

## **TOXICITY OF DIFFERENT INSECTICIDES AGAINST FRUIT FLIES UNDER LAB CONDITIONS USING TOPICAL APPLICATION BIOASSAY METHOD**

Hassan Akhtar<sup>1</sup>, \*Qaisar Hamad<sup>2</sup>, Javaid Iqbal<sup>3</sup>, Azaz Ahmad<sup>2</sup>, Jahan Zaib<sup>4</sup>, Hafiz Muhammad Zohaib<sup>5</sup>, Roshan E Zahra<sup>6</sup>, Muhammad Owais Khan<sup>7</sup>, Muhammad Abdullah<sup>2</sup>, Muhammad Salman<sup>8</sup>, Syed Zaheer Abbas<sup>2</sup>

**1** PARC-Balochistan Agriculture Research Development Center, Pakistan

**2** Department of Entomology, The University of Agriculture Peshawar, Pakistan

**3** Department of Plant Protection, MNFSR, Pakistan

**4** Directorate Agriculture Research Medicinal Plant, Kalat, Pakistan

**5** University of Karachi, Pakistan

**6** Department of Entomology, University of Agriculture Faisalabad, Pakistan

**7** Department of Zoology, Abdul Wali Khan University Mardan, Pakistan

**8** Sericulture Branch, NTFP Division, Pakistan Forest Institute Peshawar, Khyber Pakhtunkhwa, Pakistan

**Corresponding author email:** qaisarhamad97@gmail.com

### **Abstract**

The present study was conducted to evaluate the toxicity of different insecticides: imidacloprid, fipronil, cypermethrin and chlorpyrifos against the adult stage of fruit fly during 2019. *B. zonata* maggots were found vigorously feeding inside the cucurbit hosts (pumpkin, cucumber, bitter melon, watermelon, round melon, bottle gourd) collected from different localities of district Peshawar, Pakistan. Susceptibilities of *B. zonata* to insecticides were evaluated using topical method. Mortality was checked after 3, 6, 8 and 24h of exposure. Cypermethrin was most effective to kill 50% of adult stage, respectively followed by imidacloprid. Imidacloprid most effectively killed 90% of adult population respectively after 24 hours. Toxicity of each insecticide increased with exposure for longer time and increased dose.

**Keywords:** *Bactrocera diversa*, cypermethrin, fruit fly, insecticides, toxicity

## **Introduction**

Among the myriad of economically important insects, tephritid flies (Diptera: Tephritidae) stand out as a major challenge to the horticultural trade, causing significant losses both nationally and internationally (Ormbsy, 2021). The genus *Bactrocera* (Diptera: Tephritidae) alone includes about 500 known species, mostly endemic to the South Pacific Islands and Southeast Asia (Drew and Hancock, 2000). Of the approximately four thousand known Tephritidae species worldwide, approximately 350 are economically important (Plant Health Australia, 2016). Among them, about seventy species of polyphagous *Bactrocera* pose a significant threat to international trade, causing widespread damage to commercial vegetables and fruits (Doorenweerd et al., 2018). The impact of these insect pests is profound, with infestation rates as high as 89% recorded in Pakistan (Grewal and Kapoor, 1987) and as low as 20% in the Northwest Himalayan region (Gupta and Bhalla, 1990). Twig flies are estimated to cause annual losses of approximately \$200 million in Pakistan (Hussain et al., 2010), significantly affecting fruit and vegetable exports (The Express Tribune, 2017). Stonehouse et al. (2002) suggested that potential annual savings of US\$144.6 million could be realized in Pakistan by mitigating fruit fly infestations. The presence of fruit fly maggots renders commodities, especially mangoes, unfit for human consumption (Stonehouse et al., 2002). Due to the zero tolerance for such pests in national and international markets, affected commodities are often rejected, adversely affecting a country's trade and economy (Reynolds et al., 2017). In Pakistan, insecticides are commonly used to manage fruit fly populations by various means, including cover sprays for severe infestations and attractant-based techniques (FAO, 1986). Chemical control has been recognized as the most effective pest management strategy (Ullah et al., 2012), with many growers resorting to insecticide application to control fruit flies, yielding some beneficial results (Khan et al., 2022). However, the efficacy of insecticides such as organophosphates and pyrethroids declines over time (Jin et al., 2011), requiring an understanding of the factors affecting their susceptibility for effective pest management. Environmental variables, including temperature, play an important role, with organophosphates showing a positive correlation and pyrethroids a negative correlation (Gao and Zheng, 1989). Host plants also

influence insecticide toxicity by influencing the metabolism of secondary metabolites by insects (Sheets, 2000), alongside factors such as insecticide concentration and population density (Musser and Shelton, 2005). In Pakistan, various insecticides have been tested for efficacy against *Bactrocera zonata*, with studies comparing the toxicity of different compounds (Haider et al., 2021). Similarly, studies have evaluated the toxicity of various insecticides against other economically important fly species, both in field and laboratory conditions (Gazit and Akiva, 2017). Despite extensive research on various fruit fly species, there is limited literature assessing the insecticidal toxicity of *Bactrocera zonata* under field or laboratory conditions. Therefore, the present study aims to evaluate the toxicity of different insecticides against field populations of *Bactrocera zonata* while investigating the distribution and host range.

### **Materials and Methods**

Various infested fruits and vegetables including cucumber, pumpkin, tomato, mango, peach, melon, bitter gourd, and squash were gathered from untreated local farmer's fields and marketplaces in the Peshawar Division of KP during 2019. These samples were transported to the Laboratory of Entomology section of the Agriculture Research Institute Tarnab Peshawar, for rearing and taxonomic identification of fruit fly species. Each damaged sample was initially placed in a separate plastic container containing 1-2 inches of moist sand at the bottom. The containers were labeled with essential field information such as sample name, location, collection/rearing date, and collector name. To minimize the risk of fungus attack, all containers were cleaned with 75% ethyl alcohol before separating the damaged samples and covered with muslin cloth. The containers were maintained at  $25\pm 2^{\circ}\text{C}$ , 65% RH, and a 16-hour photophase for 2-3 weeks in the laboratory until all fruit flies emerged. After 10-12 days, the host samples were checked to ensure that all maggots had exited the hosts and buried into the sand for pupation. The total number of adult fruit flies emerged from pupae was recorded, and species were identified based on diagnostic morphological features. Specimens were identified under a microscope (Leica MZ6) up to the species level with the assistance of available literature (Mahmood and Hassan, 2005). Pictorial keys from Prabhakar et al. (2012) were utilized for the identification process.

## **Chemicals**

Four technical grade insecticides, namely Chlorpyrifos EC40% (Chlorpyrifos), Arrivo EC10% (Cypermethrin), imidacloprid SC25% (imidacloprid), and Agenda EC 25% (Fipronil), were utilized individually and in combination to assess their toxicity effects. These chemical samples were acquired from pesticide companies including Syngenta, FMC, and Bayer for research purposes.

## **Bioassay**

The topical method for adults, were employed in this study. The field-recommended dose of all insecticides was selected, along with five serial dilutions and a control.

The efficacy of these four insecticides and their mixtures was tested on the adult stage of fruit fly species using the topical application method. Adult fruit flies aged 2-3 days were utilized in this experiment. These collected adults were placed in Falcon tubes of 50ml volume, with five adults in each tube, and exposed to low temperatures for 20-30 seconds to reduce their flying activity. Insecticides were topically applied to the thoracic region of each adult using a Hamilton micro applicator under a dissecting microscope. Each fly was treated with 0.25 $\mu$ l of insecticide and then immediately released into a labeled Falcon tube, capped with a tight lid having a small ventilation hole. Small cotton balls soaked with sucrose solution were placed at the bottom of the tubes for adult diet, and tubes were kept under controlled laboratory conditions. Mortality counts were made after 3, 6, 8, and 24 hours of holding period. Fruit flies found at the bottom of the tubes, unable to fly or climb, were counted as dead. The same procedure was repeated to test the efficacy of mixture insecticides, and mortality data were recorded.

## **Statistical analysis**

The data was subjected for the Probit analysis with the help of statistical package (STATISTIX 8.1).

## **Results and Discussions**

The results of the study showed the efficacy of different insecticides against adult fruit flies, imidacloprid exhibited the highest effectiveness, displaying the lowest LC50 values [3.5 (1.886±0.437)] after 24 hours. This was followed by cypermethrin [17.3 (0.748±0.193)], fipronil [92.9 (1.23±60.298)], and chlorpyrifos [26.2 (0.742±0.205)], respectively (Table 3). Additionally, results from Table 3 indicate that imidacloprid was the most efficacious insecticide, with maximum mortality observed after 8 hours of application, yielding the lowest LC50 [7.3 (1.624±0.267)]. Conversely, minimum mortality was observed after 3 hours of application, with an LC50 of [80.3 (1.091±0.208)]. CR values for adult fruit flies demonstrated that imidacloprid (1) remained the most effective after 24, 8, 6, and 3 hours, followed by cypermethrin, chlorpyrifos, and fipronil (Table 3). The overall results showed that cypermethrin was the greatest effective to kill 50% of the adult stage followed than imidacloprid. Imidacloprid killed more effectively 90% adult population after 24 hours. Cypermethrin combined with chlorpyrifos more effectively killed between 50 and 90 percent of adults stage of *B. zonata* after 24 hours of exposure. Its toxicity each insecticide increased with longer exposure and increased dose. cypermethrin caused 90% mortality *B. cucurbitae* (Rana et al., 2015). Cypermethrin is also seen good efficacy against another tephritid fruit fly *B. tyroni*. Toxicity evaluation of various insecticides on *Bactrocera* invasive species also demonstrated the effectiveness of cypermethrin and chlorpyrifos (Abdullahi et al., 2020). Its toxicity cypermethrin increased with increased dose in *Bactrocera* species (Lin et al., 2013) is consistent with the results of this study. The effectiveness of different insecticides was tested in three populations of *Bactrocera minax* which proved to be higher toxicity of Chlorpyrifos (Liu et al., 2015) while in us study chlorpyrifos in combination with cypermethrin is also effective against larval and adult stages of *B. diversa*. Imidacloprid proved less effective against *B. zonata* as compared to trichlorfon,  $\lambda$ -cyhalothrin (Khan and Naveed, 2017). Imidacloprid has been reported as less effective while (Yee and Alston, 2006) reported effective results of imidacloprid against the tephritis fly while in our study Imidacloprid is effective after cypermethrin. Other than *Bactrocera* species imidacloprid killed more *Anastrepha suspensa* at 8% active ingredient after 2-72 hours of exposure (Liburd et al., 2004). *B. dorsalis* is more sensitive to fipronil than *B. cucurbitae* through the oral and topical routes of exposure (Stark et al., 2009). Fipronil is the most toxic against two populations of *B. dorsalis* from two different

provinces of China while cypermethrin and imidacloprid are less toxic respectively (Wang et al., 2013). Application of insecticide for fruit fly management should be taken as a tool in combination with other environmentally friendly techniques.

**Table 1: Toxicity of different insecticides against the adult fruit flies under lab condition**

Insecticides	Exposure Time	LC 50	LC 90	Slop $\pm$ SE	Chi square	Comparative Ratio
Imidacloprid	3hr	80.3	1201.4	109.2 $\pm$ 0.207	2.5	1
	6hr	15.6	363.1	0.935 $\pm$ 0.187	0.9	1
	8hr	7.4	45.2	1.625 $\pm$ 0.267	1.5	1
	24hr	3.4	16.7	1.885 $\pm$ 0.436	1.2	1
Chlorpyrifos	3hr	1,667.3	31,458.4	1.006 $\pm$ 0.247	1.3	20
	6hr	1176.0	22,878.2	0.995 $\pm$ 0.231	0.5	75.7
	8hr	628.7	28,074.2	0.777 $\pm$ 0.200	0.8	86.2
	24hr	26.3	1,400.3	0.743 $\pm$ 0.206	0.6	7.4
Cypermethrin	3hr	422.1	8,806.1	0.970 $\pm$ 0.251	0.4	5.3
	6hr	211.5	7,101.8	0.841 $\pm$ 0.213	0.3	13.5
	8hr	104.5	11,899.1	0.624 $\pm$ 0.195	0.8	14.2
	24hr	17.9	898.7	0.749 $\pm$ 0.194	1.1	4.8
Fipronil	3hr	1651.8	30,612.2	1.012 $\pm$ 0.221	0.8	20.4
	6hr	437.9	12,043.2	0.891 $\pm$ 0.196	2.1	28.1
	8hr	92.8	1,676.3	1.021 $\pm$ 0.208	0.2	12.6
	24hr	24.8	271.6	1.23 $\pm$ 0.297	2.1	7.2

### Conclusions and Recommendations

The results of this study offer valuable insights for managing the invasive species of *Bactrocera zonata* in the specified area. They establish a foundation for effective pest management strategies.

Specifically, the timely application of cypermethrin emerges as a promising control option for local farmers, offering them an effective means to mitigate potential losses caused by this pest.

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