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Synthesis of colloidal chitosan from sea waste and its antifeedant effect against maize fall armyworm *Spodoptera frugiperda* (J.E. Smith)

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Abstract

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is one of the most important pests of maize, suddenly appeared in India 2018 and other cereal crops. The colloidal chitosan was synthesized in the laboratory, from the waste of sea food and studied for its bio efficacy by leaf dip bioassay on FAW. The antifeedant effect was assessed on first instar larva at concentration of one, three, five and seven per cent under no choice condition. The one and 7 per cent colloidal chitosan showed 40.51 per cent and 85.38 per cent antifeedant effect over untreated check respectively. The leaf weight loss was observed at one and 7 per cent colloidal chitosan exhibited 24.95 and 11.05 per cent respectively. The per cent reduction in leaf consumption on weight basis was 66.68 per cent when fed with 7 per cent colloidal chitosan treated leaf. Among different concentration used 7 per cent colloidal chitosan showed higher antifeedant effect against first instar larva of FAW.

Keywords: Fall armyworm, *Spodoptera frugiperda*, colloidal chitosan, Antifeedants

1. Introduction

Fall Armyworm (FAW) *S. frugiperda* is a deadly and latest invasive pest which primarily feed on maize but known to feed more than 100 plant species. (FAO, 2019) [5]. FAW has a short development cycle with high reproduction rate of about 1000 eggs per female. It was first reported in Southern India during July-August 2018 and the incidence ranged from 9.0 to 62.5 per cent (Sharanabasappa *et al.*, 2018) [17]. Chitin (C₈H₁₃O₅)_n is a typical marine renewable source and also second most abundant organic molecule after cellulose in earth, having a wide range of structure and forms (Kurtia, 2006) [8]. Chitosan is a linear polysaccharide and a biomaterial obtained by deacetylation of chitin, which have biocompatibility, biodegradability, and anti-bacterial properties (Sato *et al.*, 1998, Younes *et al.*, 2015, Ganesan *et al.*, 2017, Brindha *et al.*, 2018) [15, 18, 6, 3]. Depending on degree of polymerization, chitosan exhibited insecticidal nature (Zhang *et al.*, 2003, Sahebzadeh *et al.*, 2017) [21, 14] and antifeedant effect (Zeng *et al.*, 2012) [20] against some lepidopteran larva (Rabea *et al.*, 2005, Badaway *et al.*, 2012, Sabbour *et al.*, 2016) [11, 2, 13]. Insecticide in pest management leads to resistance, resurgence in pest and residues in crops. As an alternative to insecticides, chitosan received much attention for eco-friendly pest management. Therefore, the present study is focussed on the antifeedant effect of colloidal chitosan against maize fall armyworm. Since late instar larva of fall armyworm hides in whorl covered by faecal matter, it escapes from insecticidal application. Hence, special attention was given in managing the deadly pest during early stage.

2. Materials and methods

The laboratory experiments were conducted at Natural Pesticide Laboratory and Central instrumentation laboratory, Department of Agricultural Entomology and Centre of Innovation, Department of Biotechnology, Agricultural College and Research Institute, Madurai during August 2019.

2.1 Mass culturing of fall armyworm *S. frugiperda*

The larvae emerged from the egg mass were released into the container having fresh maize leaf using camel hair brush. Initially, the larvae were reared in bulk, third instar onwards they were transferred to separate container because of their cannibalism behaviour. Every day the leaf material was changed.

When the larvae attained the pre pupal stage, they were transferred to the tray containing sawdust for pupation. Adult emerged from the pupa were transferred to the oviposition cage (56.5 x 60 x 56.5cm) and 10% honey solution containing 2 to 3 drops of vitamin E solution, was given as feed. Maize leaves were used as a substrate for collection of egg mass. Then the collected egg mass was allowed to hatch and the larvae were reared and further used for bioassay and for mass culturing.

2.2 Synthesis of colloidal chitosan

Colloidal chitosan was prepared based on the method adopted by Cruz Camarillo *et al.*, (2003) [4] with slight modification. Crude chitosan flake was procured from MATSYAFED, Kerala State Co-operative Federation for Fisheries Development Ltd. Neendakara, Kerala. Chitosan flake (10g) was dissolved in 90ml of concentrated orthophosphoric acid and continuous stirring was done for one hour with glass rod. Later, the chitosan was kept for digestion overnight in deep freezer at 5°C. After digestion, it was mixed with pre-cooled water-ethanol mix at 2:1 ratio (i.e 1.5 litre of water and 750 ml of ethanol) and the mixture was refrigerated for 12 hrs. The resulting colloidal chitosan was separated by centrifugation at 4000rpm at 4°C under refrigerated centrifuge (model: Velocity 14R) and washed with concentrated ethanol (99.9%) till it reaches pH 7.0. After centrifugation, the white

gelatinous colloidal chitosan settled at the bottom was collected and freeze dried using lyophilizer (model: Scan Vac).

2.3 Antifeedant activity

Fresh maize leaf of 3 cm² (900 sq. mm) area was used for determination of antifeedant effect of colloidal chitosan against *S. frugiperda* under no choice condition. Colloidal chitosan of 1%, 3%, 5% and 7% was taken as treatment. Glacial acetic acid (1%) was used for dissolving colloidal chitosan in water and tween 20 (0.1%) acted as adjuvant for colloidal chitosan. The glacial acetic acid (1%), tween 20 (0.1%) and distilled water served as control. Totally there were seven treatments and three replications. First instar larva (10 nos.) was used for the experiment. Maize leaf bits were dipped in all the treatments using forceps for 30 sec. The treated leaf was air dried for about 30 to 60 min. Control leaf bit was prepared by dipping in distilled water. Later larvae were allowed to feed on treated leaves. Leaf weight was recorded using electronic balance (model: Radwag AS 82/220.R2) before release of larva and the total leaf area fed by larva was assessed using graph sheet. The leaf weight and leaf area fed were recorded for all the treatments individually at 24, 48 and 72 hrs after release of larva.

The absolute antifeedant index was calculated using the following formula of Nawrot *et al.*, (1986) [9],

Absolute antifeedant index

$$= \frac{\text{Leaf area consumed by larva in control leaf} - \text{Leaf area consumed by larva in treated leaf}}{\text{Leaf area consumed by larva in control leaf} + \text{Leaf area consumed by larva in treated leaf}} \times 100$$

The per cent weight of leaf was calculated using the following formula of Ngatia and Kimondo (2011) [10],

$$\text{Per cent weight of leaf consumed} = \frac{\text{Initial leaf weight} - \text{Final leaf weight}}{\text{Initial leaf weight}} \times 100$$

2.4 Statistical analysis

The data were subjected to arc sine transformation (Gomez and Gomez, 1984) [7]. The analysis of variance (ANOVA) was carried out by using AGRES Statistical package (version 3.01) to differentiate the transformed mean values by using Fisher's Least Significant Difference (LSD) at 5% probability level.

3. Results and Discussion

3.1 Antifeedant activity

The results of the laboratory experiment reveals that the colloidal chitosan is having antifeedant activity at all the concentration tested compared to untreated check. Antifeedant activity of colloidal chitosan was assessed based on per cent weight of leaf consumed and absolute antifeedant index. Higher the antifeedant index revealed the decreased rate of feeding. In the present study, antifeedant activity varied significantly based on different concentration of colloidal chitosan as shown in Table 1. Among five concentrations tested, 7% colloidal chitosan showed high mean absolute activity antifeedant activity (85.38 %) followed by 5% (57.75%), 3% (47.28 %), and 1% (40.51 %) against first instar larva of *S. frugiperda*. The interaction effect of 7% colloidal chitosan showed 93.81 per cent antifeedant effect after 72 hours of treatment followed by 85.22 per cent and 77.09 per cent after 48 hours and 24 hours of treatment respectively. Result showed that higher absolute antifeedant activity was observed after 72 hours of treatment. There was

11.05 per cent leaf weight loss in 7% while it was 33.17 per cent in untreated check (Table 2). Typically, colloidal chitosan inhibited the larval growth in a time-dependent manner from the first day of feeding on the treated leaf.

Rabea *et al.* (2006) [12] identified growth inhibition and reduced larval length in 75% of *Spodoptera littoralis* larva when treated with chitosan derivative and maximum inhibition of growth was observed fourth day of treatment. She reported that when larva fed with treated diet there was two to three fold reduction in weight gain and length because of abnormal ecdysis process. So, the larval size was very small when compared with control.

Archana (2015) [1] reported antifeedant effect of colloidal chitosan 7% on *Diaphania indica* was 43.25 per cent. Sayed *et al.* (2011) [16] studied effect of chitosan which was processed by different step, deproteinization (DP), demineralization (DM), decolouration (DC), deacetylation (DA) against *Galleria melleonella*. He noted that chitosan prepared from chemical processing step DMCPA (Demineralization, Decolouration, Deproteinization, and Deacetylation) showed much effective than others.

Zeng *et al.* (2011) [19] also reported that chitosan derivative at 5000 ppm showed 87.24% antifeedant effect in *Maruca vitrata* followed by *Agrotis ipsilon* and *Aphis glycines* which shows about 82.89% and 80.21% respectively. Zeng *et al.* (2012) [20] extracted chitin from crab and tested antifeedant effect against *Helicoverpa armigera* and recorded 84 % antifeedant effect in chitin at 5000 ppm.

Table 1: Antifeedant activity of colloidal chitosan against first instar larva of fall armyworm, *S. frugiperda* (J E Smith) in invitro condition

Treatment	Absolute antifeedant index (%)			Mean (%)
	24 HAT	48 HAT	72 HAT	
T ₁ – 1 % of colloidal chitosan	32.73 (34.88) ^f	41.49 (40.08) ^g	47.29 (43.42) ^h	40.51 (39.51) ^D
T ₂ – 3 % of colloidal chitosan	40.71 (39.63) ^e	48.32 (44.02) ^f	52.81 (46.59) ^g	47.28 (43.43) ^C
T ₃ – 5 % of colloidal chitosan	47.17 (43.36) ^f	55.45 (48.11) ^e	70.60 (57.14) ^d	57.75 (49.44) ^B
T ₄ – 7 % of colloidal chitosan	77.09 (61.38) ^c	85.22 (67.36) ^b	93.81 (75.57) ^a	85.38 (67.49) ^A
T ₅ – Adjuvant (0.1 % tween 20)	3.24 (10.37) ⁱ	2.94 (9.86) ⁱ	2.94 (9.87) ⁱ	3.04 (10.04) ^E
T ₆ – Diluent (1 % Aqueous glacial acetic acid)	1.45 (6.93) ^j	0.8751 (5.36) ^j	1.39 (6.79) ^j	1.24 (6.04) ^F
Mean (%)	33.74 (35.50) ^C	39.05 (38.66) ^B	44.81 (42.00) ^A	
	t	h	th	
SEd	0.60	0.42	1.04	
CD (0.05)	1.21**	0.86**	2.10**	

Numeric data represents the mean value of three replications, HAT – Hours after Treatment.

Figures in the parentheses are arc sine transformed values.

Mean values followed by the same alphabet in a column are not significantly different (P = 0.05) by LSD.

Table 2: Effect of colloidal chitosan on leaf feeding behaviour of first instar larva of fall armyworm, *S. frugiperda* (J E Smith) in *in vitro* condition

Treatments	Weight of leaf consumed (%)			Mean (%)	% reduction in consumption (wt basis)
	24 HAT	48 HAT	72 HAT		
T ₁ – 1 % of colloidal chitosan	14.50 (22.37) ^c	25.67 (30.43) ^g	34.69 (36.07) ⁱ	24.95 (29.96) ^D	24.78
T ₂ – 3 % of colloidal chitosan	13.40 (21.47) ^c	21.78 (27.81) ^f	33.48 (35.34) ^j	22.89 (28.57) ^C	30.99
T ₃ – 5 % of colloidal chitosan	9.60 (18.05) ^b	22.94 (28.61) ⁱ	28.82 (32.46) ^h	20.46 (26.88) ^B	38.31
T ₄ – 7 % of colloidal chitosan	5.79 (13.92) ^a	10.55 (18.95) ^b	16.79 (24.18) ^d	11.05 (19.40) ^A	66.68
T ₅ – Adjuvant (0.1 % tween 20)	16.40 (23.88) ^d	30.62 (33.58) ^h	42.31 (40.56) ^j	29.77 (33.06) ^E	10.25
T ₆ – Diluent (1 % Aqueous glacial acetic acid)	17.01 (24.35) ^{dc}	30.12 (33.27) ^h	47.44 (43.52) ^k	31.53 (34.14) ^F	4.94
T ₇ – Untreated check	18.88 (25.74) ^e	33.38 (35.28) ⁱ	47.26 (43.41) ^k	33.17 (35.15) ^G	
Mean (%)	13.65 (21.65) ^C	25.01 (29.99) ^B	35.83 (36.75) ^A		
	t	h	Th		
SEd	0.41	0.27	0.71		
CD (0.05)	0.83**	0.54**	0.73**		

Numeric data represents the mean value of three replications, HAT – Hours after Treatment.

Figures in the parentheses are arc sine transformed values.

Mean values followed by the same alphabet in a column are not significantly different (P = 0.05) by LSD.

4. Conclusion

The result shows that colloidal chitosan has good antifeedant activity, Hence it has great to use as eco-friendly management tool against *S. frugiperda* in maize.

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