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Mortality and growth inhibition of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guen.) by buprofezin, a potent chitin synthesis inhibitor

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Abstract
In the present laboratory study, different concentrations of award 40 SC (Buprofezin) have been applied (topical, potato-dip and topical + potato-dip method) on the 2nd instars larvae of brinjal shoot and fruit borer (*Leucinodes orbonalis* Guen.) and then mortality, weight reduction or cuticular change was observed at 3 HAT (hours after treatment), 3 and 7 DAT (days after treatment) application. No mortality or weight reduction was observed at 3 HAT. The significant level of mortality and weight reduction was observed at 3DAT from all applying methods but the maximum mortality and weight reduction was found at 7 DAT. It was also observed that potato-dip method was highly effective than topical method regarding mortality and weight reduction while almost similar efficacy was found from potato-dip and combination (topical + potato-dip) methods. Cuticular abnormalities were found when larvae were treated with higher concentrations of buprofezin in comparison with that in the water-treated control. Moreover, larval mortality and weight reduction were clearly dose-dependent. The lower dose (200 ppm) was ineffective for topical application method but was effective in case of potato-dip and combination methods. Therefore, it is concluded that buprofezin is a potent IGR (insect growth regulator) molecule to reduce *L. orbonalis* populations through inhibition of chitin biosynthesis.

Keywords: Buprofezin, cuticular abnormality, *Leucinodes orbonalis*, mortality, weight reduction.

1. Introduction
Brinjal (*Solanum melongena*) is one of the most important vegetables in South and South-East Asia. In Bangladesh it is the second most important vegetable crop after potato in relation to its total production [4]. Various insects cause enormous loss to this vegetable throughout the season in Bangladesh as well as in Indian sub- continent [1, 8]. Brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. (Lepidoptera: Pyralidae) is the most destructive pest and is considered to be the limiting factor in quantitative as well as qualitative harvest of brinjal fruits. Only the larvae of this pest cause 12-16% damage to shoots and 20-60% to fruits [2, 14]. The pest is very active during the rainy and summer season and often causes more than 90% damage [3, 12]. The yield loss has been estimated up to 86% in Bangladesh [3] and up to 95% in India [16].
Chemical control is the most common practice in Bangladesh to control *L. orbonalis* as well as to produce blemish-free brinjal fruits. More accurately, 98% brinjal-growers in Jessore district (southern part of Bangladesh) relied exclusively on the use of conventional pesticides and they use those pesticides 140 times or more in the 6–7 month cropping season [6]. Brinjal being a vegetable crop, use of chemical insecticides will leave considerable toxic residues on the fruits which is certainly harmful for human health. In addition, sole dependence on chemical insecticides for controlling *L. orbonalis* has led to insecticide resistance, secondary pest outbreak, killing of non-target organisms, environmental hazards etc. Therefore, effort has been made since last 20 years with the development of natural and synthetic analogs capable of interfering with the processes of growth, development, moulting and metamorphosis of the target pests. These chemicals have been called “Insect Growth Regulators” (IGRs). The mode of action of IGRs is not central nervous system-oriented but kills insects potentially through cessation of moulting process [5].
Buprofezin, a potent chitin synthesis inhibitor (CSI) reduces pest population by preventing moulting through inhibition of chitin bio-synthesis. Moreover, buprofezin has multiple effects

on the target pests like reduction of fecundity, egg hatchability, egg sterility, production of abnormal larvae and pupae [18, 22]. Buprofezin was found to be very effective against Hemipteran pests, few Lepidopteran larvae, spiders etc [7, 11, 15, 17, 18, 21]. It was reported that buprofezin is an eco-friendly biorational pesticide that is safe for non-target organisms, highly biodegradable and action is target pest specific [20]. However, no information is available yet on the impact of buprofezin against brinjal shoot and fruit borer, *L. orbonalis*. The present investigation was, therefore, planned to evaluate the efficacy of buprofezin on the mortality, weight reduction and cuticular deformation under laboratory conditions.

2. Experimental methods

The efficacy of different doses of buprofezin was evaluated on the mortality, weight reduction and cuticular deformation of brinjal shoot and fruit borer, *L. orbonalis* (Guen) in the laboratory, Department of Entomology, Bangladesh Agricultural University, Mymensingh. The study was conducted from the period of July 2013 to June 2014.

2.1 Mass rearing of *L. orbonalis*

Brinjal shoot and fruit borer was reared in the laboratory condition to get enough larvae for experimental purpose. The infested fruits and shoots of brinjal were collected from the field and then larvae were carefully collected by dissecting them. After that larvae were transferred into different plastic jars based on the larval stages and immediately provided sliced potatoes (potato is the most favourite alternate hosts of *L. orbonalis*) as their feed. When larvae were transformed into 5th instars, they came out by making hole and were usually characterized by their large size and bright-reddish colour. After that larvae were provided with dry brinjal leaves for successful pupation. After pupation, healthy pupae were separated carefully and kept inside a mosquito net chamber for getting into adults. Then male and female adults were transferred into an artificially made oviposition chamber for mating and egg laying. The inner surface of oviposition chamber was lined with green paper and mosquito net for providing natural environment. Then a brinjal seedling was kept inside the oviposition chamber for favouring the microenvironment for more egg laying by the moths. 5% dextrose was also provided inside the chamber as the feed of moths. After mating, eggs were laid singly on the brinjal leaves, green paper and mosquito nets. Eggs containing leaves, papers and nets were separated and then kept in the hatching chamber. Eggs were hatched within 2-3 days after oviposition and neonate larvae were immediately placed on sliced potato. This way, larvae were continuously reared to get adults. 2nd instars larvae were used for further experimentation.

2.2 Specifications of treatments

Experiments consisted of three treatment combinations. Three doses of buprofezin (Award 40SC, Square Pharmaceuticals Ltd.) i.e. 200, 400 and 800 ppm were provided as treatments. Each treatment was replicated thrice and ten larvae were used for each replication.

2.3 Treatments application methods

All the treatments were applied through three methods.

2.3.1 Topical application: In this method, buprofezin was directly applied on the larvae. After that, the treated larvae were transferred on the untreated sliced-potato tubers and kept in rearing chambers.

2.3.2 Potato-dip method: In this method, sliced potato tubers were treated with different concentrations of buprofezin and then treated potato slices were dried for few minutes. After that untreated larvae were placed on treated potato slice and changes were observed.

2.3.3 Combination (topical + potato-dip) method: In this method both larvae and potato tubers were treated. After treatment, the treated larvae were then transferred on previously treated potato tubers.

2.4 Data Collection

Data on the mortality was observed at 3 HAT (hours after treatment), 3 and 7 DAT (days after treatment) application. Larval weight was measured at 3 and 7 DAT. Based on the experimental time-frame, potato slices were dissected very carefully and data were recorded. Died larvae were separated and alive larvae were further provided with fresh potato slices.

The percentage of mortality was also calculated using the following formula;

$$\textbf{\% mortality = Po/Pr X 100}$$

Where,
Po = Number of larvae died due to treatment application
Pr = Total number of treated/untreated larvae

2.5 Statistical analyses

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done with the help of computer package MSTAT. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) when necessary.

3. Results

3.1 Comparative efficacy of different doses of buprofezin through topical application method

3.1.1 Effects on the mortality

The topical effects of different doses of buprofezin on the mortality of brinjal shoot and fruit borer has been shown in the table 1 ($P<0.05$). The results clearly revealed that buprofezin is moderately effective against *L. orbonalis* and the effect was clearly dose-dependent. No mortality was found at 3 hours after treatment application, the significant effect was found at 3 DAT ($P<0.05$) which was consistent up to day 7 ($P<0.05$). The maximum 44.44% mortality was recorded from 800 ppm which was followed by 400 and 200 ppm of buprofezin respectively. The lowest mortality (16.30%) was found from 200 ppm which was comparable with water-treated control (11.11%).

Table 1: Mean percent mortality of *Leucinodes orbonalis* larvae at different time interval after treating with different concentrations of buprofezin through topical application method.

Treatments	Mean percent mortality of *L. orbonalis*		
	3 HAT	3 DAT	7 DAT
Award -800 ppm	0.00	32.64c	44.44c
Award- 400 ppm	0.00	24.58b	28.28b
Award- 200 ppm	0.00	12.91a	16.30a
Control	0.00	11.11a	11.11a
Significance level	NS	P<0.05	P<0.05

In a column, means of similar letter (s) do not differ significantly as per DMRT.
[HAT: Hours After Treatment, DAT: Days After Treatment, NS: Not Significant]

3.1.2 Effects on weight reduction

Buprofezin had dose dependent and significant effect on the larval weight reduction (P<0.05) (Table 2). The weight of larvae was gradually decreased when larvae were directly treated with buprofezin. The maximum weight reduction (37.04%) was observed from 800 ppm of buprofezin which was followed by 400 (27.29%) and 200 ppm (5.10%) respectively. The lowest concentration of buprofezin (200 ppm) had no any effect on the weight reduction and was comparable with water treated control (54.07 vs. 56.98 mg/larva respectively).

Table 2: Weight reduction of larvae of *Leucinodes orbonalis* at different time interval after treating with different concentrations of buprofezin through topical application method.

Treatments	Pre-treated weight (mg/larva)	Weight after treatment application (mg/larva)		Cumulative mean (mg/larva)	%reduction over control
		3 DAT	7 DAT		
Award -800 ppm	4.28	16.91c	54.84b	35.87c	37.04
Award-400 ppm	4.40	25.53b	57.32b	41.43b	27.29
Award-200 ppm	4.57	38.33a	69.81a	54.07a	5.10
Control	4.50	37.67a	76.28a	56.98a	
Significance level	NS	P<0.05	P<0.05		------

In a column, means of similar letter (s) do not differ significantly as per DMRT.
[DAT: Days After Treatment, NS: Not Significant]

3.2 Comparative efficacy of different doses of buprofezin through potato-dip method

3.2.1 Effects on the mortality

Significant larval mortality was found when untreated larvae were provided buprofezin-treated potato slices (P<0.01) (Table 3). It has also been shown that the potato-dip method was comparatively more effective than topical application method regarding mortality percentages. The trend of buprofezin effect was similar with that of topical application method. Specifically, the maximum 66.66% mortality was recorded from 800 ppm which was followed by 400 (43.33%) and 200 ppm (33.33%) of buprofezin respectively. It was interesting observation that the lowest concentration had significant effect on the larval mortality (33.33%) while topical application method had insignificant effect. The lowest mortality was recorded from untreated control (13.33%).

Table 3: Mean percent mortality of *Leucinodes orbonalis* larvae at different time interval after treating with different concentrations of buprofezin through potato-dip method.

Treatments	Mean percent mortality of *L. orbonalis*		
	3 HAT	3 DAT	7 DAT
Award -800 ppm	0.00	45.45c	66.66d
Award-400 ppm	0.00	23.33b	43.33c
Award-200 ppm	0.00	13.33a	33.33b
Control	0.00	11.66a	13.33a
Significance level	NS	P<0.05	P<0.01

In a column, means of similar letter (s) do not differ significantly as per DMRT.
[HAT: Hours After Treatment, DAT: Days After Treatment, NS: Not Significant]

3.2.2 Effects on weight reduction

Buprofezin had dose dependent effects on the reduction of larval weight (Table 4). The weight of larvae was gradually decreased with increasing concentration level and time. The maximum weight reduction was observed on 7 DAT. Approximately 80% weight reduction was observed when larvae fed treated potato with 800 ppm followed by 400 ppm (56.23%) and 200 ppm (29.44%) respectively. The lowest concentration of buprofezin (200 ppm) had significant effect (29.44%) on the weight reduction while topical method had no significant effect.

Table 4: Weight reduction of larvae of *Leucinodes orbonalis* at different time interval after treating with different concentrations of buprofezin through potato-dip method.

Treatments	Pre-treated weight (mg/larva)	Weight change after treatment application (mg/larva)		Cumulative mean (mg/larva)	%reduction over control
		3 DAT	7 DAT		
Award -800 ppm	4.63	5.88d	18.86d	12.37d	79.96
Award-400 ppm	4.57	16.98c	37.08c	27.03c	56.23
Award-200 ppm	4.47	32.80b	54.33b	43.57b	29.44
Control	4.6	49.22a	74.27a	61.75a	------
Significance level	NS	P<0.01	P<0.01		

In a column, means of different letter (s) differ significantly as per DMRT.
[DAT: Days After Treatment, NS: Not Significant]

3.3 Comparative efficacy of different doses of buprofezin through combination (topical + potato-dip) method

3.3.1 Effects on the mortality

The highest larval mortality was found when larvae and potato slices both were treated with buprofezin than individual treatment (Table 5). No mortality was found from 3 HAT but larvae were significantly died at 3 DAT which further increased at 7 DAT. At 7 DAT, the maximum mortality was found from 800 ppm (69.44%) of buprofezin which was followed by 400 ppm (50.0%) and 200 ppm (36.67%) respectively. The significant mortality was found even from 200 ppm (36.67%) of buprofezin in comparison with that in the water-treated control. The lowest mortality was recorded from untreated control (12.7%).

Table 5: Mean percent mortality of *Leucinodes orbonalis* larvae at different time interval after treating with different concentrations of buprofezin through combination (topical + potato-dip) method.

Treatments	Mean percent mortality of *L. orbonalis*		
	3 HAT	**3 DAT**	**7 DAT**
Award -800 ppm	0.00	49.91d	69.44d
Award-400 ppm	0.00	34.41c	50.00c
Award-200 ppm	0.00	23.33b	36.67b
Control	0.00	11.10a	12.70a
Significance level	NS	P<0.01	P<0.01

In a column, means of different letter (s) differ significantly as per DMRT.
[HAT: Hours After Treatment, DAT: Days After Treatment, NS: Not Significant]

3.3.2 Effects on weight reduction

The pattern of weight reduction was similar with that of potato-dip method although reduction level was higher than potato-dip method (Table 6). It was observed that larval weight gradually reduced with increasing time and the effect was clearly dose-dependent. Approximately 83% weight reduction was observed from 800 ppm of buprofezin which was followed by 400 and 200 ppm of buprofezin. About 35% weight was even reduced when larvae and potato both were treated with lower concentrations (200 ppm) of buprofezin. The lowest reduction was observed from water-treated control.

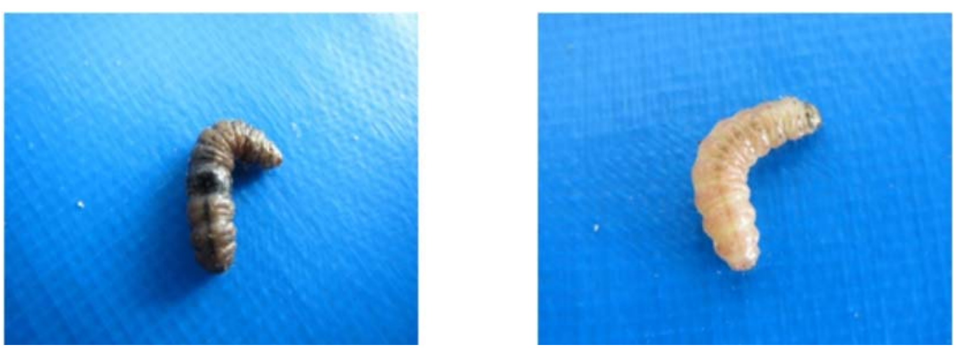
Table 6: Weight reduction of larvae of *Leucinodes orbonalis* at different time interval after treating with different concentrations of buprofezin through combination (topical + potato-dip) method.

Treatments	Pre-treated weight (mg/larva)	Weight change after treatment application (mg/larva)		Cumulative mean (mg/larva)	%reduction over control
		3 DAT	**7 DAT**		
Award -800 ppm	4.63	5.62d	16.14d	10.88d	82.78
Award-400 ppm	4.57	15.81c	32.26c	24.03c	61.97
Award-200 ppm	4.67	29.47b	52.78b	41.13b	34.91
Control	4.53	48.62a	77.75a	63.19a	-------
Significance level	NS	P<0.01	P<0.01	P<0.01	

In a column, means of different letter (s) differ significantly as per DMRT.
[DAT: Days After Treatment, NS: Not Significant]

3.4 Effects on cuticular deformations

Buprofezin is a chitin synthesis inhibitor i.e. it affects moulting process by inhibiting chitin bio-synthesis. In this study it was observed that buprofezin potently inhibits chitin synthesis and thereby the colour of cuticle was changed and cuticle was fractured (Fig. 1). The maximum deformation was observed when larvae were treated with higher concentrations of buprofezin through potato-dip and combined methods. A comparatively weak change was found from 200 ppm of buprofezin than 400 and 800 ppm.



[A] **[B]**

Fig 1: Representative photomicrographs of the larvae of *Leucinodes orbonalis* [A] treated with 800 ppm of buprofezin [B] treated with water are shown at 7 days after treatment (DAT) application. Severe cuticular deformation and colour changes [A] were observed when normal larvae were treated with 800 ppm of buprofezin while no changes were observed when treated with only water. 2nd instars larvae were treated with either buprofezin or water and changes were observed at 7 DAT. The cuticular change was gradually increased with increasing time and the severe changes were observed at 7 days after buprofezin application.

4. Discussion

In the present study it has been observed that buprofezin worked as a potent insect growth regulator (IGR) against brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guen). Buprofezin has been applied directly on the larvae (topically) and indirectly (through consumption) and it has been clearly investigated that buprofezin worked more potently when it reached to the stomach through food than contact action.

An effect of different concentrations of award 40 sc (buprofezin) has been observed on the mortality, weight reduction and cuticular deformations in the laboratory conditions. The acute (3 hrs after treatment) and chronic effects (3 and 7 days after treatment application) of buprofezin were observed on the mortality, weight reduction and cuticular deformations of *L. orbonalis* larvae.

At 3 hours after treatment application, no effect was found on the mortality of the larvae irrespective with the application method which suggests that buprofezin needs comparatively longer time to kill the lepidopteran larvae like *L. orbonalis* which has been fitted with its mode of action [10, 19, 22, 24]. Buprofezin is basically a chitin synthesis inhibitor (CSI) under the class insect growth regulator (IGR) which potently inhibits moulting [13].

Unlike traditional chemical insecticides, buprofezin reduces pest population by preventing moulting through the inhibition of chitin bio-synthesis. Chitin is a major component of the insect exoskeleton. Insects poisoned with buprofezin are unable to synthesize new cuticle, thereby preventing them from moulting successfully to the next stage and ultimately leading to death by fracturing the cuticle [23]. However, it takes at least 2-3 days for the completion of each successful moulting and accordingly no effect was found on the mortality just immediately after buprofezin application. The mortality was significantly increased at 3 days after treatment (DAT) application that further increased at 7 DAT which suggests that it takes longer time to inhibit cuticle synthesis through the interaction of buprofezin and insects intercellular molecules. It has also been found that the mortality was clearly dose-dependent which further confirmed that the inhibition of cuticle synthesis is positively correlated with the concentrations of buprofezin. Like as mortality, the growth of larvae was significantly inhibited by buprofezin and that was also dose-dependent. It raises the possibility that the buprofezin molecules interrupted the intercellular molecular action (e.g. enzymetic, peptidergic, hormonal) and thereby growth was inhibited. Moreover, larval growth may be prevented due to the inhibition of new cuticle synthesis. The cuticular deformation was clearly observed when larvae were treated with buprofezin which also suggests that the target of buprofezin was cuticle and buprofezin has blocked the cuticular pigments formations.

In this study, different concentrations of buprofezin were applied against larvae of *L. orbonalis* through direct (topical) and indirect (feeding with potato) methods to know how buprofezin works more effectively either contact or stomach action. It has been clearly observed that larval mortality and growth reductions were higher when larvae fed buprofezin-treated potato than larvae directly treated with buprofezin which raises the possibility that buprofezin worked more effectively in the stomach than contact. The present findings clearly suggested that moulting or chitin bio-synthesis is an inter-physiological process than simply cuticular process. The findings of stomach action of buprofezin might be helpful to control *L. orbonalis* in the field conditions as they are internal feeders. It is almost known that buprofezin is not a true systemic insecticide but has potential translaminar movement action [20]. Recently Jahangir *et al* (unpublished data) observed from the laboratory experiment that almost 95% brown planthoppers were died when rice plants were sprayed with buprofezin which clearly suggests that buprofezin might have systemic action.

5. Conclusion

Buprofezin has been widely using against sucking insects but until now narrowly tested against Lepidopteran pests like *Spodoptera litura*, *Spodoptera littoralis* etc. [9, 18, 21]. For the first time, the effect of buprofezin has been evaluated against *Leucinodes orbonalis*, an internal feeder of brinjal and has been found effective. This effectiveness can be increased more when buprofezin will be applied in combination with other chemical insecticides as sub-lethal doses rather than individual application.

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