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Toxicity of Chemical Insecticides and Neem Oil on Cucurbit Fruit Fly *Bactrocera cucurbitae*

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ABSTRACT

Background: The popular vegetable sweet gourd (*Cucurbita moschata*) is cultivated throughout Bangladesh. However, its yield is low owing to the severe infestation by fruit fly *Bactrocera cucurbitae*. This study investigated the toxicity of chemical insecticides and neem oil on fruit flies infesting sweet gourd.

Methods: The repellency of adult fruit flies and larvae and the body weight and mortality of the larvae of the fruit fly were evaluated using three chemical insecticides- Karate 2.5 EC (cyhalothrin), Ripcord 10 EC (cypermethrin) and Shobicron 425 EC (profenofos Q+cypermethrin) and neem oil (azadirachtin).

Result: The repellency rate of the fruit fly larvae varied from 20.0±0.0 to 86.7±6.7% and that of the adult fly from 13.3±6.7 to 73.3±6.7%; Shobicron 425 EC, at 2000 ppm, exhibited the best results in both the cases. The treatments negatively affected the weight of larvae compared to the control, where Shobicron 425 EC caused the lowest weight (7.8±0.2 mg). The insecticides at 24, 48 and 72 h after treatment demonstrated toxic effects on the larvae, where the LC_{50} values ranged from 742.5 (550.4-1060.5) to 2476.3 (2150.6-2954.7) ppm at 72 h after treatment. On the whole, Shobicron 425 EC demonstrated better efficacy than the other insecticides against fruit flies.

Key words: *Bactrocera cucurbitae,* Body weight, *Cucurbita moschata,* Mortality, Repellency.

INTRODUCTION

The cucurbit fruit fly *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) is a polyphagous pest of cucurbit crops affecting more than 125 host plants worldwide and 81 crop plants in Bangladesh (Khatun *et al*., 2016). The yield losses due to fruit fly infestation vary in different fruits and vegetables in the country, up to 19.2% in cucumber and as high as 71.5% in sweet gourd (Amin *et al*., 2011). Therefore, the high incidence of fruit fly infestation is a serious obstacle to the yield and quality of sweet gourd.

Adult female flies usually insert eggs just below the epidermis of the young fruit and once hatched, the maggots feed on the fruit's internal pulp (Khatun *et al*., 2016). Controlling this pest is difficult owing to the larvae's internal feeding patterns.

Farmers in Bangladesh rely primarily on chemical insecticides to control its infestation. Fencord 10 EC (Cypermethrin) @ 2 ml/L of water, Shobicron 425 EC (Profenofos Q + Cypermethrin) @ 2 ml/L of water, Sumithione 50 EC (Fenitrothion) @ 1.12 L/ha (PTAC, 2020), Qiothion 57 EC (Malathion) @ 0.5 ml/L of water, Karate 2.5 EC (Cyhalothrin) @ 1 ml/L of water (Sarkar *et al*., 2017) and Ripcord 10 EC (Cypermethrin) @ 1 ml/l of warer (Nahar, 2021) are already in use against fruit fly in Bangladesh. Among the botanicals, neem products are widely used as they are naturally available materials and cheaper (Biswas, 2013), but there has been some evidence of the development of resistance of purified azadirachtin (Ascher, 1993). Due to the lack of awareness and information, it is easy to overdose or underdose insecticides, leading to negative consequences such as the development of insecticide resistance in insects.

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Thus, the importance of being aware of the performance of the currently used insecticides is undeniable. In this study, we selected four insecticides that are easily available in local market and are being used by the farmers for several years. To this end, this study was performed to determine the efficacy of selected insecticides and Neem oil against fruit flies *via* mortality and repellency tests.

MATERIALS AND METHODS

Insecticidal treatments

The study was conducted in the laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, maintained at 25°C and 80% RH. The insecticides Karate 2.5 EC, Ripcord

10 EC, Shobicron 425 EC and neem oil were used as treatments. Detailed information on the insecticides used in this study is presented in Table 1.

Observation of the repellency effects of insecticides on fruit flies

Larval repellency

Maggots of fruit flies were collected from infested sweet gourds. Fresh sweet gourd fruits were brought to the laboratory, cut into pieces, treated with insecticides at the recommended doses and air-dried. The surface of the Petri dish (diameter 14.5 cm) was divided into two halves, where treated and untreated pieces of fruit were placed separately at the periphery (Fig 1). Ten maggots were released at the center of the Petri dish. The maggots present on each half were counted at hourly intervals for up to 3 h. The observation for each treatment was repeated three times and the percent repellency was calculated using following formula (McDonald *et al*., 1970).

Repellency (%) =

$$\frac{\text{Number of leave present in untreated half - Number of leave present in treated half}}{\text{Number of leave present in untreated half + Number of leave present in treated half}} \times 100$$

Adult repellency

The treated and untreated fruit pieces were placed in separate Petri dishes (diameter 9.5 cm). These Petri dishes were then kept on two sides of an insect-rearing cage (height 32.5 cm, length 25.0 cm, breadth 21.5 cm), which was divided with a hard paper with the upper portion open so, that the adult insect was free to change its side. Ten adult fruit flies were released into each cage (Fig 2). The number of fruit flies on each side of the cage was counted at 1, 2 and 3 h after treatment (HAT). The observation for each treatment was repeated thrice; thereafter, the repellency data were recorded.

Observation of larval weight

Small pieces of fresh sweet gourd fruit were treated with insecticides at the recommended doses and then transferred to Petri dishes. The individual weight of the larva was measured using a digital weighing balance and placed in a Petri dish containing insecticide-treated fruit pieces. The untreated control was made with the larva by placing them in Petri dishes containing fresh fruit. The larval weight was measured for each treatment after three days. The weight of the Petri dish was first measured and then, the Petri dish containing one larva was weighed to obtain the individual larval weight. A total of 10 maggots were used in each treatment throughout the experiment.

Observation and calculation of larval mortality

Fresh sweet gourd fruits were collected from the field, cut into pieces and then treated with insecticides at five different doses. The applied doses for Karate 2.5 EC and Ripcord 10 EC were 1200, 1000, 750, 500 and 300 ppm and for Shobicron 425 EC were 2200, 2000, 1500, 1000 and 700 ppm. In the case of neem oil, the doses applied were 3200, 3000, 2000, 1000 and 700 ppm. The fruit pieces were then transferred to Petri dishes with maggots. Each Petri dish had 10 maggots and the fruit pieces were used as their diet. Only fresh fruits were provided as the larval diet in the untreated control. The number of dead larvae in each Petri dish was recorded at 24, 48 and 72 HAT and then, the percent mortality was calculated. Observations for each dose were repeated thrice and the means were used in the probit analysis. As per the WHO (2016) protocol, when the observed mortality in the untreated control exceeded 5%, the observed mortality under insecticide treatments was corrected using the formula proposed by Schneider-Orelli as outlined by Puntener (1981).

$$\text{Mortality (\%)} = \frac{\text{Number of dead larve per Petri dish}}{\text{Number of total larve per Petri dish}} \times 100$$

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality \% - Control mortality \%}}{\text{100 - Control mortality \%}} \times 100$$

Statistical analysis

One-way analysis of variance was performed followed by the Tukey's HSD post hoc test (at a 5% level of significance) to determine the variation in the effects of the insecticides on the repellency of larvae and adults, larval weight and mean larval mortality of fruit flies. Probit analysis was performed to analyze the dose-mortality response at 95% fiducial confidence intervals and the LC_{50} values with their fiducial limits were estimated. The toxicity ratios (TR) were calculated using the formula described by Gusmao *et al*. (2013), where TR = LC_{50} of the insecticide with less toxicity divided by LC_{50} of the other insecticides, individually. The statistical software package IBM SPSS 20.0 was used for data analysis.

RESULTS AND DISCUSSION

Repellency effects of insecticides on fruit fly

The repellency effects of Karate 2.5 EC, Ripcord 10 EC, Shobicron 425 EC and neem oil are presented in Table 2, with their respective recommended doses on *B. cucurbitae* maggots at different HAT. The repellent rates of the insecticides at 1 HAT demonstrated significant differences ($F_{3,8}$=12.3, p<0.01). Shobicron 425 EC demonstrated the highest repellent rate (86.7±6.7%) at 1 HAT and the lowest repellent rate (33.33±6.7%) was observed in the larvae treated with neem oil. The repellent rates of the insecticides on the maggots at 2 HAT also demonstrated significant differences ($F_{3,8}$=4.4, p<0.05). Shobicron 425 EC demonstrated the highest repellency (66.7±6.7%) of the maggots. By contrast, neem oil demonstrated the lowest repellent rate (26.7±6.7%). The repellent rates of different insecticides at 3 HAT varied significantly ($F_{3,8}$=15.2, p<0.01). The highest result (60.0± 0.0%) at 3 HAT was observed in Shobicron 425 EC-treated larvae and the lowest repellency effect (20.0±0.0%) at 3 HAT was observed under neem oil treatment.

The repellency effects of Karate 2.5 EC, Ripcord 10 EC, Shobicron 425 EC and neem oil on adult fruit flies at different HAT are presented in Table 2. The results at 1 HAT varied significantly ($F_{3,8}$=7.0, p<0.05). The highest repellent rate (73.3±6.7%) was observed in Shobicron 425 EC, which was statistically similar to the repellency effect of Karate 2.5 EC (66.7±6.7%). The lowest repellent rate in adults (33.3±6.7%) at 1 HAT was observed in the neem oil treatment. The repellent rates of the different insecticides at 2 HAT varied significantly ($F_{3,8}$=7.0, p<0.05). Shobicron 425 EC obtained the highest repellent rate (53.3±6.7%), which was statistically similar to that of Karate 2.5 E (46.7±6.7%). Neem oil demonstrated the lowest repellent rate (13.3±6.7%) at 2 HAT. The repellent rates at 3 HAT also showed significant variations ($F_{3,8}$=6.0, p<0.05). Shobicron 425 EC and Karate 2.5 EC showed the highest and similar repellency effects. The lowest repellent rate (13.3±6.7%) at 3 HAT was observed under neem oil treatment. Mawtham *et al.* (2019) reported that the repellent activity of neem seed kernel extract against cucurbit fruit fly was 64.6% at 1 hour after exposure and 31.3% at 48 h after exposure, with an overall mean of 47.9%. Shafiullah *et al.* (2016) found that 4% neem extract demonstrated the highest repellent rate in fruit flies at 1, 2 and 3 HAT. Although neem was reported to have a high repellent effect on fruit flies, in the current study, where the repellent efficacy of neem oil against fruit flies was tested along with synthetic insecticides, it showed the lowest efficacy.



Fig 1: Repellency test of fruit fly larvae using the tested insecticides.



Fig 2: Repellency test of adult fruit fly using the tested insecticides.

Fruit fly larval weight

The effects of different insecticides on the larval weight of fruit flies are presented in Table 3. The insecticides negatively affected the larval weight compared to that of the untreated control ($F_{4,10}$=28.4, p<0.001). The larvae treated with Shobicron 425 EC demonstrated the lowest weight (7.8±0.2 mg), which was statistically similar to that of Karate 2.5 EC (8.2±0.3 mg). The highest and lowest weight reductions were found in the Shobicron 425 EC (28.4%) and neem oil (11.0%) treatments, respectively. So, synthetic insecticides demonstrated a higher larval weight reduction than the neem oil treatment.

Mortality rate of fruit fly larvae

The tested insecticides at 24, 48 and 72 HAT revealed significant differences in the mortality of the larvae of *B. cucurbitae* (24 HAT: $F_{3,8}$=4.0, p<0.05; 48 HAT: $F_{3,8}$=13.3,

Table 1: Details of insecticides used in the experiment for their comparative effectiveness.

Trade name	Active ingredient	Mode of action	Recommended dose (ppm)
Karate 2.5 EC	λ-Cyhalothrin	Contact	1000
Ripcord 10 EC	Cypermethrin	Contact and stomach action	1000
Shobicron 425 EC	Profenofos Q+Cypermethrin	Contact and stomach action	2000
Neem oil	Azadirachtin	Systemic and contact	3000

Table 2: Effect of different insecticides (at the recommended dose) on the repellent rates of *B. cucurbitae* with sweet gourd.

Treatment	% Larval repellency			% Adult repellency		
	1 HAT	2 HAT	3 HAT	1 HAT	2 HAT	3 HAT
Karate 2.5 EC	66.7±6.7 ab	53.3±6.7 ab	46.7±6.7 ab	66.7±6.7 a	46.7±6.7 a	46.7±6.7 a
Ripcord 10 EC	46.7±6.7 bc	40.0±11.5 ab	26.7±6.7 bc	53.3±6.7 ab	33.3±6.7 ab	26.7±6.7 ab
Shobicron 425 EC	86.7±6.7 a	66.7±6.7 a	60.0±0.0 a	73.3±6.7 a	53.3±6.7 a	46.7±6.7 a
Neem oil	33.33±6.7 c	26.7±6.7 b	20.0±0.0 c	33.3±6.7 b	13.3±6.7 b	13.3±6.7 b

Data are presented as the mean±S.E. Means within a column followed by no common letter(s) are significantly different using the Tukey's post hoc test at p≤0.05 (HAT: hours after treatment).

p<0.01; 72 HAT: $F_{3,8}$=13.7, p<0.01; Fig 3). The highest and lowest mortality rates were found in larvae treated with Shobicron 425 EC and neem oil, respectively. Shobicron 425 EC showed the highest mortality which was statistically similar to the mortality under Ripcord 10 EC (48.1±3.7%). The lowest mortality was observed in the larvae treated with neem oil. The mortality at 72 HAT ranged from 54.5±0.0% to 81.1±3.8% and the best result was seen with Shobicron 425 EC.

Table 3: Effect of different insecticides (at the recommended dose) on the weight of *B. cucurbitae* larvae.

Treatment	Larval weight (mg)	% Weight reduction over control
Karate 2.5 EC	8.2±0.3 cd	24.8
Ripcord 10 EC	8.9±0.3 bc	18.3
Shobicron 425 EC	7.8±0.2 d	28.4
Neem Oil	9.7±0.3 b	11.0
Control	10.9±0.1 a	-

Data are presented as the mean±S.E. Means within a column followed by no common letter(s) are significantly different using the Tukey's post hoc test at p≤0.05.

The different insecticides at 24, 48 and 72 HAT demonstrated toxic effects on the maggots of cucurbit fruit flies (Table 4). The toxicity data revealed LC_{50} values ranging from 1147.5 (1036.4-1316.9) to 5573.0 (4298.2-8763.2). Ripcord 10 EC and neem oil showed the highest TR_{50} and lowest toxicity, respectively. The LC_{50} of the treatments at 48 HAT ranged from 950.0 (834.0-1128.7) to 3964.2 (3210.8-5529.9). Ripcord 10 EC and neem oil exhibited the highest and lowest results, respectively. LC_{50} values at 72 HAT ranged from 742.5 (550.4-1060.5) to 2476.3 (2150.6-2954.7). Ripcord 10 EC and neem oil demonstrated the highest TR_{50} (3.3) and lowest toxicity, respectively. A higher

Table 4: Toxicity of different insecticides on *B. cucurbitae* larvae.

Insecticide	Slope±S.E	LC_{50} (95% fl)	TR_{50}	x^2 (df)	P
		24 HAT			
Karate 2.5 EC	2.6±0.3	1217.3 (1062.0-1468.0)	4.6	6.5 (3)	0.091
Ripcord 10 EC	3.4±0.4	1147.5 (1036.4-1316.9)	4.9	1.1 (3)	0.590
Shobicron 425 EC	2.9±0.3	1856.6 (1679.1-2112.1)	3.0	2.6 (3)	0.465
Neem oil	2.1±0.3	5573.0 (4298.2-8763.2)	-	7.6 (3)	<0.05
		48 HAT			
Karate 2.5 EC	2.4±0.3	1234.8 (1065.2-1538.5)	3.2	7.8 (3)	<0.05
Ripcord 10 EC	2.2±0.3	950.0 (834.0-1128.7)	4.2	7.0 (3)	0.073
Shobicron 425 EC	2.8±0.3	1732.0 (1568.1-1956.7)	2.3	7.8 (3)	<0.05
Neem oil	1.8±0.3	3964.2 (3210.8-5529.9)	-	7.0 (3)	0.071
		72 HAT			
Karate 2.5 EC	2.9±0.3	857.4 (649.8-1351.5)	2.9	9.2 (3)	<0.05
Ripcord 10 EC	2.8±0.3	742.5 (550.4-1060.5)	3.3	8.4 (3)	<0.05
Shobicron 425 EC	3.8±0.3	1331.3 (972.5-1760.7)	1.9	11.8 (3)	<0.01
Neem oil	2.0±0.2	2476.3 (2150.6-2954.7)	-	5.1 (3)	0.162

Data are presented as the mean of three replicates, with each setup containing 10 larvae. The concentrations were expressed in ppm, where fl. represents the fiducial limit.

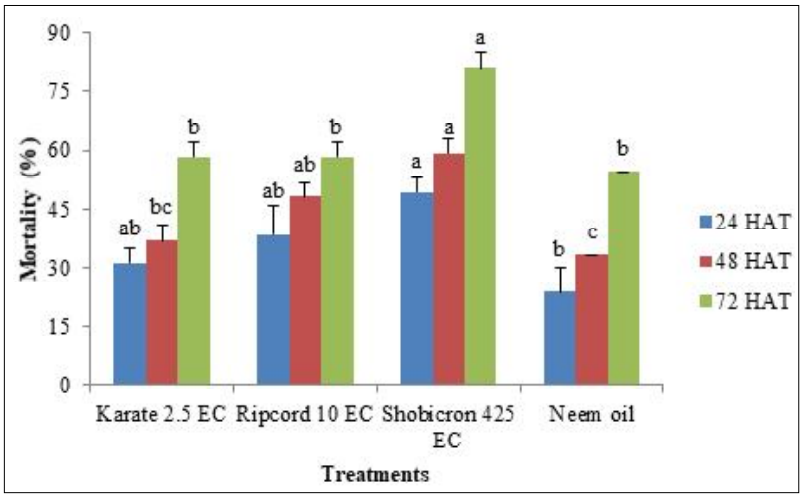


Fig 3: Effects of different insecticides on the mortality rate of *B. cucurbitae* larvae.

slope value indicated that small variations in concentrations induced greater responses to mortality.

The toxicity of the insecticides clearly showed that the mortality of the maggots of the cucurbit fruit fly varied with the insecticides, their concentrations and exposure periods. These insecticides contain broad-spectrum toxic substances that interrupt the normal physiology of insects, including their feeding and survival. A lower mortality rate indicates lower insecticide toxicity in insects. According to the current findings, although Ripcord 10 EC demonstrated the highest toxicity, based on mean larval mortality, it was observed that Ripcord 10 EC did not show high efficacy at its recommended dose. By contrast, with moderate toxicity, Shobicron 425 EC showed the maximum larval mortality at the recommended dose.

In the present study, LC_{50} revealed that none of the tested insecticides performed satisfactorily against fruit fly larvae indicating that the insects started to display resistance to these insecticides. These findings support the results of Nadeem *et al*. (2014), who reported that most field populations of *B. zonata* have developed resistance toward trichlorfon, malathion (organophosphates), bifenthrin, lambda-cyhalothrin (pyrethroids) and spinosad (microbial). The current findings also support the results of Jin *et al*. (2011), who provided evidence of the evolution of trichlorphon, β-cypermethrin and avermectin resistance in *B. dorsalis* populations at an alarming pace. The application of insecticides while ignoring the recommended dose may be the reason for insecticide resistance of insects.

CONCLUSION

The results obtained in the present study revealed that Shobicron 425 EC, Ripcord 10 EC, Karate 2.5 EC and neem oil demonstrated toxic and repellent effects on the larvae of *B. cucurbitae*, affecting their growth and development. Shobicron 425 EC exhibited the highest comparative efficacy against cucurbit fruit fly. However, the larval mortality under laboratory conditions showed that fruit flies began to display resistance to the applied insecticides.

Conflict of interest

The authors declare no conflict of interest among them.

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