^2			Slope±S.E	X^2		
Talstar 10 EC	7.03	1.451±0.413	0.859	17.494-51.538	33.65	0.941±0.304	0.088	78.597-33.643	4.79
Confidor 70 WS	14.44	0.945±0.275	1.773	2.134-27.738	219.58	1.164±0.26	1.304	133.967-340.214	15.20
Curacron 50 EC	17.45	1.384±0.306	0.436	7.039-27.415	267.59	1.25±0.279	0.650	137.451-403.919	15.34
Deltamethrin 2.5 EC	0.25	3.597±0.81	1.141	0.169-0.317	0.69	1.92±0.352	2.283	0.419-0.949	2.74
Diptrex 80 WP	1.75	3.515±0.936	0.655	0.957-2.253	57.18	1.587±0.35	1.813	22.595-89.397	32.71
Proclaim 1.9 EC	13.48	1.531±0.443	0.420	1.43-26.998	19.97	1.664±0.416	0.679	5.291-33.854	1.48
Karate 2.5 EC	14.43	1.605±0.355	1.485	5.779-22.432	56.75	1.449±0.332	2.147	19.985-91.718	3.93
Malathion 57 EC	0.46	1.289±0.45	0.679	0.013-0.962	2.23	0.983±0.296	1.550	0.164-4.852	4.90
Tracer 240 SC	120.62	1.182±0.262	0.727	58.04-184.756	127.44	0.828±0.253	0.018	35.18-227.071	1.06
Indoxacarb 360 SC	157.02	0.952±0.256	1.306	68.441-260.162	278.44	0.928±0.252	1.908	156.727-529.442	1.77

There is no comparable study for resistance of Karate against fruit flies but present results are in agreement with those of Urbaneja *et al.* (2009) who found it least effective against *C. capitata*. The fruit flies also showed very low level of resistance against Deltamethrin with 2.35 and 2.74 RR after 24 and 48 h, respectively. The Deltamethrin caused high mortality to fruit flies. These results are in agreement with the results of Raga and Sato (2005), who reported that Deltamethrin gave high mortality in *C. capitata* and *Anastrepha fraterculus* after two days of treatment. Three insecticides including Proclaim, Indoxacarb and Tracer were proved to be effective as field population was susceptible against these insecticides. These insecticides gave high mortality in field population of fruit flies. These results are also in direct conformity with those reported elsewhere (Raga & Sato, 2005, 2006; Burns *et al.*, 2001; Magana *et al*., 2007; and El-Aw *et al.*, 2008). Most of above authors found Tracer to be highly effective against *B. cucurbitae* (coq.), *B. dorsalis* (Hendel) and *C. capitata* (Wiedemann) females. Unfortunately, because of the current review of organophosphate insecticides under the FQPA regulations, it appears likely that many organophosphate insecticides will either be removed from registration for use on fruits or the usage patterns of any remaining materials will be altered to prevent late season use and development of resistance. Because of these potential problems with the future registration of organophosphates, both laboratory and field tests have been conducted to evaluate the effectiveness of several new insecticides against fruit flies: Spintor (Tracer), Indoxacarb and Proclaim (Reissig *et al.*, 2001).

CONCLUSION

Fruit flies developed resistance against some insecticides, which also includes Diptrex in Pakistan. Further studies with synergists will indicate the type of mechanism, operating in fruit flies for resistance to the insecticides reported herewith. There is a need to use insecticides wisely and according to the methods prescribed by the experts and also integrate the insecticides in IPM program.

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