RESEARCH ARTICLE

INSECTICIDAL AND INHIBITORY POTENTIAL OF TWO CHITIN SYNTHESIS INHIBITORS, BUPROFEZIN AND LUFENURON ON OKRA SHOOT AND FRUIT BORER, *EARIAS VITTELLA*

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ARTICLE DETAILS

ABSTRACT

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In this laboratory study, insecticidal and inhibitory potential of two chitin synthesis inhibitors (CSIs) viz. Buprofezin and Lufenuron were evaluated against okra shoot and fruit borer (OSFB). CSIs were applied on three days old larvae through different bioassay methods like topical or direct, okra-dip or indirect and combined (topical + leaf-dip). Data were collected on larval mortality, weight reduction and deformations of larvae and adults. Results showed that larval mortality and weight reduction were clearly dose, application methods and time dependent. In case of both CSIs, the highest mortality was found @ 1.0 ml/L that was followed by 0.75 ml and 0.50 ml/L respectively. Likewise, the highest body weight reduction was also found from 1.0 ml/L. In both cases, the concentration 0.5 ml/L was found less effective. In case of application methods, combined bioassay method was found superior than that of rest of the two methods. Moreover, a significant percentage of deformed larvae and adults were developed when 3 days old larvae were treated with either Buprofezin or Lufenuron @ 1.0 ml/L through combined bioassay method. The concentration 0.5 ml/L had very less efficacy on the development of deformed larvae or adults. Therefore, based on the laboratory findings Buprofezin and Lufenuron may be the potential component in IPM programmed, rather than their individual application, for controlling okra shoot and fruit borer as well as production of safe okra.

KEYWORDS

Buprofezin, Lufenuron, Mortality, Reduction, Deformation

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an important summer vegetable. It is belonging to the family Malvaceae of tropical and subtropical regions. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. In Bangladesh, it is an important vegetable grown in kharif season when rainfall, humidity, temperature is favorable for cultivation (Reddy et al., 2017; Acharya, 2010). Okra is a nutritious vegetable which plays an important role to meet the demand of vegetables of the country when vegetable are scanty in the market (Rahman et al., 2017). Green okra is the important sources of vitamins (A, B, C), fat, carbohydrates and minerals like potassium, magnesium, calcium and iron. Okra plant is highly susceptible to various biotic factors and among them insect infestation is more destructive one. Some of the important insect pests are shoot and fruit borer, aphids, jassids, leaf roller, white flies, mealy bug etc. Among these pests, okra shoot and fruit borer (OSFB) (*Earias vittella*) is considered as the most notorious one which causes both quantitative and qualitative losses of okra (Butani and Jotwani, 1984; Acharya, 2010). In case of severe infestation OSFB causing upto 55% yield loss of the crop (Patel et al., 2012).

Various control strategies such as biological, chemical, cultural methods have been evaluated against this insect pest (Patra et al., 2009; Parmar et al., 2013; Pardeshi et al., 2011). Conventionally, farmers are using various types of synthetic chemical insecticides to control OSFB in field condition

but it creates several problems in agro-ecosystem and develop high level of resistance. Conventional insecticides are fast-acting, convenient and economical, making them the most powerful tools in pest management (Misra and Senapati, 2003). But injudicious and indiscriminate uses of insecticides in many cases causes or accelerates insecticide resistance, pest resurgence, secondary pest outbreak, environmental contamination, persistent residual toxicity and destruction of beneficial insects (Qayyum et al., 2015; Ratanpara and Bharodia, 1989; Sinha and Singh, 2009).

Attempts are therefore being made to develop more specific agents that will do work without leading further environmental degradation, safe for bio-control agents and human health and will not develop resistance or develop slower resistance. The recent discovery of a new class of chemicals, the chitin synthesis inhibitor (CSI), an specific analogue of insect growth regulators (IGRs) may be a step towards achieving this goal. Chitin, a polysaccharide, is a major components of insect cuticle. As insects develop from immature stage to adults, they undergo several molts during which they shed their old cuticle and form new one (Haroardottir et al., 2019; Uchida et al., 1985). Diflubenzuron, the main chemical of CSIs, disrupt moulting process by interfering chitin synthesis and kill insects before attaining maturity (Tail et al., 2008). This chemical compound is also highly effective to inhibit the growth and development of insect by reducing body weight and develop deform larvae or adults (Nasr et al., 2010).

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Underweight or abnormal insects gradually dies and alive insects become unable for further reproduction. Higher animals that do not produce chitin might not be affected by this chemical (Wright, 2010). Several studies have been conducted on the effect of CSIs on the mortality as well body weight reduction of sucking insects like jassids, whitefly, aphids etc (Gogi et al., 2006; Saleem and Akbar, 2019). But very few studies were done on the effects of CSIs on lepidopteran insect mortality or deformations. Therefore, the present study was designed to determine the insecticidal and inhibitory effects of two chitin synthesis inhibitors viz. Buprofezin and Lufenuron on larval mortality, growth and development as well as larval and adult deformity of OSFB under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Mass rearing of *E. vittella*

Okra shoots and fruit borer infesting pods were collected from okra field and then kept in sterilized glass jars until pupal development. Once developed, pupae were collected and kept in glass jar until adult formation. After 5-7 days, adults were formed. Then male and female adults were carefully released inside the mylar cage along with previously grown okra plants and immediately covered the upper face of the mylar cage through mosquito net to prevent escaping. After mating, the female moths were started to laying eggs on the fresh leaf, tender stem, buds as well as on mosquito net. After that egg-laid leaves, stem, bud and mosquito nets were carefully collected and kept in sterilized petridishes. After 3-4 days, the eggs were hatched and newly hatched larvae come out. Fresh okra fruits were supplied to the neonate larvae for feeding and this help the larvae for its proper growth and development. 3-4 days old with uniform sized larvae were used in all experiments. This rearing technique was continued until the end of the experiments to get enough larvae for conducting experiments smoothly (Figure 1).

Figure 1: Protocols of mass rearing of okra shoot and fruit borer under laboratory condition (A-I). A: Infested okra fruit along with larvae were collected from field, B: Pupae are formed on the fruit surface, C: Collected pupae, D: Adult of OSFB, E: Seedlings of okra were raised on plastic pot, F: Male and Female moths were released inside the plastic cage along with okra seedling and top was covered with mosquito net, G-H: Eggs were laid on tender leaves and shoots, I: 1st and 2nd instar larvae inside the petridish

2.2 Chitin synthesis inhibitors and their concentrations

Two chitin synthesis inhibitors viz. Award 40 SC (*Buprofezin*) and Heron 5 EC (*Lufenuron*) were evaluated with three concentrations of each like 0.50, 0.75 and 1.0 ml/L. The CSIs were purchased from local pesticide dealer shop of Mymensingh town, Bangladesh.

2.3 Bioassay methods

Treatments were applied through three application method viz topical or direct, okra-dip or indirect and combine (topical + okra-dip) method.

2.4 Direct or topical method

In this method, different concentrations of Award 40SC and Heron 5EC were carefully applied on the cuticular surface of neonate larvae (n=15 per replication) using micro-pipette (Eppendorf, Germany). Then treated larvae were gently transferred on untreated fresh okra pods and finally placed in petridishes. Concurrently, untreated insects were placed on fresh untreated okra fruits as control treatment.

2.5 Indirect or okra-dip method

In this method, tender okra fruit was dipped in different concentrations of Buprofezin and Lufenuron for 15-20 seconds. Then okra fruit were taken out from the solution and dried on tissues. Then untreated larvae (n=15 per replication) were transferred gently on treated okra fruits. Then larvae with okra fruits were kept in petridishes for observation. Moist cotton is placed inside the petridish to avoid desiccation of okra fruits. Concurrently, untreated insects were placed on fresh untreated okra fruits as control treatment.

2.6 Combined (direct + indirect) method

In this method, both larvae (n=15 per replication) and okra fruits were treated with different concentrations of selected CSIs. After that treated fruits were air dried and then transferred in Petri dishes. Then, treated larvae were carefully transferred on treated okra fruits. Moist cotton is placed inside the Petri dish to avoid desiccation of okra fruits. Concurrently, untreated insects were placed on fresh untreated okra fruits as control treatment.

2.7 Data collection

Data were collected on larval mortality, larval weight reduction and deformity of larvae and adults at different time interval. Mortality data was collected at 3 and 7 DAT, larval weight reduction and deformation data were collected at 7 DAT. Adult deformity data was collected when larvae reached into adulthood. Then, percent larval mortality and weight reduction over control was calculated using following formulae:

$$\% \text{ Mortality} = \frac{Po}{Pr} \times 100$$

Where,

Po=Number of died larvae

Pr=Number of treated or untreated larvae provided

$$\% \text{ weight reduction over control} = \frac{Wt - Wc}{Wt} \times 100$$

Wt = Mean weight of jassid in treated condition

Wc = Mean weight of jassid in untrated or control condition

2.8 Statistical analysis

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 Effect of Award 40 SC on the mortality of *E. vittella* larvae

Insecticidal potential of Award 40 SC on the percent mortality of OSFB larvae has been shown in table-1. Mortality trend was clearly time, concentrations and application methods dependent. In case of both CSIs, the highest mortality was found at 7 DAT from 1.0 ml/L through combined bioassay method. 64.12% mortality over control was found at 7 DAT @ 1.0 ml/L through combined bioassay method that was followed by 61.74% and 53.33% through okra-dip and topical bioassay method. Comparatively lower percentages of mortality were found from 0.75 ml/L through combined, okra-dip and topical application methods respectively. Least efficacy was found from the concentration 0.50 ml/L. On the other hand, poor mortality was found at 3DAT from all three concentrations and bioassay methods (Table 1).

Table 1: Mean percent mortality of OSFB larvae at different time interval following treated with different concentrations of Award 40 SC through different bioassay methods.

Treatments	% mortality in different bioassay methods					
	Direct or topical		Leaf-dip		Combined	
	3 DAT	7 DAT	3 DAT	7 DAT	3 DAT	7 DAT
Award 40 SC @ 0.50 ml/L	13.09c	17.85c	13.69b	18.26c	13.88b	22.22c
Award 40 SC@ 0.75 ml/L	22.19b	42.85b	28.89a	46.67b	33.33a	50.00b
Award 40 SC@ 1.00 ml/L	30.00a	53.33a	32.95a	61.74a	37.43a	64.12a
Control	4.76d	10.04d	4.76c	10.04d	4.76c	10.00d
CV (%)	7.67	9.17	8.32	6.36	7.80	5.86
LSD _{0.05}	2.80	3.12	1.63	2.78	2.20	2.25

In a column, means followed by different letters are significantly different 5% level of probability. DAT = Days After Treatment.

3.2 Effect of Heron 5EC on the mortality of *E.vittella* larvae

Weeds are more persistent, adaptive and aggressive than crops, which causes a serious threat to crop production as they are able to survive under adverse conditions and they take more water and nutrient from the soil which ultimately reduces crop yield (Singh and Singh, 2000). The weed may reduce the yield of rice by 70-80% in direct seeded condition

by competing with various requirements of crop including water (Dass et al., 2016). Transpiration by weeds can be reduced by breeding crop varieties that are effective weed suppressants. The most practical and efficient means of reducing transpiration and saving water for crop use is eliminating the weeds from the crop (Kaur et al., 2017). So weed management could be a better managerial option to reduce water stress to some extent.

Table 2: Mean percent mortality of OSFB larvae at different time interval following treated with different concentrations of Heron 5EC through different bioassay methods.

Treatments	% mortality in different bioassay methods					
	Direct or topical		Leaf-dip		Combined	
	3 DAT	7 DAT	3 DAT	7 DAT	3 DAT	7 DAT
Heron 5EC @ 0.50 ml/L	9.89b	14.4 c	12.00c	15.26c	13.53c	17.78c
Heron 5EC @ 0.75 ml/L	13.27b	25.11b	19.99b	35.11b	24.34b	40.12b
Heron 5EC @ 1.00 ml/L	21.86a	36.45a	25.13a	49.48a	29.43a	53.78a
Control	4.76c	8.67d	4.76d	8.67d	4.76d	8.67d
CV (%)	7.67	9.17	8.32	6.36	7.80	5.86
LSD _{0.05}	2.12	2.78	3.50	2.67	2.90	2.25

In a column, means followed by different letters are significantly different 5% level of probability. DAT = Days After Treatment.

3.3 Effect of Award 40 SC and Heron 5 EC on the weight reduction of *E. vittella* larvae

Effect of different concentrations of Award 40 SC (*Buprofezin*) and Heron 5EC (*Lufenuron*) on the weight reduction of OSFB larvae over control through different bioassay methods has been shown in table 3. Weight reduction data was determined at 7 DAT. Like as mortality data, more superior efficacy was found from Award 40 SC than that of Heron 5EC. 56.93% weight reduction over control was found when 3 days old larvae

were treated with Award 40 SC @ 1.0 ml/L through combined bioassay method while 47.64% reduction was found from Heron 5 EC (Fig.2). Likewise, 49.60% and 41.94% weight reduction was observed from Award 40 SC and Heron 5EC @ 0.75 ml/L through combined bioassay method respectively. Lower efficacy was found from the concentration of 0.5 ml/L of both CSIs (Figure 2). It has also been shown that okra-dip and topical methods were found less effective than that of combined bioassay method regarding weight reduction of larvae over control.

Table 3: Percent weight reduction of OSFB larvae over control at 7DAT following treated with Award 40 SC (*Buprofezin*) through different bioassay methods

Concentrations	Award 40 SC			Heron 5 EC		
	Direct	Indirect	Combined	Direct	Indirect	Combined
0.50 ml/L	13.78c	28.46c	31.13b	12.18b	24.55b	27.28b
0.75 ml/L	34.50b	43.93a	49.60a	30.33a	36.04a	41.94a
1.00 ml/L	41.77a	50.53a	56.93a	37.88a	40.97a	47.64a
LSD _{0.05}	2.45	4.34	4.67	4.56	3.89	4.11
CV(%)	7.89	6.89	7.70	5.67	7.12	6.90

In a column, means followed by different letters are significantly different at 5% level of probability

Figure 2: Gradual weight reduction of larva with increasing age following treated with *Buprofezin* and *Lufenuron* through combined bioassay methods. Treatment was applied on 3rd day of larval age

3.4 Larval and adult deformations

Cuticle is the outer exoskeleton of insect and that is the main target of chitin synthesis inhibitor's molecule. When three days old larvae were treated with CSIs like Buprofezin or Lufenuron through different bioassay methods, then diflubenzuron molecules enters inside the insect body that stop chitin synthesis. As a result new cuticle is not developed, growth and development of different organs become arrested and finally deformed larvae or adults developed. In this study, 45.65% and 52.25% larval deformations, 20.45% and 27.35% adult deformations were found when 3 days old larvae were treated with *Lufenuron* and *Buprofezin* respectively (Figure 3 & Figure 4). They're had significant differences between the efficacy of *Buprofezin* and *Lufenuron* regarding larval and adult deformity. Very scant abnormality was found in case of untreated control (Figure 3 & Figure 4).

Figure 3: Effect of *Buprofezin* and *Lufenuron* @ 1.0 ml/L through combined bioassay method on the development of larval deformation (B-D). A: Untreated larva without deformations. E: Percent larval deformation at 7 DAT. Different letters on each bar indicates significant at 5% level of probability.

Figure 4: Effect of *Buprofezin* and *Lufenuron* @ 1.0 ml/L through combined bioassay method on the development of adult deformation at 7 DAT. Different letters on each bar indicates significant difference at 5% level of probability

4. DISCUSSION

As alternate of synthetic broad-spectrum insecticides, two potential chitin synthesis inhibitors were tested against the lepidopteran insect, okra shoot and fruit borer under laboratory condition. It is well reported that insect growth regulators are highly potential against sucking insects like jassids, whiteflies, aphid etc but very limited works has been found on lepidopteran larvae (Das and Islam, 2014; Saleem et al., 2018; Saleem and Akbar, 2019). This study clearly documented that AWARD 40 SC (*Buprofezin*) and Heron 5 EC (*Lufenuron*) are moderately effective against okra shoot and fruit borer larvae. About 65% larval mortality and 55% weight reduction over control was found when neonate larvae were treated with AWARD 40 SC @ 1.0 ml/L through combined bioassay method. Almost similar result was found from Heron 5EC treated experiment. Our data clearly showed that the mortality and weight reduction trend was related with concentrations, exposure time and bioassay method.

In case of both CSIs, the concentration 1.0 ml/L was most effective than that of 0.75 ml/L and 0.5 ml/L which indicates that higher the diflubenzuron molecules in endocrine system then maximum inhibition of chitin formation and resulting higher mortality. On the other hand, data were collected at 3 and 7 days after treatment application but the highest mortality and weight reduction was found at 7 DAT than that of 3 DAT which confirms the slower mode of action of CSIs against lepidopteran caterpillar (Das and Islam, 2014). Synthetic insecticides are usually fast-acting molecules while CSIs are slower in action and therefore, more than 7 days need to wait to get the maximum efficacy from CSIs after application in the field. It is well reported that CSIs works by inhibiting chitin synthesis in cuticle but it is not yet confirmed how the CSIs enters inside the insect body (Uchida et al., 1985).

Therefore, caterpillars were exposed to *Buprofezin* and *Lufenuron* solutions by three different bioassay methods like topical, okra-dip and their combination. The highest mortality and weight reduction was found

when both larvae and okra fruits were treated with CSIs solution (combined) that was closely followed by okra-dip and topical methods. This interesting result confirmed that both *Buprofezin* and *Lufenuron* are able to enter in endocrine system of insects through cuticular pores (topical entry) as well as through foods (stomach entry). It was also observed that both of the tested CSIs had translaminar or systemic actions as growth and development was significantly arrested through okra-dip bioassay method. In case of okra-dip method, untreated larvae were released on CSIs treated okra and finally weight was reduced when diflubenzuron molecules of CSIs entered inside the larval body through consumed tissues.

Moreover, due to the effect of *Buprofezin* and *Lufenuron*, about 50% and 30% deformed larvae and adults were found respectively. Insect growth and developments occurs by consecutive moulting and if moulting process become stop then abnormal or deformed insects are develop. Our present results further confirmed that the tested CSIs are highly potential to stop moulting process by inhibiting chitin formation in cuticle (Haroardottir et al., 2019). Deformed adults are not able for mating with opposite sexes and eventually not able to produce new progeny (Khatun et al., 2019). Our present findings are in close agreement with some previous findings where they reported that *Buprofezin*, *Lufenuron* and *Pyriproxifen* are potent CSIs to kill insects as well as in reducing body weight (Gogi et al., 2006; Saleem et al., 2019; Chakraborty et al., 2019a; Chakraborty et al., 2019b; Shoma et al., 2020).

5. CONCLUSION

Both *Buprofezin* and *Lufenuron* were found moderately to highly effective in controlling lepidopteran larvae with their maximum concentrations. Therefore, both of the tested CSIs can apply against okra shoot and fruit borer in field condition. As efficacy was found moderate to high, therefore, it would be better to apply in integration with IPM packages rather than their sole or individual application.

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