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Efficacy of Buprofezin on the reduction of pupal growth and development of S*podoptera Litura* (Fabricius)

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Abstract
A laboratory experiment was conducted to evaluate different application methods and three different concentrations of Buprofezin 200, 400 and 600 ppm against *Spodoptera litura* at Department of Entomology, Bangladesh Agricultural University. Treatments were applied on 2nd instar larvae as topical or direct, leaf-dip or indirect and combined application methods. Data of pupae were recorded after completion of pupation and these data were compared with control. Results obtained show that the Buprofezin was effective in controlling *Spodoptera litura* by hampering the growth and development of pupae regarding weight reduction and inhibition of length & width of pupae. The results were clearly dose, time and method dependent. The highest percent of weight reduction (26.05%), length & width inhibition (19.85% & 18.29%) was recorded from 600 ppm followed by 400 and 200 ppm through combined application method. The 2nd best application method was leaf-dip which was followed by topical method. From this study it can be concluded that Buprofezin (IGR) have the potentiality to reduce the population of *Spodoptera litura* by inhibiting the growth and development of pupae.

Keywords: *spodoptera litura*, buprofezin, growth and development, pupae, laboratory

1. Introduction
The *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), is known as one of the most notorious polyphagous insect pest that causes heavy losses in many agricultural crops including tobacco, tomato, cotton, chilly, okra, cauliflower, castor, groundnut, soybean, maize black gram etc. and thus, deprives the farmers from getting high yield [4]. *Spodoptera litura* (Fabricius) is now considered as one of the major threats to the present-day intensive agriculture and changing cropping patterns worldwide. This leafworm, *Spodoptera litura* (Fabricius), a serious but sporadic insect pest causes economic losses of crops from 25.8-100% [9] based on crop stage and its infestation level in the field. It has a large host range of more than 120 host plants including crops, vegetables, weeds and ornamental plants [21]. The major ones include tobacco, cotton, groundnut, jute, maize, rice, soybeans, tea, cauliflower, cabbage, capsicum, potato and castor [22]. It feeds gregariously on leaves at earlier stage leaving midrib and veins but feeds whole leaf along with midrib and vein in case of severe infestation. On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, flower buds and occasionally on bolls. In tomatoes, larvae bore into the fruit, which is thus rendered unsuitable for consumption [10].

At present it is very difficult to control this noctuid insect because of the development of high level of resistance to almost all conventional insecticides. Widespread development of resistance to chemical insecticides including the widely used pyrethroids has been reported in (*Spodoptera litura* (F.) [2]. Beside this resistance development, conventional insecticides have negative effects on non-target organisms, including humans, and the environment. In addition to their deleterious influence on the environment, the synthetic insecticides are more hazardous to handle, toxic residues in food products, and are not easily biodegradable. All these factors are threatened for environmental sustainability [8].

To overcome resistance problems target-based eco-friendly insecticides like Insect growth regulators (IGRs) offer good control option to combat this problem. In general, insect growth regulators (IGRs), which act as chitin synthesis inhibitors, have been regarded as excellent integrated control insecticides because of their specificity to the target pest, their relative non-toxicity to beneficial organisms and their general safety to vertebrates, mollusks and plants [5, 11, 14, 24]. Chitin synthesis inhibitors (CSIs) are broadly IGRs those specially do functions in the cuticle. Among different chitin synthesis inhibitors (CSIs), Buprofezin is one the potential chitin synthesis inhibitor (CSI) that reduces the population of *Spodoptera litura* by inhibiting the bio-synthesis of chitin during moulting process. Buprofezin also affects the population of *Spodoptera litura* by reducing fecundity, egg hatchability, egg sterility, production of abnormal larvae and pupae [20]. Buprofezin was found to be effective against hemipteran pests, some lepidopteran larvae, spiders etc [6, 15, 19]. The use of IGRs compounds in insect control is known as insect developmental inhibition, which inhibits or prevents normal metamorphosis of immature stages to the adult stage. These compounds have been tested successfully against several insect species e.g. *S. litura* [23] and *S. littoralis* [12]. The selected molecules of Buprofezin (CSI) will be evaluated on the growth and development of *Spodoptera litura* pupae. This is the demand of the current situation to evaluate these bio-rational molecules against *S. litura* as the alternatives of conventional insecticides to combat resistance development, effective control, inhibition of growth and development of *Spodoptera litura*, to keep the natural enemies safe and fit these molecules in IPM packages individually or combindly.

2. Materials and Methods
Experiments were conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural

University, from July 2015 to June 2016.

2.1 Mass Rearing of *Spodoptera Litura* (Fabricius)

The egg masses of *Spodoptera litura* (Fab.) were collected from soybean field and were kept in petridishes for hatching. After hatching, fresh soybean leaves were supplied to the neonate larvae for feeding and this helped the larvae for its proper growth. This feeding was continued until the larvae became matured and when the larvae reached to the final instar, they were transferred to the plastic container filled with soil for pupation. After emergence of adult from pupa, male & female moth was kept in a rearing chamber with previously growth aroid plants. After mating, female moths laid eggs in masses on the lower and upper surface of the aroid leaves. The leaves containing egg masses were cut & then kept in sterilized petridishes with wet cotton to prevent the drying of leaves. After 3-4 days, the eggs were hatched and neonate larvae were come out. Fresh and insecticides free soybean leaves were provided every day for larval rearing. When the larvae reached to 2nd instar with uniform size were used for treatment applications. Rearing was continued until the end of the experiments to get sufficient larvae for the experiments.

2.2 Specifications of Treatments

In the laboratory three treatments of Buprofezin, Award 40 SC 200, 400 and 600 ppm along with control were used in different application methods. Each treatment was replicated thrice and ten 2nd instar larvae of *Spodoptera litura* (Fab.) were used for each replication.

2.3 Methods of Treatment Application

For this experiment three different application methods were used. Descriptions are presented below:

2.3.1 Topical Application Method (Direct Method):

In this method, the larvae were directly treated (using micropipette) with different concentrations of Award 40 SC (Buprofezin). Then the treated larvae were immediately transferred into a sterilized petridish using a sterilized fine brush. In this case, fresh soybean leaves were supplied into the petridish for feeding the larvae. A moist filter paper was placed into the petridish to avoid desiccation. Then the petridish was covered with sterilized lid allowing proper air circulation & avoiding larvae from escape. As the larvae became bigger in size later it was transferred in a sterilized plastic box with lid that was perforated and covered with net avoiding larvae from escape.

2.3.2 Leaf-Dip Method (Indirect Method)

In this case, soybean leaves were treated with Award 40 SC (Buprofezin) with different concentrations as experimental specifications. Following that the treated leaves were kept open to avoid wetting of the leaves. The proper dried leaves were placed on moist filter paper in a sterilized petridish. Then the untreated larvae of definite number were transferred into the sterilized petridish using fine sterilized brush and covered with sterilized lid. As the larvae became bigger in size later it was transferred in a sterilized plastic box with lid that was perforated and covered with net avoiding larval escape.

2.3.3 Combination Method (Direct Method + Indirect Method)

In case of combination method, both larvae and soybean leaves were treated with different concentrations of Award 40 SC (Buprofezin). After that the treated leaves were properly dried & placed on moist filter paper in a sterilized petridish. The petridish & brushes, hand and working surface that were used in this experiment all were sterilized with alcoholic solution before work. The treated larvae were then transferred on treated leaves using fine brush & covered with sterilized lid. As the larvae became bigger in size later it was transferred in a sterilized plastic box with lid that was perforated and covered with net avoiding larvae from escape.

2.4 Data Collection

Pupal data were recorded after completion of pupation. The percentage of pupal weight reduction, length & width inhibition were calculated using the following formulae;

$$\% \text{ Weight reduction} = (Po - Pr)/ Po \times 100$$

Where,
Po = Mean weight of a single pupa in control condition
Pr = Mean weight of a single pupa in a treated condition

$$\% \text{ Length/ Width inhibition} = (Po - Pr)/ Po \times 100$$

Where,
Po = Mean length/width of a single pupa in control treatment
Pr = Mean length/width of a single pupa in a specific treatment

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).

3. Results

3.1 Efficacy of different concentrations of Buprofezin (Award 40 SC) on the weight changes of *Spodoptera litura* pupae through topical application method

Pupal weight reduces due to directly application of different concentrations of Award 40 SC on the 2nd instars larvae of *S. litura* shown in (Table 1) and it's being accumulated in larval body although later no Award 40 SC was applied. Buprofezin an important growth regulator, its hamper the growth & development of insects through multiple targets like deformation, weight reduction, length & width inhibition of larva, pupa, adult, preventing moulting etc [20]. To evaluate this we carefully observed the growth & development of larva, pupa and adult of *Spodoptera litura*. In case of direct application method the highest mean pupal weight reduction 11.80% was recorded from 600 ppm followed by 400 ppm (6.10%) and 200 ppm (3.48%) respectively that was statistically significant ($P<0.01$) in comparison with control. The potentiality of low dose, 200 ppm was comparatively weaker for pupal weight reduction than 400 and 600 ppm but the result was statistically significant. From the results it was cleared that pupal weight reduction was dose dependent.

Table 1: Effect of different concentrations and topical application method of Award 40 SC on the weight changes of *S. litura* pupae

Treatments	Weight (Mg/Pupa)	% Reduction Over Control
Award 40 SC @ 200 Ppm	254.52b	3.48
Award 40 SC @ 400 Ppm	247.60c	6.10
Award 40 SC @ 600 Ppm	232.56d	11.80
Control	263.70a	---
SD	13.11	
P-Level	**	
CV (%)	0.56	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

3.2 Efficacy of Different Concentrations of Buprofezin (Award 40 SC) On the Weight Changes of *Spodoptera Litura* Pupae through Leaf-Dip Application Method

Pupal weight reduced with the increasing concentrations of Award 40 SC through indirect application method was given in (Table 2). In case of 600 ppm the highest pupal weight reduction was 20.61% followed by 400 ppm (12.34%) and 200 ppm (9.21%) respectively that was statistically significant (P<0.01) compared to control and this result clearly revealed that Buprofezin, Award 40 SC had significant effect on pupal weight reduction. Maximum pupal weight reduction from direct application method was 11.80% which was found from 600 ppm but from leaf-dip application maximum weight reduction was 20.61% and its indicates indirect application method was more effective than direct application method for pupal weight reduction of *Spodoptera litura*.

Table 2: Effect of different concentrations and leaf-dip application method of Award 40 SC on the weight changes of *S. litura* pupae

Treatments	Weight (Mg/Pupa)	% Reduction Over Control
Award 40 SC @ 200 Ppm	240.16b	9.21
Award 40 SC @ 400 Ppm	231.87c	12.34
Award 40 SC @ 600 Ppm	210.00d	20.61
Control	264.53a	---
SD	22.52	
P-Level	**	
CV (%)	0.77	

In a column, means of similar letter (s) do not differ significantly. DAT = Days after Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

3.3 Efficacy of different concentrations of Buprofezin (Award 40 SC) on the weight changes of *Spodoptera litura* pupae through combined application method

The highest pupal weight reduction were recorded when both the larvae & soybean leaves were treated with Award 40 SC (Table 3). From combination application method maximum mean pupal weight reduction 26.05% was observed from 600 ppm (P<0.01) followed by 400 ppm (17.29%) and 200 ppm (13.88%) respectively that was statistically significant and it was higher than indirect (20.61%) and direct (11.80%) method. According to the experimental results, direct application method and low dose 200 ppm of Award 40 SC was less effective for pupal weight reduction but the effectiveness was gradually increased with the increasing concentrations 400 ppm & 600 ppm of Award 40 SC and time. This result also indicates that indirect application method was more effective than direct application method but the combination application method was the best as it was more effective for both larval & pupal weight, length and width compared to individual method.

Table 3: Effect of Different Concentrations and Combined Application Method of Award 40 SC on the Weight Changes of *S. Litura* Pupae

Treatments	Weight (mg/pupa)	% reduction over control
Award 40 SC @ 200 ppm	228.96b	13.88
Award 40 SC @ 400 ppm	219.91c	17.29
Award 40 SC @ 600 ppm	196.60d	26.05
Control	265.89a	---
SD	28.80	
P-level	**	
CV (%)	0.67	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

3.4 Efficacy of different concentrations of Award 40 SC on the length and width changes of *Spodoptera litura* pupae through topical application method

As a growth regulator Buprofezin, Award 40 SC had significant (P<0.01) and dose dependent effects on the length

& width changes of *S. litura* pupae shown in (Table 4). From the table it was cleared that maximum mean length and width inhibition was observed from 600 ppm 10.39% (length) & 9.6% (width) followed by 400 ppm 7.31% (length) & 7.8% (width) and 200 ppm 5.53% (length) and 4.8% (width) respectively that was statistically significant (P<0.01) compared to control. But in case of lower dose, 200 ppm of Award 40 SC mean pupal length and width inhibition was weak compared to 400 and 600 ppm.

Table 4: Effect of Different Concentrations and Topical Application Method of Award 40 SC on Pupal Length and Width of *Spodoptera Litura*

Treatments	Length of Pupa (Mm)	% Inhibition Over Control	Width Of Pupa (Mm)	% Inhibition Over Control
Award 40 SC @ 200 Ppm	15.36b	5.53	4.76b	4.8
Award 40 SC @ 400 Ppm	15.07b	7.31	4.61c	7.8
Award 40 SC @ 600 Ppm	14.57c	10.39	4.52c	9.6
Control	16.26a	---	5.00a	----
SD	0.71		0.21	
P-Level	**		**	
CV (%)	2.17		0.67	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

3.5 Efficacy of different concentrations of Award 40 SC on the length and width changes of *Spodoptera litura* pupae through leaf-dip application method

Significant (P<0.01) pupal length and width inhibition was observed when soybean leaves treated with different concentrations of Award 40 SC and supplied to the untreated larvae for feeding shown in (Table 5). It was observed that the leaf-dip method was more effective than topical application method regarding pupal length and width inhibition compared to control. The maximum mean pupal length and width inhibition 12.38% and 11.80% were recorded from 600 ppm which was followed by 400 and 200 ppm respectively that was greater than direct application method. From the experimental result it was cleared that pupal length and width inhibition was increased with the increasing concentrations of Award 40 SC.

Table 5: Effect of Different Concentrations and Leaf-Dip Application Method of Award 40 SC on Pupal Length and Width of *Spodoptera Litura*

Treatments	Length of pupa (mm)	% inhibition over control	Width of pupa (mm)	% inhibition over control
Award 40 SC @ 200 ppm	15.85b	5.65	4.71b	5.80
Award 40 SC @ 400 ppm	15.31c	8.86	4.62b	7.60
Award 40 SC @ 600 ppm	14.72d	12.38	4.41c	11.80
Control	16.80a	---	5.00a	---
SD	0.88		0.25	
P-level	**		**	
CV (%)	0.74		0.58	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

3.6 Efficacy of different concentrations of Award 40 SC on the length and width changes of *Spodoptera litura* pupae through combined application method

The highest mean pupal length and width inhibition was recorded when both the larvae and soybean leaves were treated with different concentrations of Award 40 SC shown in (Table 6, P<0.01). The maximum mean pupal length and width inhibition 19.85% and 18.29% were recorded from 600 ppm which was followed by 400 ppm 14.58% & 13.12% and 200 ppm 9.98% and 7.55% respectively that was statistically significant (P<0.01) compared to control and it was higher than leaf-dip and topical application method. From this result it was cleared that weight reduction, length & width inhibition of pupa were dose dependent and combination application method was more effective for these abnormalities following indirect and direct method of application.

Table 6: Effect of Different Concentrations and Combined Application Method of Award 40 SC on Pupal Length and Width of *Spodoptera Litura*

Treatments	Length Of Pupa (Mm)	% Inhibition Over Control	Width Of Pupa (Mm)	% Inhibition Over Control
Award 40 SC @ 200 Ppm	16.05b	9.98	4.65b	7.55
Award 40 SC @ 400 Ppm	15.23c	14.58	4.37c	13.12
Award 40 SC @ 600 Ppm	14.29d	19.85	4.11d	18.29
Control	17.83a	---	5.03a	---
SD	1.50		0.40	
P-Level	**		**	
CV (%)	0.26		0.57	

In a column, means of similar letter (s) do not differ significantly. DAT = Days After Treatment, ** = Significant at 1% level of probability, NS = Not significant, P-level = Probability Level, CV = Co-efficient of Variation, SD = Standard Deviation.

4. Discussion

In this study three different concentrations of Buprofezin (Award 40 SC) have been evaluated in the laboratory on the growth and development of *S. litura* pupae as the 2nd instar larvae were treated with this concentration through topical, leaf-dip and combined application method. The present findings clearly showed that Buprofezin is able to inhibit the growth of pupa regarding pupal weight, length and width compared to control. The inhibition was found to be significant. As *Spodoptera litura* (Fab.) already developed resistance to many chemical insecticides [16] therefore, it is a demanding issue to combat this resistance problem with using selective bio-insecticides.

Buprofezin possesses a novel insecticidal mechanism not based on a neurotoxic action as they are IGRs. As IGRs are not neurotoxic their efficacy is increased with time because of the accumulation of insecticides in insect body. They disrupt the physiology and development of target pest insects and show no/low toxicity towards non-target organisms, making them ideal in combination with biological control and also to circumvent insecticide resistance [7]. Buprofezin chitin synthesis inhibitors caused appreciable effect in pupal growth and development of *S. litura.* The experimental results indicated that in different application methods all the three concentrations of Buprofezin, Award 40 SC i.e. 200, 400, 600 ppm were significantly effective against *S. litura* pupae in terms of weight reduction, length & width inhibition and abnormalities of pupa. The highest percentage of pupal weight reduction over control was found when larvae were treated with 600 ppm of Award 40 SC through different application methods (11.80%, 20.61%, and 26.05% weight reduction in case of topical, leaf-dip and combination application methods respectively) followed by 400 ppm (6.10%, 12.34% and 17.29%) and 200 ppm (3.48%, 9.21% and 13.88% weight reduction respectively).

The present work showed that the growth hampered by inhibiting chitin formation of *S. litura* pupa as a result some of larvae can't able to form pupal case and ultimately some pupae died & some pupae become deformed and this results related to the development and growth also are in harmony with those obtained by [3[who study the effect of Chitin synthesis inhibitors (flufenoxuron) on some biological and biochemical aspects of the cotton leaf worm *S. littoralis* and the results showed that the tested IGR significantly increased the larval and pupal durations, on the other hand decrease the percentages of pupation, adult emergency, fecundity and fertility of the eggs produced by the adult progeny and induced some morphogenic abnormalities in larval, larval-pupal and pupal stages, as well as pupal-adult intermediate. The obtained results also agree with the results of [1, 13, 18] as this was happened in larvae. Furthermore higher concentrations of Buprofezin have antifeeding effect that eventually inhibit the growth and development of *S. litura* pupa. Like as pupal weight reduction, pupal length & width was also significantly inhibited by Buprofezin and that was also dose-dependent. The highest percentage of pupal length and width inhibition over control was found when larvae were treated with 600 ppm of Award 40 SC through different application methods (length 10.39%, 12.38%, and 19.85%, width 9.6%, 11.80%, and 18.29% length and width inhibition in case of topical, leaf-dip and combination application methods respectively) followed by 400 ppm (length 7.31%, 8.86%, and 14.58%, width 7.8%, 7.60%, and 13.12% length and width inhibition and 200 ppm (length 5.53%, 5.65%, and 9.98%, width 4.8%, 5.80%, and 7.55% length and width inhibition in case of topical, leaf-dip and combination application methods respectively). The obtained results also agree with the results of a research findings [17] in which he observed because of the application of Lufeneuron an insect growth regulator pupal deformation occurred. From these experimental results it can be concluded that Buprofezin is a potential IGR that can reduce the population of *S. litura* by hampering the growth and development of *S. litura* pupa and it can be an important part of IPM for controlling this devastating *S. litura.*

5. Conclusion

From this study, it was cleared that Buprofezin has significant effect on the growth and development of *S. litura* pupa regarding weight changes as well as length and width inhibition. The effect was clearly dose, method and time dependent. The best efficacy was found from 600 ppm which was followed by 400 and 200 ppm respectively. On the other hand, the combination method was found to be the best compared to leaf-dip or topical application methods. So, from this study it can be concluded that Buprofezin have the potentiality to reduce the population of *S. litura* by disrupting the growth and development of this insect life stage. Considering all results, the application of Buprofezin would be more effective when it will apply in integration with other components of IPM rather than its individual application.

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