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COLLOIDAL CHITIN SYNTHESISED FROM MARINE WASTE AS NOVEL ECOFRIENDLY ANTIFEEDANT FOR THE FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J E SMITH)

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ABSTRACT

Colloidal chitin is a natural compound shown to have antifeedant activity against insects. In this study, the antifeedant activity of colloidal chitin was investigated against the first instar larva of *Spodoptera frugiperda* (J E Smith). The results showed that colloidal chitin had antifeedant activity, with the highest activity observed at 7% concentration. The antifeedant effect was enhanced with increase in concentration and also with increase in time after treatment. Specifically, colloidal chitin 7% showed 90.43% antifeedant effect after 72 hr of treatment, followed by 87.43 and 81.76% after 48 hr and 24 hr of treatment, respectively. The results suggest that colloidal chitin could be a potential candidate for use as an insect repellent or pesticide.

Key words: Colloidal chitin, marine waste, *Spodoptera frugiperda*, synthesis, standardization, FTIR, antifeedant, ecofriendly, deacetylation, solubility, β- glucosidic bonds, concentration, time

Chitin is the major component in Arthropoda exoskeleton, available in large proportion, especially in the crustacean exoskeleton. It is one of the most ubiquitous natural long-chain biopolymers. It consists of N-acetyl glucosamine, which is a derivative of glucose. It is not readily soluble in water. This polysaccharide consists of several functional groups like acetyl, amino, and hydroxyl, which have several inter and intramolecular hydrogen bonds. The solubility of chitin increased due to the deacetylation process with the addition of a basic solution (Zeng et al., 2012). The deacetylation increases its solubility due to the hydrophilic nature of the introduced amino group. These are new functional biomaterials with the greatest potential in numerous fields. There are copious opportunities for further development. Recently, the exploitation of chitin and its derivatives in IPM of agricultural pests has invited much attention. Maize fall army worm (FAW), *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is recently invaded insect pest, causing threat to grain/ fodder production. It is a long-distance migratory insect pest reported in India at Karnataka in July 2018, and later it has spread all over India (Sharanabasappa et al., 2018). It is a highly destructive polyphagous pest attacking > 300 agricultural and horticultural crops; more significantly damages maize, sorghum, cowpea, and millets (Montezano et al., 2018). Hence, to manage maize this pest, chitin can be included as one of the ecofriendly tools in IPM for maize.

MATERIALS AND METHODS

Colloidal chitin was prepared adopting the standard method (Danai-Tambhale, 2018), with slight modification. The chitin flakes (10 g) were taken in a 1000 mL beaker and 50 mℓ of concentrated ortho-phosphoric acid was added slowly in fume hood chamber, along the sides of the beaker and stirred with glass rod for one min at an interval of every five min for one hour at room temperature. Later, the chitin-acid mixture was incubated overnight in freezer at 5°C, for digestion. After dissolving the chitin completely, the impurities were removed by filtering through glass wool in Buchner funnel fitted with vacuum filtration flask, which contained precooled water-ethanol mix at 2:1 ratio (i.e., 1.5 ℓ of water and 750 ℓ of ethanol). The milky white colour colloidal substance was precipitated, while the filtrate was mixing with ice-cold water-ethanol mix. This process was repeated several times until the mixture becomes neutral (pH 7.0). The precipitated colloidal chitin was separated by centrifugation at 5000 rpm in refrigerated centrifuge at 4°C. The precipitate in the bottom of the centrifuge tube was freeze dried in lyophilizer, powdered and stored in refrigerator at 4°C, until used for bioassay. The infrared spectral analysis of the crude and colloidal chitin was recorded

by Fourier Transform Infrared Spectrometry (FTIR) using FT/IR-4700.

Maize leaf of 4 cm^2 (1600 sq mm) area was used for determination of antifeedant effect of colloidal chitin against *S. frugiperda* based on both area and weight basis under no choice condition. Colloidal chitin was dissolved in the solvent, aqueous glacial acetic acid (1%) and tested at 1%, 3%, 5% and 7%. Tween 20 (0.005%) was used as surfactant and the glacial acetic acid (1%) and distilled water served as control. Totally there were seven treatments and three replications for colloidal chitin. Treatment concentrations were fixed based on preliminary studies. Maize leaf bits were dipped in the respective treatments using forceps for 30 sec. The treated leaf was air dried for about 30 min. Control leaf bit was prepared by dipping in distilled water. Later the first instar larva (10 nos./ replication) was allowed to feed on treated leaves individually. Leaf weight was recorded using electronic balance (model: Radwag AS 82/220.R2) before/ after release of larva and the total leaf area fed by larva was assessed using graph sheet. The weight of leaf fed (mg/ larva) and leaf area fed (sq.mm/ larva) were recorded for all the treatments individually at 24, 48 and 72 hr after release of larva. One set of leaf was maintained in similar condition without larval release, to record the reduction in leaf weight due to moisture loss, which was used to nullify the weight loss due to evaporation, while estimating the weight of leaf fed. The absolute antifeedant index was calculated using the formula of Nawrot et al. (1986). The % weight of leaf consumed was calculated using the formula Ngatia and Kimondo (2011). The % reduction over control was calculated using the formula of Abbott, (1925). All the experiments were conducted under completely randomized block design (CRBD). Data were statistically analyzed using SPSS for Windows (version 22) (IBM Corp. Released 2013) software to carry out ANOVA. Grouping of data was done using Tukey's HSD (honestly significant difference) test (Tukey, 1977; p= 0.05).

RESULTS AND DISCUSSION

The colour and texture of the synthesized product was different from crude chitin. The crude chitin was white and texture was rough, whereas, the colloidal chitin was milky white and colloidal. Dry yield of colloidal chitin was 6.3 g out of 10 g of crude chitin flakes. The FTIR spectral analysis of crude chitin revealed the following eight characteristic absorption bands viz., 3433, 2927, 1635, 1560, 1378, 1261, 1156 and 896 cm^{-1}, which showed respective functional group of N-H, C-H, C=C, C=C-C, CH_3, C-OH, C-H and C-H deformation. FTIR spectrum of laboratory synthesized colloidal chitin showed four bands viz., 3891 (O-H), 3493 (N-H), 3218 (Amide I), and 1651 cm^{-1} (C=C). The result showed the presence of two bands from parent compound 3433 and 2927 cm^{-1} remain undisturbed. There was a formation of alcoholic group in colloidal chitin at 3891 cm^{-1} which showed increase in solubility over crude chitin. At absorbance band 3218 cm^{-1} overlapping of N-H with hydroxyl group occurred in colloidal chitin and no such band was found in crude chitin. The typical character of colloidal chitin was absence of methyl (CH_3) group. C-H deformation at 896 cm^{-1} showed breakage of β- glucosidic bonds in colloidal chitin.

Cardenas et al. (2004) reported chitin absorbance band of 3479 cm^{-1} due to the intra molecular hydrogen bond involving the OH functional group. Kumirska et al. (2010) also reported the IR spectra of β-chitin at band 1630 cm^{-1} and α-chitin at band 1562 cm^{-1}. He also report the band shifts from 890 cm^{-1} in β-chitin to 895 cm^{-1} 1 in α-chitin represent CH deformation of the β-glycosidic bond. Cardenas et al. (2004) also reported band at 3268 cm^{-1} assigned in the α-chitin to the vibrational modes of the NH of the amide (intermolecular hydrogen bond C=O, H-N). The results indicated that the solubility of chitin derivative in 1% glacial acetic acid enhanced upon chemical modification. The crude chitin exhibited lesser solubility as 10.00%, whereas conversion of chitin to colloidal chitin enhanced the solubility to 73.30%, in 1% glacial acetic acid. These present findings on the solubility of chitin are supported by the findings of the several scientists. Crawfish chitin showed lower solubility (26.40%) compared with other chitins (Nawrot et al., 1986). Shah et al. (2018) also reported solubility colloidal chitosan of silver nanoparticles was 63%. The solubility depends on the degree of deacetylation. The result also indicates that there was increase in solubility with the increase in the degree of deacetylation (DD) of chitin and its derivatives. The DD was less in crude chitin (37.00%), and improved to 77.30% in colloidal chitin. This is apparently evidenced by the improvement in the affinity for solvent (1% glacial acetic acid). The degree of deacetylation of chitin obtained from prawn shell and silver scale was 48.59 and 47.59%, respectively (Alabaraoye et al., 2018). Fernandez-Kim (2004) reported that 68% of degree of deacetylation when chitosan obtained from DPMCA (deproteinized, demineralized, decolorized and deacetylated) process. According to Di Martino et al. (2005) DD of chitosan range from 30 to 90%. There

exists a need to improve the solubility and Degree of deacetylation to enhance its applicability in agriculture.

The results revealed that the colloidal chitin was having antifeedant activity and varied significantly at different concentrations. Higher antifeedant index indicates the decreased rate of feeding. The antifeedant effect was enhanced with increase in concentration and also with time after treatment (Table 1). Among the four concentrations tested, colloidal chitin 7% showed 90.43% antifeedant effect after 72 hr of treatment followed by 87.43% and 81.76% after 48 and 24 hr of treatment of the same treatment, respectively. Result showed that higher absolute antifeedant activity was observed after 72 hr of treatment. Day by day, there was an increase in antifeedant index. The chitosan at 5% showed 87.24% antifeedant effect on *Maruca vitrata* Geyer. followed by *Agrotis ipsilon* Hufnagel. and *Aphis glycines* Matsumura. which exhibited about 82.89 and 80.21% antifeedant effect, respectively (Zeng and Mei, 2011). The present findings are consistent with those of AL-Khazraji and Shaher (2020), who showed that larvae treated with chitosan had lower food intake rates and altered metabolism. This led to a slower overall growth rate and the development of small pupae with insufficient food reserves. This, in turn, has a direct impact on the reproductive capacity and lifespan of adults. When Zeng et al. (2012) extracted and tested antifeedant effect of the chitin from crab at 5%, there was 84 % antifeedant effect on *Helicoverpa armigera* (Hubner) due to chitin. This finding was supported by Paulraj et al. (2017) that nanopesticide formulation developed using chitosan nanoparticles (CSNs) with cross-linking agent tripolyphosphate (TPP) and a botanical pesticide PONNEEM® (CSNs-TPP-PONNEEM) recorded 88.5% antifeedant activity against *H. armigera* followed by CSNs-TPP combination (76.44%) respectively.

Badawy and El-Aswad (2012) reported that lower molecular weight chitosan (2.27 x 10^5 g/ mol) showed 76% antifeedant activity against *Spodoptera littoralis* (Boisduval) larva. He also reported 90 and 86% in antifeedant activity in chitosan-Ni and chitosan-Hg complexes. Uddin et al. (2021) also reported that a concentration of 3000 ppm of chitosan inhibited larval growth by the highest amount (27.61%), followed by the topical method (13.81%) and leaf-dip method (12.27%) against *S. litura*. This result is consistent with present findings. Also Abdullah and Sukar (2021), reported chitosan mixture (chitosan with secondary metabolites of *Beauveria bassiana* Balsamo.) showed antifeedant activity of 74.51% against third instar larva of *S. littoralis*. Higher % reduction in consumption of leaves on weight basis indicates lesser consumption by larva. When the leaves were treated with colloidal chitin 7%, the first instar larva of *S. frugiperda* consumed lesser leaf, and it was 69.90% reduction. It was followed by colloidal chitin 5% (57.14%). With the increase in time, the weight of leaf consumed increased, as there was increase in age of the larvae (Table 1). There was two-fold increase in weight of leaf consumed on every 24, 48 and 72 hr after treatment at all the treatments. Interaction study showed lowest % weight of leaf consumed on 24 hr of treatment at 7 (3.66%) and 5% (4.01%). Typically, colloidal chitin inhibited the larval growth in a time-dependent manner from the first day of feeding on the treated leaf (Table 1). Ding et al. (1998), confirmed that the larvae reared on plants deficient of *Manduca* chitinase.

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AUTHOR CONTRIBUTION STATEMENT

Moorthy A V, Shanthi M performed idea for the study. Selva Rani S helped in synthesis of colloidal chitin. Moorthy A V did preliminary and main bioassay and wrote the manuscript. Shanthi M guided the whole study including correction of the article.

CONFLICT OF INTEREST

No conflict of interest.

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Table 1. Antifeedant activity and weight of leaf consumed by first instar larva of *S. frugiperda* treated with colloidal chitin

Treatment	% weight of leaf consumed			Absolute antifeedant index (%)[#]			% reduction in consumption over untreated check (wt basis)
	24 HAT	24 HAT	24 HAT	24 HAT	48 HAT	72 HAT	
T_1 –Colloidal chitin 1%	34.99± 3.70 (36.25)^{c}	34.99± 3.70 (36.25)^{c}	34.99± 3.70 (36.25)^{c}	7.33± 0.49 (15.70)^{b}	14.60± 0.70 (22.46)^{cd}	28.99± 1.40 (32.56)^{c}	38.62
T_2 –Colloidal chitin 3%	50.35± 4.64 (45.18)^{b}	50.35± 4.64 (45.18)^{b}	50.35± 4.64 (45.18)^{b}	6.05± 0.45 (14.23)^{b}	13.47± 0.91 (21.53)^{bc}	29.97± 1.00 (33.18)^{c}	40.32
T_3 –Colloidal chitin 5%	53.65± 2.18 (47.07)^{b}	53.65± 2.18 (47.07)^{b}	53.65± 2.18 (47.07)^{b}	4.01± 0.29 (10.54)^{a}	10.99± 1.20 (19.35)^{b}	21.21± 1.53 (27.41)^{b}	57.14
T_4 –Colloidal chitin 7%	81.76± 2.18 (64.69)^{a}	81.76± 2.18 (64.69)^{a}	81.76± 2.18 (64.69)^{a}	3.66± 1.72 (11.03)^{a}	7.28± 1.32 (15.65)^{a}	14.03± 1.02 (21.99)^{a}	69.90
T_5 – Surfactant (Tween 20- 1 %)	2.69± 0.35 (9.44)^{d}	2.69± 0.35 (9.44)^{d}	2.69± 0.35 (9.44)^{d}	9.85± 0.73 (18.29)^{c}	17.62± 1.08 (24.81)^{de}	41.08± 0.94 (39.85)^{d}	17.35
T_6 – Solvent (aqueous glacial acetic acid 1%)	1.94± 0.79 (8.00)^{d}	1.94± 0.79 (8.00)^{d}	1.94± 0.79 (8.00)^{d}	9.38± 1.15 (17.83)^{c}	18.50± 0.86 (25.64)^{ef}	47.55± 1.24 (43.58)^{de}	9.07
T_6 – Control	-	-	-	11.77± 0.34 (20.06)^{d}	22.03± 1.62 (27.98)^{f}	49.15± 2.43 (44.49)^{e}	-
SEd*	1.46	0.94	1.58	1.07	0.80	1.10	-
F value	466	2177	2370	23.28	51.31	212.1	-
P value**	0.00	0.00	0.00	0.00	0.00	0.00	-

[#]Mean values of three replications represented as mean± standard deviation; Figures in parentheses arc sine transformed values; Means followed by same letter not significantly different from each other, Tukey's test ($p \leq 0.05$); SEd: Standard error of the difference; HAT- hours after treatment

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