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Insecticidal and growth inhibitory potentiality of Chitosan, a chitin-derived biopesticide against *Spodoptera litura* (Fabricius)

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Abstract : Chitosan is a chitin-derived bio-molecule. Its insecticidal possibility was evaluated at concentrations of 1.0, 2.0, and 3.0 g/L against the common cutworm or armyworm, *Spodoptera litura* (Fab.) larvae under laboratory conditions in Bangladesh during the period from July 2016 to June 2017. The 2nd instars larvae were treated with selected concentrations through different application methods viz. topical (direct), leaf-dip (indirect), and combined. The efficacy of different techniques and concentrations on larval mortality and growth inhibition was observed. The larval mortality was recorded at 1, 3, 5 and 7 days after treatment (DAT) application. The mortality and growth were dose, method, and time dependent. The highest larval mortality was found in 3.0g/L and maximum mortality (62.72%) was recorded in combined approach followed by topical (52.51%) and leaf dip (46.14%) method at 7 DAT. With increasing time larval mortality increased and at 7 DAT it was maximum followed by 5, 3 & 1 DAT. The highest (27.61%) growth inhibition was obtained from the combination method at 3.0 g/L dose of chitosan. The results of the present study revealed that chitosan has a insecticidal activity to control *Spodoptera litura* (Fab.). However, it might be used as a insecticide after commercialization or could be a component of Integrated pest management for the management of *Spodoptera litura* (Fab.).

Keywords : Biopesticide; Chitosan; Growth inhibition; Larval mortality; *Spodoptera litura*.

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Introduction

World population is increasing and it has been projected that world population will reach around 10 billion in 2050. To ensure the food security of that huge population there is no alternative to secure the food crops from biotic and abiotic stresses. Application of chemicals is one of the quick and effective method to reduce the crop losses from attacks of insects, weeds, microbial diseases, and other pests menace [3,7]. FAO reported that globally 20-40% crop yield decreased by the attack of various insect pests and pathogenic organisms, which results in loss of about US $120 billion [28].

Food and fibre crops are damaged by more than 10,000 species of insects, with an estimated annual loss of 13.6 % globally. Insecticides have saved millions of human and animal lives since the date of their synthesis and use. They have played an important role that brought revolution in the field of agriculture and human health on control of insect pests of crops and vector-borne diseases. Discovery and use of insecticides saved the millions of human and animal lives from the hunger but on the other hand excessive use of insecticide causes a threats like toxic residual effect in food, air, soil, water, resistance and resurgence of insect pests [12, 16, 25]. Moreover, humans are most likely to develop diseases after pesticide intoxication which include cancer, asthma, diabetes, Parkinson's disease, leukemia, endocrine disorders, and many others [11].

More or less 70,000 different insect species found responsible for food crop damage around the world. Among them, the Lepidopteran species are the major and appeared as destructive for crop losses [17]. *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an insect of polyphytophagous nature, damaging many vegetables and field crops reported in Asian countries [8, 22, 23]. About 112 plant species belonging to 44 families marked as hosts plants for this insect species [15]. Based on the infestation level at different crop stages, *S. litura* causes from 25.8-100% economic loss [5]. Nowadays, available market insecticides failed to control *S. litura* effectively [2, 10]. The frequent use of conventional insecticides such as organophosphates, carbamates and synthetic pyrethroids against *S. litura* has provided an ideal environment for its evolution of resistance [2]. Not only that more than 645 species of insects and mites have developed resistance to insecticides with 542 species of arthropods resistant to at least one compound. Approximately 7,470 cases of tolerance were reported in insects to a specific insecticide; 16 arthropod species accounted for 3,237 (43 %).

In light of the above facts, the selection of biorational insecticides might be a reliable alternative of chemical pesticides. Insect growth regulators (IGRs), a new era of minimizing resistance problem which act as chitin synthesis inhibitors and an elevated component due to their specificity to the target pest, non-toxicity to beneficial organisms and environment friendly properties [4, 6, 9, 24].

Chitosan is chitin derived copolymer of D-glucosamine and N-acetyl- D-glucosamine with β-(164) linkage, is obtained by alkaline or enzymatic deacetylation process [21]. Chitin, the raw material of chitosan, was isolated from the exoskeleton of the different crustaceous [1]. It is considered environment-friendly for agricultural uses as it is easily degraded in the environment, and nontoxic to humans. The chitosan showed high insecticidal activity for lepidopterous and homopterous insect pests. Chitosan had 70–80% insecticidal activity against *Aphis gossypii* [27]. The conjugate structure of Avermectin-grafted-N,O-carboxymethyl chitosan (NOCC) confirmed 100% lethal rate against armyworms, carmine spider mites, black bean aphids, and brown plant hoppers [26]. Chitosan inhibits 7% growth of *S. littoralis* larvae where its derivatives like; O-(decanoyl) chitosan inhibits 64% growth after 5 days of feeding on a treated artificial diet [14].

Despite insecticidal efficiency of citosan, not so much study has been found around the world and in Bangladesh no insecticidal activity of chitosan has been reported yet. However, insecticidal activity of chitosan encouraged to conduct this experiment to test it efficacy against the *Spodoptera litura* (Fabricius) which is one of the great threat for crop production in Bangladesh.

2. Materials and methods

2.1. Specification of chitosan

Chitosan, an Insect Growth Regulator (IGR) have been used in this experiment against *Spodoptera litura* in the laboratory condition. Chitosan was purchased from Sigma Aldrich Ltd., Germany. The trade name, chemical name, formula, properties, and mode of actions of Chitosan is given below in brief:

Trade name: Chitosan

Chemical name: (1,4)-2-Amino-2-deoxy-beta-D-glucan

Empirical formula: $(C_6H_{11}NO_4)_n$

2.2. Treatments and statistical design

Chitosan is insoluble in water but utterly soluble in glacial acetic acid. At first, chitosan was mixed with 1.0% glacial acetic acid and then stirred using a magnetic stirrer at 5000 rpm. When the Chitosan was found to be mixed clearly, then finally mixed with water. This way, 1.0, 2.0, and 3.0% Chitosan solution was prepared for experimental use. Three methods i.e. topical (direct application to larval body), leaf–dip (mixed with food and then allowed for insect feeding) and combined approach (direct application to larval body and mixed with feed of the larvae) were used for tested the concentration against the *S. litura*. Complete Randomized Design (CRD) with three replication of each treatment was followed to generate the experimental data

2.3. Test pests

Egg masses of the common cutworm *S. litura* were collected from the infested cabbage field. Clean and sterilized petri dishes were used for hatching larvae. The egg hatched colony was reared under laboratory conditions at the Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh. A natural diet of pesticide-free cabbage leaves was used to feed the colony. Continuous feeding was provided from the larval stage to just before the pupal stage. The final instar larva, which was ready for pupation carefully transferred to the soil-filled plastic container. After completing the pupal stage, male & female moth was emerged and set up in a rearing chamber. As per the natural process, mating was done between two adults, and female moths laid eggs in masses on the lower and upper surface of the bean leaves. Eggs containing leaves were collected in sterilized petri dishes with wet cotton to prevent the drying of leaves. The eggs were hatched after 3-4 days, and neonate larvae have come out. Fresh and insecticides free bean leaves were provided daily for larval rearing. All the treatments of three different methods were applied on 2^{nd} instar larvae of uniform size. The rearing process was continued until the end of the experiments to get sufficient caterpillars for the tests.

2.4. Insecticidal and growth inhibitory assay against *S. litura* by topical application method

The worms were directly treated with three dosages of chitosan solution. Immediately, the treated larvae were transferred into a sterilized petri dish. Then, untreated country bean leaves were provided in the petri dish for feeding.

2.5. Insecticidal and growth inhibitory assay against *S. litura* by leaf-dip method

In this case, country bean leaves were treated with three concentration of chitosan solutions. The treated leaves were air-dried and placed in a sterilized petri dish. Then, untreated larvae were placed on treated leaves using a fine brush.

2.6. Insecticidal and growth inhibitory assay against *S. litura* by combination method

In combination method, both larvae and country bean leaves were treated with three concentrations of chitosan solution. After that, treated leaves were thoroughly dried in the air. Then, treated larvae were placed on treated leaves using a fine brush.

2.7. Data collection

Data on larval mortality was observed at 1, 3, 5, and 7 DAT (days after treatment) application. Died larvae were separated carefully and alive were provided with fresh or treated country bean leaves based on the treatment application method.

The larval mortality percentage was calculated using the following formula:

% Mortality = Po/Pr × 100

Where,

Po = Number of larvae died
Pr = Number of treated or untreated larvae provided
The growth inhibition was calculated from the following equation:

Growth inhibition (%) = $(C_L - T_L)/C_L \times 100$

Where,

C_L- larval weight gained in the control
T_L- larval weight gained in the treatment

The pupal mean weight reduction (%) over control was calculated from the following equation:
Reduction over control (%) = $(C_P - T_P)/C_P \times 100$

Where,
C_P- pupal weight gained in the control,
T_P- pupal weight gained in the treatment

2.8. Statistical analysis

The recorded data were compiled and tabulated for statistical analysis. Analysis of variance (ANOVA) was done with the help of Statistical Tool for Agricultural Research (STAR). The mean differences among the treatments were adjudged with Duncan`s Multiple Range Test (DMRT) and Least Significant Difference (LSD).

3. Results

3.1. Insecticidal activity against larvae of *Spodoptera litura*

The systemic, contact and combined toxicity effect of different Chitosan concentrations against third instar larvae of *S. litura* are shown in table 1, 2 and 3. Significant mortality started after 3 days of treatment in case of every methods. Also gradual increment of mortality was observed with increased of the concentration and time. Thus the chitosan effect was clearly dose and time-dependent. For each application method highest mortality recorded by the dose of 3.0 g/L. Chitosan scored 52.51% larval mortality with 3.0 g/L at the end of the experiment (after 7 days) through topical methods. The lowest mortality was exhibited by leaf-dip application method. Combined application of chitosan concentrations showed high toxicity compared to the other application methods against *S. litura* larvae. Through combined application effect of chitosan highest larval mortality (62.72%) was found over control larvae with 3.0 g/L concentration at 7 DAT.

Table 1: Insecticidal activity of different concentrations of Chitosan against third instar larvae of *S. litura* through Topical application methods

Treatments	**Mean percent of larval mortality ± SE at different Days After Treatment (DAT)**			
	1	**3**	**5**	**7**
Control	0.0±0.0	0.00 c ±0.0	3.78 c ±0.2	3.96 d ±0.4
Chitosan @ 1.0 g/L	0.0±0.0	5.90 a ±1.5	17.51 b ±1.7	26.97 c ±3.6
Chitosan @ 2.0 g/L	0.0±0.0	8.87 b ±4.4	33.32 a ±4.8	42.85 b ±2.5
Chitosan @ 3.0 g/L	0.0±0.0	9.52 b ±4.8	38.09 a ±4.8	52.51 a ±2.4
P-level	-	0.13 (P>0.05)	0.00 (P<0.01)	0.0 P<0.01)

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
**P-Level* = significance of the *F* ratio

Table 2: Insecticidal activity of different concentrations of Chitosan against third instar larvae of *S. litura* through Leaf-dip application methods

Treatments	**Mean percent of larval mortality ± SE at different Days After Treatment (DAT)**			
	1	**3**	**5**	**7**
Control	0.0±0.0	0.00 c ±0.0	3.78 d ±0.2	3.96 d ±0.4
Chitosan @ 1.0 g/L	0.0±0.0	4.65 b ±0.4	14.71 c ±0.7	20.07 c ±0.7
Chitosan @ 2.0 g/L	0.0±0.0	6.36 b ±3.2	24.89 b ±2.3	35.44 b ±2.0
Chitosan @ 3.0 g/L	0.0±0.0	12.23 a ±1.2	33.59 a ±3.2	46.14 a ±2.1
P-level	-	0.00 (P<0.01)	0.00 (P<0.01)	0.00 (P<0.01)

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
**P-Level* = significance of the *F* ratio

Table 3: Insecticidal activity of different concentrations of Chitosan against third instar larvae of *S. litura* through Combined application methods

Treatments	**Mean percent of larval mortality ± SE at different Days After Treatment (DAT)**			
	1	**3**	**5**	**7**
Control	0.0±0.0	0.00 c ±0.0	3.78 c ±0.2	3.96 d ±0.0
Chitosan @ 1.0 g/L	0.0±0.0	7.90 b ±0.5	21.85 b ±3.2	33.49 c ±4.2
Chitosan @ 2.0 g/L	0.0±0.0	12.28 a ±0.6	35.96 a ±3.8	42.70 b ±2.5
Chitosan @ 3.0 g/L	0.0±0.0	14.92 a ±2.0	43.69 a ±5.1	62.72 a ±2.2
P-level	-	0.00 (P<0.01)	0.00 (P<0.01)	0.00 (P<0.01)

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
**P-Level* = significance of the *F* ratio

3.2. Growth inhibitory activity against larvae of *Spodoptera litura*

The growth inhibitory potentiality of chitosan was observed during the experimental period (Table 4, 5 and 6). The systemic and contact effect of Chitosan against *S. litura* larvae in case of growth inhibition was lower than that of combination effect. At 7 days after treatment 3.0 g/L dose showed highest larval growth inhibition 27.61% through combination method which was followed by 13.81% (topical method) and 12.27% (leaf-dip method) respectively.

The Chitosan concentrations also reduced the mean weight of *S. litura* pupa. As shown in the table 6, Chitosan significantly affect pupal weight where 167.47 mg/pupa was found with a concentration of 3.0 g/L compared to 265.78 mg/pupa in the control. The highest pupal weight reduction over control was scored by combination application method (37.00%) which was followed by topical application method (32.36%) and leaf-dip method (31.75%) respectively.

Table 4: Growth inhibition and pupal weight reduction (%) of different concentrations of Chitosan against third instar larvae of *S. litura* through Topical application method

Treatments	Mean weight gained (mg/larvae) ± SE during the observation time		Growth inhibition (%) ± SE after 7 days	Mean pupal	
	3 DAT	7 DAT		Weight (mg/pupa)	reduction (%) over control
Control	67.80 ±0.4	350.08 a ±0.7	**0.00 d ±0.0**	262.30 a ±0.6	**0.00 c ±0.0**
Chitosan @ 1.0 g/L	65.55 ±1.4	325.71 c ±2.3	**6.96 c ±0.6**	189.91 b ±2.3	**27.60 b ±0.9**
Chitosan @ 2.0 g/L	63.96 ±2.2	307.44 b ±0.3	**12.18 b ±0.1**	180.45 c ±0.8	**31.20 a ±0.3**
Chitosan @ 3.0 g/L	62.82 ±2.0	301.72 b ±1.1	**13.81 a ±0.3**	177.42 c ±1.2	**32.36 a ±0.5**
P-level	0.27(P>0.05)	0.00 (P<0.01)	**0.00 (P<0.01)**	0.00 (P<0.01)	**0.00 (P<0.01)**

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
*DAT = Days after treatment
**P-Level* = significance of the *F* ratio

Table 5: Growth inhibition and pupal weight reduction (%) of different concentrations of Chitosan against third instar larvae of *S. litura* through Leaf-dip application method

Treatments	Mean weight gained (mg/larvae) ± SE during the observation time		Growth inhibition (%) ± SE after 7 days	Mean pupal	
	3 DAT	7 DAT		Weight (mg/pupa)	reduction (%) over control
Control	65.10 ±0.2	357.41 a ±2.9	**0.00 d ±0.0**	260.70 a ±2.4	**0.00 c ±0.0**
Chitosan @ 1.0 g/L	63.99 ±0.7	335.35 bc ±2.8	**6.17 c ±0.8**	192.62 b ±3.1	**26.11 b ±1.2**
Chitosan @ 2.0 g/L	62.26 ±0.8	324.16 ab ±1.1	**9.30 b ±0.3**	183.87 c ±3.0	**29.47 a ±1.2**
Chitosan @ 3.0 g/L	60.39 ±0.6	313.56 b ±3.0	**12.27 a ±0.8**	177.93 c ±0.5	**31.75 a ±0.2**
P-level	0.01 (P=0.01)	0.00 (P<0.01)	**0.00 (P<0.01)**	0.00 (P<0.01)	**0.00 (P<0.01)**

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
*DAT = Days after treatment
**P-Level* = significance of the *F* ratio

Table 6: Growth inhibition and pupal weight reduction (%) of different concentrations of Chitosan against third instar larvae of *S. litura* through Combination application method

Treatments	Mean weight gained (mg/larvae) ± SE during the observation time		Growth inhibition (%) ± SE after 7 days	Mean pupal	
	3 DAT	7 DAT		Weight (mg/pupa)	reduction (%) over control
Control	70.15 ±0.5	366.01 a ±0.5	**0.00 d ±0.0**	265.78 a ±1.7	**0.00 d ±0.0**
Chitosan @ 1.0 g/L	64.53 ±0.8	310.00 c ±0.6	**15.30 c ±0.5**	182.12 b ±1.4	**31.48 c ±0.5**
Chitosan @ 2.0 g/L	60.38 ±0.4	277.56 b ±0.3	**24.17 b ±0.4**	172.27 c ±1.0	**35.18 b ±0.4**
Chitosan @ 3.0 g/L	57.72 ±0.9	264.94 b ±0.8	**27.61 a ±0.1**	167.47 d ±0.3	**37.00 a ±0.1**
P-level	0.26 (P>0.05)	0.00 (P<0.01)	**0.00 (P<0.01)**	0.00 (P<0.01)	**0.00 (P<0.01)**

*means in a column followed by same letter(s) are not significantly different
*SE = standard error
*DAT = Days after treatment
**P-Level* = significance of the *F* ratio

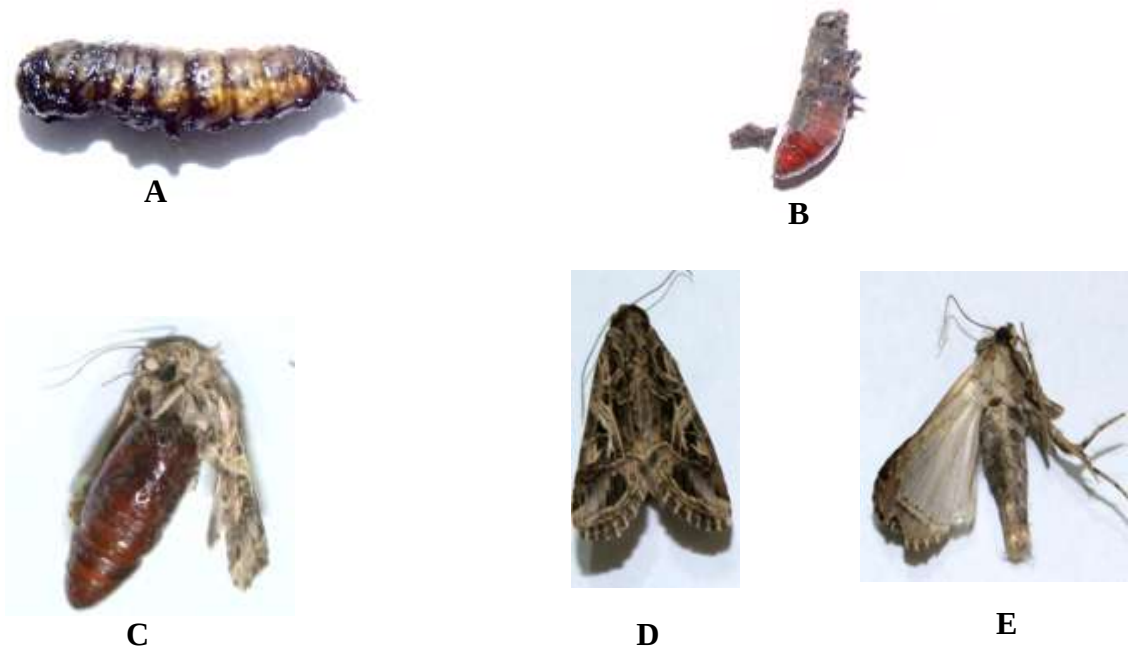

Figure.1 Representative photomicrographs of different stages of *S. litura* that were treated with Chitosan: [A] A treated *S. litura* larva with cuticular deformations [B] Treated larva failed to switch on a normal pupal stage [C] A intermediate stage with incomplete hatching of moth (pupal-adult) [D] A control or untreated moth [E] An abnormal moth that was treated at larval stage.

4. Discussion

Researchers around the world are thinking to build up a synthetic pesticide free agriculture systems. To get more crop productivity without any loss chemical insecticides are being used in the field. Frequent use of synthetic chemicals against major crop insects resulted pesticide resistant in insect. Moreover, residual effect of insecticide hinder human health as well as reduce the biodiversity of the environment. *S. litura* is polyphagous insect and is one of the major bottleneck of crop production around the world. Chitosan is a chitin derivatives molecule and possess insecticidal activity. In this study highest larval mortality (62.27%) and growth inhibition (27.6%) were found in combined approach method compared to others. In this method chitosan was applied both directly on larval body and by mixed with insect feed which might be cause of higher action of chitosan as well as for highest larval mortality and growth inhibition of larvae. Chitosan and its derivatives confirmed a number of mortality effect as well as growth inhibitor of different lepidopterian and homopteran insects [27]. Latterly IGRs are attracted researchers for their selective toxicity and less hazardous effect as residue. After experimental analysis, a conclusion may be like that individual chitosan solutions showed lower toxicity against *S. litura* larvae. And in case of growth inhibitory performance, it contains an emerging potentiality. An early study of chitosan nanoparticles against some soybean insects reported that insect growth was significantly decreased from 99% in semifield control to 22% (77.8% decrease) in treated insects under semifield condition [20]. In another study, chitosan derivative (*N*- (2- chloro- 6- fluorobenzyl) was found effective against *Spodoptera littoralis* with an LC_{50} of 0.32 g kg^{-1} diet and 100% mortality at ≥0.625 g kg^{-1} [18]. In addition, the activity of chitosan with different molecular weight and chitosan-metal complexes against *S. litura* was higher, with 97.3% mortality. The insecticidal activity of chitosan complexes against another insects *Aphis nerii*, was 84.4% mortality after 48 h [13]. The present study indicated that the unmodified chitosan was active against *S. litura* larvae, with 62.72% mortality after 7 days of treatment. It also reduced the larval growth, with more than 27% after 7 days. Comparing with previous studies we found that to make chitosan more effective it should be modified. Although chitosan showed less killing ability than the chemical pesticide phoxim, due to its low toxicity and environment compatibility, it could be used on vegetables and fruits as a substitute for the toxic chemical pesticides now being used [27]. Chemical insecticides provide the primary means for controlling agricultural insect pests. Uninterrupted use of synthetic compounds has faced two major hindrances: increasing public concern regarding the defilement of perishables with pesticide residues and the development of

resistance in the pest populations. The ultimate aim of recent research in this area to reduce dependence on synthetic pesticides through finding alternatives. Recently, the exploitation of Chitosan, a chitin derived molecule, to control agricultural pests has received more attention. From the experimental findings, it can be concluded that the doses of Chitosan along with application methods had a significant effect on the growth and development of *S. litura.* All the doses showed a significant effect against all the parameters studied although 3.0 g/L provided the best efficacy which was followed by 2.0 g/L and 1.0 g/L respectively. Considering the application methods, the combined application method was found to be the best which was followed by topical and leaf-dip application methods respectively. Thus it has been shown that chitosan was found to be moderately effective both as insecticides as well as inhibitory molecules. Therefore, Chitosan can be used as a component of the IPM program rather than its individual or sole application

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