

EFFECTS OF CYPERMETHRIN ON FECUNDITY, FERTILITY, PUPATION, ADULT EMERGENCE AND SURVIVAL RATE OF MELON FRUIT FLY *BACTROCERA CUCURBITAE* (COQ.)

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ABSTRACT

Effects of cypermethrin on melon fruit fly, *Bactrocera cucurbitae* (Coq.) were studied. Third instar larvae were treated with different doses of cypermethrin i.e. 0.0016%, 0.0032%, 0.0063%, 0.0125% and 0.025% by contact method. LD₅₀ against larvae of melon fruit fly after 24 hours of treatment was found to be 0.00647%. Treated insects were left for 24 hours for the evaluation of toxic effects and for the determination of lethal dose concentration. Surviving insects were kept for the observation of percent pupal formation, emergence of adults, egg laying (fecundity) and hatching (fertility). The lowest concentration of cypermethrin i.e. 0.000245% showed 1% mortality, while 0.0354% concentration of cypermethrin caused 90% mortality. As a result of a high dose 0.025% of cypermethrin against 50 larvae, only 9.66 larvae were succeeded to pupate among them only three pupae succeeded to emerge as adult. Similarly, the insects treated with the same dose i.e 0.025% showed 69.95% fecundity and 30.05% fecundity inhibition while 75.67% fertility and 24.33% fertility inhibition. Fecundity inhibition was not reciprocal to ascending concentration of cypermethrin.

Key words: cypermethrin, melon fruit fly, *Bactrocera cucurbitae*, fertility, inhibition, fecundity

INTRODUCTION

Synthetic pyrethroids have great tendency to affect the various biological functions of insects and they also act to disturb the neuro-endocrine functions (Rowland, 1995). In addition, their neuro-toxic function resembles with the DDT (Beeman, 1982). Pyrethroids have capability to paralyze the nervous system of insects (Elliott, 1977). They show insecticidal and larvicidal activity, knockdown and lethal effects, repellency and antifeeding activity as well. Pyrethroids are widely used in plant protection (Hirano and Nagao, 1989; Mujeeb *et al.*, 2012), and are generally characterized as substances affecting the insect's metabolic system. Since the pyrethroids are esters, they are inactivated by cleavage by esterases (Khan *et al.*, 2003).

Among the food and vegetable pests, melon fruit fly, *Bactrocera cucurbitae* (Coq.) is one of the economic importances. It causes particular damage to family cucurbitaceae and the loss to vegetables is estimated about 30-100% in the season. More than four thousand species of fruit flies are known, out of which seven hundred species belong to Dacine fruits (Fletcher *et al.*, 1990). About 250 species have enormous economic worth in temperate regions, (Christenson and Foote, 1960), while more than 80 plants species have been documented as its host species, the most of them belong to cucurbitaceae (Ellwood *et al.*, 1999). Fruit fly lays eggs within the fruit. Developing larvae of fruit fly survive and feed within the fruit and forms eating chambers in it. Occasionally fruit fly punctures the fruit without laying egg; this punctured spot reduces the market price of the fruit. Beside that fruit fly also lays eggs in flower buds where larvae can develop efficiently (Singh, 2003).

Present study was aimed to evaluate the effects of cypermethrin on melon fruit fly. The experiment will help in understanding the chemical control provision of the test organism at the lower doses, which can be helpful in IPM strategy, so as to avoid environmental hazards.

MATERIALS AND METHODS

Rearing of melon fruit fly

Initial strain of melon fruit fly was obtained from the Nuclear Institute of Agriculture (NIA) Tando jam, and further rearing was done in the laboratory of entomology, MAHQ Biological Research Center (BRC), University of Karachi. Glass cages of $32 \times 32 \times 32$ cm. size were used for rearing purpose; front and top of the cages were provided with 18 cm. hole covered with the muslin cloth to serve the insects with food and adequate aeration. Cages were placed at 26 ± 2 °C and 65 ± 5 % relative humidity. Provided photoperiods was 12:12 hours light and dark. Adult melon flies were served with 6% honey solution, sugar, and protein-hydrolysate and yeast extract. Water was served in waterlogged foam pads. The adult melon fruit flies select cucurbits suitable for oviposition site. Thus ready to egg laying fruit flies were provided bottle gourd as oviposition media. Thereafter, eggs were hatched and larvae flourished thereon. Popped out third-instar larvae of *B. cucurbitae* were collected for the toxicity treatment.

Preparation of cypermethrin doses

Cypermethrin (10 EC) was obtained from the market. Initially stock solution of 1% concentration was prepared which was further dissolved in distilled water and a series of dilutions of 0.016%, 0.0032%, 0.0063%, 0.0125% and 0.025% were prepared for further experiments.

Data Analysis

All the tables contain concentration of compound, mean mortality, standard deviation, standard error and 95% confidence limit. The observed mortality was corrected by formula described by Tatters field and Morris (1924) and Abbot (1925). SPSS Version-14 and Bio stat 2009 software were used to analyze the data. Solutions of selected samples were prepared in different concentrations using following equation.

$$C_1V_1 = C_2V_2$$

Where,

C_1 = Initial concentration of solute

V_1 = Initial volume of solute

C_2 = Final concentration of solvent

V_2 = Final volume of solvent

The relative fecundity and fertility was calculated by using following formula:

$$\text{Relative fecundity} = [a - A/A] \times 100$$

Where:

a: percentage of eggs laid in the treatment

A: percentage of eggs laid in the controls

Fecundity inhibition (F_1) was calculated by applying following formula

$$F_1 = 100 - B$$

Where:

Measurement of fertility inhibition,

100: formula constant

B: percent fertility

Experimental Design

Experiments were conducted on third instar larvae of melon flies. Melon fruit flies were reared in the laboratory as described earlier. Five concentrations of sample were used for LD₅₀ determination. Contact method was applied for the treatment. A control batch (with no treatment) was kept as reference with the assay. The treated insects were left for 24 hours for the evaluation of toxic effects and for the determination of lethal dose concentration. Alive insects were kept for the observation of percent pupation, emergence of adults, egg laying (fecundity), hatching (fertility) and biological functions as well.

Study scheme

After preliminary trials five different doses were selected for different under test samples. During the experiments insects of uniform size and age were used. For the treatment and accuracy in results five replicates were treated along with a batch of control insects. Mortality count was recorded after 24 hours of treatment and calculations were made. Each experiment was repeated five times; while the whole experiment was discarded and repeated in case of mortality observed above ten percent of any experiment.

RESULTS AND DISCUSSION

Toxic effects of cypermethrin against melon fruit fly

By plotting the mean mortality values through the concentrations of compound through probit analysis (Table 1), cypermethrin LD₅₀ against 3rd instar larvae of melon fruit fly was found to be 0.00604%, The lowest concentration of cypermethrin i.e. 0.000245% showed 1% mortality, while 0.0354% concentration of cypermethrin caused 90% mortality. Table 2 shows 21.33% mean mortality at lowest concentration 0.0016% of cypermethrin, while 87.00% mean mortality was recorded at the highest dose i.e. 0.025%.

Effects of cypermethrin on pupation and adult emergence

Table 3 shows when the larvae of melon fruit fly were treated with the low dose 0.0016% of cypermethrin, only 44.66 were succeeded to pupate, out of fifty. Table 4 shows that out of 44.66 pupae only 39.33 succeeded to emerge as adult. when 50 larvae were treated with high dose 0.025% of cypermethrin, only 9.66 larvae were succeeded to pupate and only three succeeded to emerge as adult.

Effects of cypermethrin on fecundity fertility

Adults emerged from the treated larvae were collected, and pairs were made as an untreated male and a treated female then released in five replicates in plastic containers and were observed for fecundity (egg laying), fecundity inhibition, fertility (egg hatching) and fertility inhibition (Table 5). Five pairs of untreated flies were also kept as control under the same laboratory conditions. Considering the given data in table-5, it was recorded that less number of eggs were laid by all the treated adult females at all the applied concentrations. The group of females treated with cypermethrin at 0.0016 % dose showed 80.82% fecundity and 19.88% fecundity reduction while 84.86% fertility and 15.14% fertility inhibition. Similarly, the insects treated with highest dose i.e 0.025% showed 69.95% fecundity and 30.05% fecundity inhibition while 75.67% fertility and 24.33% fertility inhibition.

Zhang *et al.*, 2000 worked to evaluate the effects of alpha- cypermethrin and deltamethrin against mosquito larvae, *C.quinquefasciatus* and house fly, after the inclusive experiments they reported the LC₅₀ for alpha cypermethrin as 0.0018mg/L and 0.00138 mg/L for deltamethrin in case of mosquito. While the LD₅₀ in case of alpha-cypermethrin was 0.00359mg/female and 0.00203mg/female in case of deltamethrin against *Musca domestica*. The cited findings are in line with the present study. A lot of the reports have been presented on topical application of insecticides against dipterous flies (Gunjima & Saito, 1992); Keiding, 1995). The insecticide having the low LD₅₀ value in fewer times may be much effective. Pyrethroids have been applied with the belief that they were the least dangerous to the atmosphere as reported by (Mostafa & Zayed, 1999; Kocisova, 2001; Hu *et al.*, 2001). Pyrethroids applied in aerosols offer an immediate eradication of the house flies but it is not apparent whether pyrethroids can be a successful, long lasting and continual control. Ahmed *et al.*, (2004) worked on house flies and concluded the effect of cypermethrin along with some other synthetic insecticides, they observed that cypermethrin had showed the lowest LC₅₀ i.e. 183 ppm. To assess the toxicity, Knockdown and mortality data they permitted the house flies to nourish on the insecticide coated sugar for 1, 2, 4, 6, 8, 12, 24, and 48 hours. They observed that KD₅₀ values were increased during 1st, 2nd and 4th hours but at 6th, 8th and 12th the KD₅₀ value were dropped down. They observed that there was no mortality in the 1st hour after treatment. LC₅₀ values were very high after 2nd and 4th hours. In the present investigations cypermethrin stood very effective against the test organisms which suggest that the insecticide disrupted the normal functions of nervous tissues. Similar findings have been reported by (Dyek *et al.*, 1985). Frampton, (1999) worked to investigate the effects of insecticides chlorpyrofos, cypermethrin and primicarb against *Collembola* in winter wheat fields. They mainly worked on cypermethrin and primicarb and incorporated chlorpyrofos as poisonous standard. They recorded that the number of specimens decreased after the application of chlorpyrofos while number of specimens were increased after the treatment of cypermethrin. They also concluded that application of cypermethrin in winter was not harmful for the predator arthropods. Soderlund and Knipple, (2003) investigated the development of resistance against pyrethroids in the *Musca domestica*. They concluded pyrethroids were still effective in those strains of *Musca domestica* which were showing more resistance against DDT. Byford *et al.*, (1998) examined the development of resistance pattern of pyrethroid by using horn fly as test organism. They selected field colonies at different places and in the laboratory as well. They carried out their experiments up to several successive generations. After collecting a remarkable data they concluded that in the laboratory experiments, resistance developed against pyrethroids after 21st generation, but the magnitude of resistance was ranging from less than 3 folds, whilst field studies explained the product failure after 3-4 years of consecutive application of pyrethroids. Whereas, discontinuous use of pyrethroids in the laboratory can exhibit the postponed commencement of resistance and also diminish the magnitude of pyrethroid resistance. These contrasting results of postponed resistance against pyrethroids suggest the alternated use of pyrethroids in the field. In this way efficacy of product will not be decreased even after so many years. These reports are in support to the toxic effects of cypermethrin in the present study. In the present experiment group of females treated with cypermethrin at 0.0016

% dose showed 80.82% fecundity and 19.88% fecundity reduction while 84.86% fertility and 15.14% fertility inhibition. Similarly, the insects treated with highest dose i.e 0.025% showed 69.95% fecundity and 30.05% fecundity inhibition, while 75.67% fertility and 24.33% fertility inhibition. Kryger *et al.*, (2006) worked to determine the non target effects of cypermethrin and cymiazol on different beetles. During their investigation, they concluded that the dung beetle species *Euoniticellus intermedius* appeared as unaffected by the dung of treated cattle. Similarly, Khalequzzaman *et al.*, (2006) and Ullah *et al.*, (2006) reported the disturbance in the developmental stages may be seen by the toxic effects of cypermethrin, these reports are in favour of the present study.

Table 1. Probit analysis of mortality data of 3rd instar larvae of melon fruit flies under the toxic effects of cypermethrin.

Log10 Dose	Probit Percent (%)	Insects exposed	Insects Killed	Difference	Chi-square	Probit (Y)
0.0016	0.166879	100	20	1.656047	0.328681	4.158543
0.0032	0.321424	100	30	1.071191	0.071398	4.475998
0.0063	0.511051	100	48	1.552571	0.094334	4.949981
0.0125	0.700131	100	68	1.006521	0.028941	5.467275
0.025	0.847937	100	88	1.603145	0.060619	6.175091

Table 2. Toxicity of cypermethrin against 3rd instar larvae of melon fruit flies.

Conc.% of compound	Doses µg/insect	Mean mortality	Standard deviation	Standard error	Range at 95% confidence limit	
Control	00	2.3333	1.15470	0.66667	0.5351	5.2018
0.0016	0.0002	21.3333	1.52753	0.98192	17.5388	25.1279
0.0032	0.0004	30.3333	1.52753	0.87190	26.5388	34.1279
0.0063	0.0006	49.3333	1.52753	0.86122	45.5388	53.1279
0.0125	0.0013	68.6667	2.51661	1.45297	62.4151	74.9183
0.025	0.0025	87.0000	1.73205	1.00000	82.6973	91.3027

Table 3. Toxic effects of cypermethrin on pupation of melon fruit flies.

Conc.% of compound	Doses µg/insect	No. of larvae developed into pupae	Standard deviation	Standard error	Range at 95% confidence limit	
Control	0.000	47.3333	1.15470	0.66667	44.4649	50.2018
0.0016	0.0002	44.6667	1.52753	0.88192	40.8721	48.4612
0.0032	0.0004	38.6667	2.08167	1.20185	33.4955	43.8378
0.0063	0.0006	32.6667	1.52753	0.88156	28.8721	36.4612
0.0125	0.0013	9.6667	1.52753	0.77192	5.8721	13.4612
0.025	0.0025	1.0000	0.00000	0.00000	1.0000	1.0000

Table 4. Toxic effects of cypermethrin on adult emergence of melon fruit flies.

Conc.% of compound	Doses µg/insect	No. of pupae emerged as adult	Standard deviation	Standard error	Range at 95% confidence limit	
Control	0.000	42.0000	1.00000	0.57735	39.5159	44.4841
0.0016	0.0002	39.3333	1.52753	0.88192	35.5388	43.1279
0.0032	0.0004	31.0000	1.00000	0.57735	28.5159	33.4841
0.0063	0.0006	20.0000	2.00000	1.15470	15.0317	24.9683
0.0125	0.0013	3.0000	1.00000	0.57735	0.5159	5.4841
0.025	0.0025	0.0000	.00000	0.00000	0.0000	0.0000

Table 5. Effects of cypermethrin on fecundity and fertility melon fruit flies.

% Concentration	Mean No. of eggs ± S.E (fecundity)	% relative fecundity	Fecundity inhibition %	Mean fertility	% relative fertility	Fertility inhibition
0.0016	199±3.33	93.42	6.58	160±3.65	86.48	13.53
0.0032	171±2.88	80.82	19.18	157±2.54	84.86	15.14
0.0063	163±3.99	76.52	23.48	150±4.13	81.08	18.92
0.0125	161±2.79	75.75	24.25	153±3.95	82.70	17.3
0.025	149±3.55	69.95	30.05	140±1.99	75.67	24.33
Control	213±2.16	00	00	185±3.55	00	00

REFERENCES

- Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- Ahmed, S., W. Akbar and M.A. Riaz (2004). Effect of crop rotation and intercropping on subterranean termites in wheat at Faisalabad. *Pak. Entomol.*, 26: 25-30.
- Beeman, R.W (1982). Recent advances in mode of action of insecticides. *Ann. Rev. Ento.*, 27: 253-281.
- Byford, R. L., M. E. Craig, S. M. DeRouen, M. D. Kimball, D. G. Morrison, W. E. Wyatt, and L. D. Foil (1998). Influence of permethrin, diazinon and ivermectin treatments on insecticide resistance in the horn fly (Diptera: Muscidae). *Int. J. Parasitol.*, 29: 125-135.
- Christenson, L.D. and R.H. Foote (1960). Biology of fruit flies. *Ann. Rev. Ento.*, 171-192.
- Dyek, P.J., S. Masatake, P.S. Gerald, and C.S. Alfred (1985). The evaluation of a new synthetic pyrethroid (permethrin) for neurotoxicity. *J. Environ. Pathol. Toxicol. Oncol.*, 11: 109-118.
- Elliot, M. (1977). Synthetic Pyrethroid. *ACS Symposium Series NO. 42* American Chemical Society Washington. pp 1-128.
- Ellwood, A.J., A. Chinajariyawong, R. Drew, E.L. Hamacek, D.L. Hancock, C. Hengsawad, J.C. Jinapin, M. Jirasurat, K.C. Kong, S. Kritsaneepaiboon, Leong, C.T.S and S. Vijaysegaran (1999). Host plant records for fruit flies (Diptera: Tephritidae) in South-East Asia. *Raffles Bul Zool (Supplement.)*, 1-92.

- Fletcher, R.C., Axtell and R.E. Stinner (1990). Longevity and fecundity of *Musca domestica* (Diptera: Muscidae) as a function of temperature. *Entomol Soc of Ameri.*, 922-926.
- Frampton, G.K (1999). Spatial variation in non-target effects of the insecticides chlorpyrifos, cypermethrin and pirimicarb on Collembola in winter wheat. *Pestic Sci.*, 875-886.
- Gunjima, K and K. Saito (1992). Oral toxicities of some pyrethroids to the house fly, *Musca domestica* L. (Diptera: Muscidae). *Appl Entomol Zool.*, 27: 319-24.
- Hirano, S and N. Nagao (1989). Effect of chitosan, pectic acid, lysozyme and chitinase on growth of several Phytopathogens. *Agric Biol Chem.*, 53: 3065-3066.
- Hu, X., M.S. Wu and R.M. Li (2001). Study on the resistance against ten insecticides of house fly in slaughter house of Hefei. *Chinese J Vector Biol Control.*, 12: 352-59.
- Keiding, J (1995). Lessons provided by the house fly on evaluation of resistance (R) to insecticides. *Phytoparasitica.*, 23: 97-100.
- Khalequzzaman M and S. Sultana (2006). Insecticidal activity of *Annona squamosa* L seed extracts against the red flour beetle *Tribolium castaneum* (Herbst.). *J of Boil Sc.*, 107-112.
- Khan, M.F and S.M. Ahmed (2003). Comparative toxicity of neem fruit extract and cypermethrin against *Tribolium confusum* Hbst.(Coleoptera: Tenebrionidae) and *Papilio demoleus* Linn. (Lepidoptera: Papilionidae). *Philipp. J of Sci.*, 132: 109-114
- Kocisova A (2001). The stability of resistance in a field house fly population, *Musca domestica*, over 60 generations is following the interruption of insecticides selection pressure. *Czech J nim Sci.*, 46: 281-8.
- Kryger. U., C. Deschodt, A.L. Davis and C.H. Snocholtz (2006). Effects of cattle treatment with a cypermethrin / cymiazol spray on survival and reproduction of the dung beetle species *Euoniticellus intermedius* (Coleoptera: Scarabaeidae). *Bull of Entomol Res.*, 6: 3597-603.
- Mostafa, A.A and A.B. Zayed (1999). Resistance of *Musca domestica* (L.) (Diptera: Muscidae) from Gamasa city to some insecticides. *J Egypt Soc Parasitol.*, 29: 193-201.
- Mujib, A., A. Ilah, J. Aslam, S. Fatima, Siddiqui, Z.H and M. Maqsood (2012). *Catharanthus roseus* alkaloids: application of biotechnology for improving yield. *Plant Growth Reg.*, 68:111-127
- Rowland, A.S (1995). Pesticides and birth defects. *Epidemiology*, 6: 6-7.
- Tattersfield, F. and R.M. Morris (1924). An apparatus for testing the toxic values of contact insecticides under controlled conditions. *Bull. Entomol. Res.*, 14: 223-224.
- Singh, S (2003). Effects of aqueous extract of neem seed kernel and azadirachtin on the fecundity, fertility and post-embryonic development of themelonfly, *Bactrocera cucurbitae* and the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *J Appl Entomol.*, 127: 540-547.
- Soderlund, D and D. Knipple (2003). The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem Mol Biol.*, 33:563-577.
- Ullah, M.S., M. Ahmad, N. Ahmad, Khan, M.Z and I. Ahmad (2006). Toxic effects of cypermethrin in female Rabbits. *Pakistan Vet J.*, 26: 193-196.
- Zhang, Z., L. Lin and W. Lu (2000). Efficacy of Alpha-Cypermethrin against Mosquito, *Cx. quinquefasciatus* and Housefly *Musca domestica*. *Chi. J. Vec. Biol. and Cont.*, 271-272.

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