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Efficacy of lufenuron, a chitin synthesis inhibitor on the mortality of *Spodoptera litura* (Fabricius) under laboratory conditions

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Abstract
Effectiveness of Heron 5 EC (Lufenuron) was evaluated against 3rd instar larvae of *Spodoptera litura* (Fab.) under laboratory conditions for time-oriented mortality with three different concentrations through different treatment methods. The larvae were treated with three different concentrations like 25, 50 and 75 ppm of lufenuron through three different treatment methods *viz*. direct or topical, indirect and combined. The highest mortality (about 100%) was observed from 75 ppm which was followed by 50 and 25 ppm respectively. The larval mortality was recorded at 6 hours after treatment (HAT) and 1-5 days after treatment (DAT) application. Considering the efficacy as well as mortality-duration among three treatment application methods, the combined method proved the best (100% mortality at 3 DAT in all concentrations of lufenuron) which was closely followed by indirect (100% mortality at 4 DAT in all concentrations of lufenuron) and topical method (83.30 to 100% mortality at 5 DAT in different concentrations of lufenuron). From this study, it can be concluded that Heron (Lufenuron) is very much effective against *S. litura* while stomach action is more effective compared to the direct or topical action.

Keywords: *Spodoptera litura*, lufenuron, mortality, laboratory.

1. Introduction
The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), is an economically important and regular polyphagous pest which seriously harms cabbage, soybeans, cotton and other vegetables and cash crops [5]. It has been reported to attack 112 species of plants belonging to 44 families [8]. It has wide distribution throughout tropical and temperate Asia, Australia and Pacific basin [20]. After intensive use of broad spectrum insecticides, *S. litura* populations have developed high levels of resistance to almost all conventional insecticides and cypermethrin (61 to 148 folds) [1, 6]. Therefore, now it has become necessary to search for an alternative means of pest control which can minimize the use of these synthetic chemicals. Along with synthetic insecticides, increasing use of bio-pesticides like insect growth regulators (IGRs), botanicals, entomopathogens and microbial derived pesticides in an agroecosystem is now emerging as one of the prime means to protect crops and the environment from pesticidal pollution [2, 9].
Insect Growth Regulators (IGRs) are now becoming very popular and dominant molecules in reducing pest populations by multiple effects like decreasing fecundity, egg hatchability, increasing sterility, production of abnormal larvae and pupae etc. [19]. Heron (Lufenuron), basically a chitin synthesis inhibitor (CSI) specifically acts on the incorporation of N-acetyl glucosamine monomer into chitin in the integument, resulting in the formation of abnormal new cuticle and death of the insect [10, 11, 12]. It has also been reported that lufenuron has the properties of juvenile hormone (JH) as well as ecdysteroid agonists [18]. Buprofezin, an another prominent CSI which has effectively worked on the weight reduction of brinjal shoot and fruit borer [4] as well as inhibition of progeny of rice weevil [3]. It has also been reported that Buprofezin caused weight loss of *S. littoralis* and pyriproxyfen decreased body weight, extended the duration of larval and pupal development, and reduced the pupation of *S. littoralis* at doses without significant mortality [13]. Therefore, studies of effects of chitin synthesis inhibitors against insects can influence application of synthetic insecticides and potentially reduce negative environmental effects. It is also reported that CSIs are safe for non-target organisms, highly biodegradable and action is target pest specific [17]. The present investigation was, therefore, planned to evaluate the efficacy of lufenuron (Heron 5 EC) against *S. litura* and its incorporation in integrated pest management (IPM) system.

2. Material and methods

The experiment was conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural University, during February-March, 2015.

2.1. Rearing of *Spodoptera litura*

Egg masses of *S. litura* were collected from soybean field and kept in petridishes for hatching. After hatching, fresh soybean leaves were provided regularly for feeding. When the larvae reached to the final instar, they were transferred to the plastic container with soil for pupation. After emergence from the pupa, adults were kept in a rearing chamber with a soybean plant for mating. After mating, egg masses were laid by the female moths and then masses were collected from the plant and kept in sterilized petridishes. 3-4 days later the eggs hatched with the emergence of neonate larvae. Fresh and insecticide free soybean leaves were provided every day for larval feeding. Third instar larvae with uniform size were used in all the experiments as treatment specifications.

2.2. Specifications of treatments

The experiment consisted of three treatment combinations. Three doses of Heron 5 EC (Lufenuron), (Haychem Bangladesh Ltd.) i.e. 25, 50 and 75 ppm were tested against third instar larvae. Each treatment was replicated thrice and 10 larvae were used for each replication.

2.3. Application methods

All the treatments were applied through three methods.

2.3.1. Topical application: In this method, larvae were directly treated (using micropipette) with different concentrations of lufenuron and soybean leaves were left untreated. Unsprayed soybean leaves were collected from the field, washed and dried on tissues. The treated larvae were then transferred on the untreated soybean-leaf and finally placed in petridishes having a moist filter paper to avoid desiccation. Concurrently, water treated larvae were placed on fresh untreated soybean leaves as control treatment.

2.3.2. Leaf-dip/Indirect method: In this method, unsprayed soybean leaves were collected from the field, carefully washed and dipped into different concentrations of lufenuron for 10 seconds with a gentle agitation and dried on tissues. The untreated larvae were then transferred on the treated soybean-leaf and finally placed in petridishes having a moist filter paper to avoid desiccation. Concurrently, water treated larvae were provided with fresh untreated soybean leaves as control treatment.

2.3.3. Combination (topical + leaf-dip) method: Collected soybean leaves were washed, dipped into different concentrations of lufenuron for 10 seconds with a gentle agitation and dried on tissues. After that, larvae were treated with different concentrations of lufenuron using micropipette. Finally, treated larvae were transferred on treated soybean-leaf and placed in petridishes having a moist filter paper. At the same time, water treated larvae were placed on fresh untreated soybean leaves as control treatment.

2.4. Data Collection

Data on the mortality was observed at 6 HAT (hours after treatment) as well as 1, 2, 3, 4 and 5 DAT (days after treatment) application. Died larvae were separated immediately and alive larvae were further provided with fresh treated/untreated soybean leaves. The percentage of mortality was also calculated using the following formula;

% 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 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. Comparative efficacy of different doses of lufenuron on the mortality of *S. litura* through topical application method

The topical/direct effect of different doses of lufenuron on the mortality of *S. litura* has been shown in the table 1 ($P<0.01$). The results clearly revealed that lufenuron had significant effect on the mortality of *S. litura* and the effect was clearly dose-dependent as well as time-dependent. It was confirmed that all the doses had no acute effect on *S. litura* larvae i.e. no mortality was found at 6 hours after treatment application. Considering all the doses, the mortality level increased significantly at 1 DAT in comparison with that in the control, then increased gradually and reached at the peak level by 5 DAT. The larval mortality reached to the 100% by 5 DAT when larvae were directly treated with 50 and 75 ppm of lufenuron while about 83% mortality was found at 25 ppm.

Table 1: Mean percent larval mortality of *Spodoptera litura* at different time intervals after treating with different concentrations of lufenuron through topical/direct application method.

Treatments	Mean percent mortality of *S. litura*					
	6 HAT	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT
25 ppm	0.00	30.00c	40.00d	73.30c	83.30c	83.30c
50 ppm	0.00	40.00c	53.30c	83.30b	93.30b	100.00b
75 ppm	0.00	60.00b	73.30b	90.00b	93.30b	100.00b
Control	0.00	0.00a	4.11a	4.11a	5.23a	5.34a
Significance level	NS	P<0.01	P<0.01	P<0.01	P<0.01	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. Comparative efficacy of different doses of lufenuron on the mortality of *S. litura* through indirect method

The results indicated that all the doses had significant effect on the mortality of *S. litura* and the mortality was dose-dependent ($P<0.05, 0.01$) (Table 2). Like as direct method, no mortality was found at 6 HAT. Unlike direct method, very low mortality (3 - 10%) was found at 1 DAT but mortality reached to 40, 50 and 60% by day 2 in the doses of 25, 50 and 75 ppm, respectively. It was also observed that almost 100% larvae died by day 3 at the dose 75 ppm which was followed by 90% and 73% mortality using the doses 50 and 25 ppm, respectively. The larval mortality reached to 100% by day 4 in all applied doses.

Table 2: Mean percent larval mortality of *Spodoptera litura* at different time interval after treating with different concentrations of lufenuron through indirect method.

Treatments	Mean percent mortality of *S. litura*				
	6 HAT	1 DAT	2 DAT	3 DAT	4 DAT
25 ppm	0.00	0.00a	43.33c	73.33c	100.00b
50 ppm	0.00	3.33a	56.73b	90.00b	100.00b
75 ppm	0.00	10.00b	63.33b	100.00b	100.00b
Control	0.00	0.00a	4.11a	4.11a	5.23a
Significance level	NS	P<0.05	P<0.01	P<0.01	P<0.01

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

3.3. Comparative efficacy of different doses of lufenuron on the mortality of *S. litura* through combination method

The results showed that combination method was much effective than topical and indirect method (Table 3, P<0.01). Approximately 40, 60 and 80% larvae died by day 1 after treatment application with the doses of 25, 50 and 75 ppm lufenuron, respectively while the mortality level reached to 100% by day 2 with the doses 50 and 75 ppm. On the other hand, the lower dose (25 ppm) worked slowly i.e. four days were required to get 100% larval mortality. The mortality was clearly dose-dependent as well as time-dependent. Very low mortality (about 4%) was found from untreated-control.

Table 3: Mean percent larval mortality of *Spodoptera litura* at different time interval after treating with different concentrations of lufenuron through combined treatment method.

Treatments	Mean percent mortality of *S. litura*			
	6 HAT	1 DAT	2 DAT	3 DAT
25 ppm	0.00	40.00d	76.67c	100.00b
50 ppm	0.00	66.70c	100.00b	100.00b
75 ppm	0.00	80.00b	100.00b	100.00b
Control	0.00	0.00a	4.11a	4.11a
Significance level	NS	P<0.01	P<0.01	P<0.01

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

4. Discussion

S. litura, is a severe polyphagous pest which is highly capable to develop resistance against insecticides [6]. Different broad-spectrum synthetic insecticides are commonly used against this pest but the outcome is not so satisfactory. In this study, efficacy of lufenuron (an insect growth regulator) on the mortality of *S. litura* was evaluated under laboratory conditions. Three doses *viz*. 25, 50 and 75 ppm were tested using three different methods like, topical, leaf-dip/indirect and combination. Lufenuron was found very much effective on the mortality of *S. litura* (Table 1-3), especially when applied in combination.

The mortality was clearly dose and time-dependent. The maximum mortality was recorded from 75 ppm regarding all application methods which was followed by 50 and 25 ppm of lufenuron, respectively. In this study, the acute and chronic effect of lufenuron was evaluated on the mortality of *S. litura*. No mortality was found at 6 hours after treatment application which has fitted with the mode of action of insect growth regulator especially chitin synthesis inhibitor [18]. The action of chitin synthesis inhibitor (CSI) is comparatively slow than neurotoxic insecticides as CSI disrupt moulting process successively. One day after treatment application, the mortality was significantly increased in comparison with that in the water-treated control and almost all larvae died (100%) by 2-5 days based on the methods after treatment application. These findings suggested that more time is required for IGR than neurotoxic insecticides to kill larvae successfully. More clearly, insects poisoned with lufenuron 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 [19].

Larval mortality was observed through three methods like direct, indirect and combination. Considering the percent mortality as well as time needed for larval killing, the combination method was found to be the best among these three methods which was followed by indirect and direct method, respectively. It was also observed that approximately 30-60% and 40-80% larvae were died by day 1 after treatment application by direct and combination methods, respectively. In contrast with these methods, only 3-10% larvae were died by day 1 through leaf-dip or indirect method which raised the possibility that lufenuron needed more time to initiate its action when it reached to the stomach through food but once action was initiated larval mortality occurred much quickly than contact action (direct method). It was also interesting observation that when larvae were poisoned through stomach and cuticle (combination method) about 80% larval mortality occurred by day 1 and all larvae were died by day 2 (except 25 ppm, about 75% mortality occurred) which suggested that lufenuron worked much faster and effectively when contact and stomach actions were initiated simultaneously rather than individual action. The reason was not clear why more than 50% larvae were died at 24 h of exposure (except indirect method) although the actions of IGRs are comparatively slow. The possible reasons might be that the applied IGR (Heron 5 EC) had insecticidal properties, selected doses were higher and treatment application methods were different compared to other studies [16]. Lingaraj *et al.* (2009), Rao and Subbaratnam (2000) reported the increase in mortality of larvae with increasing dose as well as methods of treatment application [7, 15]. However, the current study clearly indicates that the mortality-duration is dependent on the methods of application of IGR i.e. larval mortality reached to the 100% by day 5, 3 and 2 through direct, indirect and combination method respectively. Therefore, previous and current findings have indicated that the IGRs works more quickly and efficiently when stomach and cuticular action (combination method) initiates at the same time [4].

5. Conclusion

Insect growth regulators are used widely in the world as a potential tool of bio-intensive IPM (BIPM) considering their efficacy against target pests as well as safety to the natural enemies and environment. Lufenuron, a chitin synthesis inhibitor, was evaluated against *Spodoptera litura* under laboratory conditions and found very much effective. The dose 50 ppm was more effective considering its mortality level, time needed to kill larvae and costs of pesticides when applied through combination method. In conclusion, the overall results of this study suggest that larvae of *S. litura* and target plants both must be sufficiently treated with lufenuron to control *S. litura* successfully while spraying in the field.

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