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EVALUATION OF COLLOIDAL CHITOSAN AGAINST DIAMOND BACK MOTH *PLUTELLA XYLOSTELLA* L. ON CAULIFLOWER

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ABSTRACT

An ecofriendly colloidal chitosan byproduct synthesized from crude chitosan was evaluated for its chronic toxicity against second instar larvae of *Plutella xylostella* L. by leaf dip bioassay. The consumption of 10000 ppm colloidal chitosan treated leaf caused 100% mortality after the 4th day, with least larval weight (0.02 mg). Whereas, the consumption of 8000 ppm treated leaves reduced the larval (0.65 mg), pupal (1.26 mg), and adult weights (0.75 mg), with 10% adult malformation and extended larval duration. This is compared to untreated larval duration of 10.66 days. Thus, colloidal chitosan exhibits chronic toxicity and growth inhibition effect on *P. xylostella* larvae.

Key words: *Plutella xylostella*, cauliflower, chitosan, synthesis, colloidal chitosan, glacial acetic acid, chronic toxicity, growth inhibition, malformation, larval period, larval weight

Diamond back moth (DBM) *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a widespread pest of cultivated and wild Brassicaceae viz., cabbage, cauliflower, broccoli, Brussel sprouts, radish, and field crops such as turnip, mustard, and rape. The pest can cause a high yield loss of 91.2% (Elizeu et al., 2020). Farmers apply insecticides at 6 to 10 days interval for 6 to 8 times to control this, and thus there is indiscriminate usage of insecticides. This results in residues, resurgence, resistance, and environmental hazards, warranting use of alternative ecofriendly insecticides. Recently, chitosan is gaining momentum in plant protection. Chitin is the most abundant biopolymer in marine environments (Souza et al., 2011), isolated from crustaceans such as crab and shrimp as well as from fungi (Kurita et al., 2000) and derived by deacetylation of chitin. It's a poly-b-(1™4)-D-glucosamine, and has attracted considerable attention for its potential applications in food, agriculture, medicine, pharmaceuticals, cosmetics, and wood preservation, due to its interesting physicochemical and biological properties (Eikenes et al., 2005; Torr et al., 2005; Du et al., 2009). It may also serve as a good alternative to pest control because of its insecticidal and antimicrobial properties (Rabea et al., 2003; Badawy et al., 2005). Its insecticidal effect against lepidopteran and homopteran insect pests is known, especially against *P. xylostella, Helicoverpa armigera, Spodoptera exigua,* and aphids *Hyalopterus pruni* (Zhang et al., 2003). Chitosan is an active insecticide against 4th instar larvae of *Spodoptera littoralis* (Sayed et al., 2014). The insect mortality can be achieved at its low dosage levels, and it is non-toxic to vertebrates and humans. Chitosan treatments are effective against herbivorous insect pests, but it has been used successfully as an ingredient in the artificial diet fed to carnivorous insects being reared for use in the biological control of chitinous pests (Tan et al., 2010). The present study focused on the synthesis of colloidal chitosan from chitosan and evaluation of its chronic toxicity and growth inhibition against diamond back moth *P. xylostella*.

MATERIALS AND METHODS

The synthesis and evaluation of colloidal chitosan were conducted in the Natural Pesticide Laboratory, Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai during 2020-2021. Colloidal chitosan was synthesized based on the method adopted by Cruz et al. (2004) with slight modification. Crude chitosan flakes were procured from MATSYAFED, Kerala State Co-operative Federation for Fisheries Development Ltd., Neendakara, Kerala. Crude chitosan flakes (10 g) were dissolved in 1500 ml of

0.2 N Hydrochloric acid for one hour by intermittent stirring and digested overnight. After digestion, pH was adjusted to 5.5 by using 0.2 N NaOH and 0.1 N HCl. After neutralization, the chitosan was centrifuged (model: Velocity 14R) at 7000 rpm for 5 min, the sediment was collected and freeze-dried in a lyophilizer (model: Scan Vac), and designated as colloidal chitosan.

Plutella xylostella culture was maintained by following the procedure described by Justin (1996). The effect of colloidal chitosan on the growth and development of *P. xylostella* was estimated by allowing the larvae to feed chronically on the colloidal chitosan-treated leaves (leaf dip bioassay) from second to final instar. The colloidal chitosan was dissolved in the solvent (1% glacial acetic acid), mixed with surfactant (Tween 80 0.05%), and prepared at different concentrations viz., 3000 ppm, 5000 ppm, 8000 ppm, 10000 ppm. Tween 80 0.05% and glacial acetic acid 1% were kept as negative checks, azadirachtin 1 EC @ 2 ml/ l was used as a treated check, in comparison with untreated check. The leaf bits (4x 4 cm) were prepared from young cauliflower leaves and used for the bioassay. Each treatment was replicated thrice, with each consisting of 10 second instar larvae. Larval weight was recorded daily. Pupal and adult weights were also noted. The % reduction in weight of larva, pupa, and adult over untreated check was estimated. If there were any malformations or mortalities during any life stages, these were also recorded. Larval, pupal period, adult life span, and the number of adults emerged were recorded. Adult emergence was estimated in % (Tian et al., 2020). All the experiments were conducted under a 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 by using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The mass of the colloidal chitosan (20 g) prepared from the raw material, called crude chitosan (10 g) was increased by two times. But, when the colloidal chitosan was freeze-dried, the mass was reduced to 5 g. It shows that the recovery yield of colloidal chitosan from crude chitosan was 50%. The raw chitosan was in the form of flakes and insoluble in water, hence it was transformed into colloidal form by deacetylation process, which had increased solubility in aqueous acidic solution, water binding capacity, and degree of deacetylation (%) (Selva Rani et al., 2021). The chronic feeding of *P. xylostella* on colloidal chitosan-treated leaves, at all concentrations, resulted in an efficient reduction in the growth and development of larva compared to the untreated check. The weight of the larva fed on colloidal chitosan 10000 ppm and azadirachtin 1 EC @ 2 ml lit^{-1} was very minimum after the third day of feeding (D7) (0.10 mg and 0.06 mg, respectively). The next effective treatment was colloidal chitosan 8000 ppm (0.37 mg), while the untreated check had 1.19 mg on the same day. The maximum larval weight attained in 8000 ppm and the untreated check was 0.92 mg and 5.97 mg per larva, respectively. Subsequently, there was a significant reduction in pupal and adult weight also (Table 1).

This significant reduction in larval, pupal, and adult weight after feeding on colloidal chitosan 8000 ppm was noticed (91.57%, 74.38% and 66.32%, respectively). Consequently, the larval period of *P. xylostella* was prolonged by two days (12.66 days) compared to untreated check (10.66 days). In the case of pupal period and adult life span, no significant difference was found among the treatments. From the chronic feeding study, it was found that 10000 ppm of colloidal chitosan and azadirachtin 1 EC caused 100% larval mortality on the fourth day after treatment, hence no adults were emerged in these treatments. While, 8000 ppm colloidal chitosan caused larval mortality to an extent of 63.33% and malformations of larva, pupa and adult to a level of 23.33, 3.33, and 10.0%, respectively and no normal adults were emerged (Table 1). These results revealed the efficacy of the colloidal chitosan at 10000 and 8000 ppm in causing chronic toxicity and growth inhibition in *P. xylostella*. At the same time, there was no mortality and growth inhibition of larva, pupa, and adult in the untreated check.

Chitosan caused 72% of mortality of *P. xylostella* due to the formation of the film on the surface of insects, which block the air while breathing that resulting in asphyxiation and ultimate death (Zhang et al., 2003). In another study, chitosan derivative (*N*- (2- chloro- 6- fluorobenzyl) was found effective against *S. littoralis* with an LC50 of 0.32 g kg^{-1} diet and 100% mortality at ≥0.625 g kg^{-1} (Badawy et al., 2012). Typically, colloidal chitosan inhibited the larval growth in a time-dependent manner from the first day of feeding on the treated leaf. Unmodified chitosan caused 62.72% mortality and also 27% reduction of *S.litura* larval growth after 7 days of treatment (Uddin et al., 2021). An early study of *N*-alkyl chitosan (NAC) derivatives against *S.litura* reported that insect growth was significantly decreased and the

Table 1. Effect of chronic feeding of colloidal chitosan on *P. xylostella.*

Treatments	Reduction in weight over untreated (%)*$			Developmental period (days)*#			Larval morality (%)*$		Pupal morality (%)*$		Adult emergence (%)*$	
	Larval weight	Pupal weight@	Adult weight@	Mean Larval Period+	Mean pupal period+	Mean adult life span+	Normal@	Malformed@	Normal@	Malformed@	Normal@	Malformed@
T1– Colloidal chitosan - 3000 ppm	75.82± 0.74 (60.52)^{d}	47.07± 5.38 (43.32)^{c}	39.64± 2.75 (39.02)^{c}	11.00± 0.00 (3.31)^{b}	5.00± 0.00 (2.23)	14.66± 0.57 (3.82)	33.33± 0.00 (35.26)^{d}	6.66± 0.57 (14.96)^{b}	-	0.00± 0.00 (0.91)^{b}	53.33± 0.57 (54.73)^{b}	6.66± 0.57 (14.96)^{a}
T2 – Colloidal chitosan -5000 ppm	81.98± 3.06 (64.85)^{c}	58.60± 1.61 (49.95)^{b}	53.37± 1.34 (46.93)^{b}	11.00± 0.00 (3.31)^{b}	5.33± 0.57 (2.30)	14.00± 0.00 (3.74)	43.33± 0.57 (41.16)^{c}	6.66± 0.57 (14.96)^{b}	-	3.33± 0.57 (10.51)^{a}	40.00± 0.57 (39.23)^{c}	6.66± 0.57 (14.96)^{a}
T3 – Colloidal chitosan - 8000 ppm	91.57± 1.87 (73.09)^{b}	74.38± 0.82 (59.59)^{a}	66.32± 4.46 (54.53)^{a}	12.66± 0.57 (3.55)^{a}	5.33± 0.57 (2.30)	0.00± 0.00 (0.91)	63.33± 0.57 (52.73)^{b}	23.33± 0.57 (28.88)^{a}	-	3.33± 0.57 (10.51)^{a}	0.00± 0.00 (0.91)^{d}	10.00± 0.00 (18.43)^{a}
T4 – Colloidal chitosan - 10000 ppm	99.63± 0.43 (86.49)^{a}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)	0.00± 0.00 (0.91)	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	0.00± 0.00 0.91)^{d}	0.00± 0.00 (0.91)^{b}
T5 – Acetic acid 1%	2.88± 0.44 (9.76)^{e}	2.46± 3.03 (9.02)^{d}	1.55± 0.61 (7.16)^{d}	10.33± 0.57 (3.21)^{b}	5.00± 0.00 (2.23)	14.33± 0.57 (3.78)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
T6 –Tween80 0.05%	4.67± 2.02 (12.48)^{e}	1.69± 1.17 (7.46)^{d}	0.62± 0.31 (4.52)^{d}	10.66± 0.57 (3.26)^{b}	5.00± 0.00 (2.23)	14.66± 0.57 (3.89)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
T7 – Azadirachtin 1 EC @ 2 ml/ l	99.24± 0.86 (84.97)^{a}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{e}	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)	0.00± 0.00 (0.91)	100.00± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	0.00± 0.00 (0.91)^{d}	0.00± 0.00 (0.91)^{b}
T8 – Untreated check	-	-	-	10.66± 0.57 (3.26)^{b}	5.00± 0.00 (2.23)	14.33± 0.57 (3.78)	0.00± 0.00 (0.91)^{c}	0.00± 0.00 (0.91)^{c}	-	0.00± 0.00 (0.91)^{b}	100± 0.00 (90.00)^{a}	0.00± 0.00 (0.91)^{b}
SEd	1.23*	1.86*	1.76*	0.33*	NS(0.24)	NS(0.43)	0.28	0.43	-	0.27	0.16	0.27

Treatments	Mean fresh weight of larvae (mg)+						Cumulative daily larval mortality (%)@			
	Initial weight	3 DAT	4 DAT	5 DAT	6DAT	7DAT	1DAT	2DAT	3DAT	4DAT
T1– Colloidal chitosan - 3000 ppm	0.11± 0.01 (0.33)^{a}	0.79± 0.02 (0.89)^{c}	1.13± 0.04 (1.06)^{b}	1.36± 0.11 (1.17)^{c}	1.42± 0.09 (1.19)^{b}	1.52± 0.04 (1.23)^{ab}	0.00± 0.00 (4.05)^{d}	13.33± 0.57 (21.40)^{d}	23.33± 0.47 (28.88)^{c}	23.33± 0.57 (28.88)^{d}
T2 – Colloidal chitosan -5000 ppm	0.11± 0.02 (0.33)a	0.72± 0.09 (0.85)^{c}	1.10± 0.02 (1.05)^{b}	0.71± 0.18 (0.84)^{b}	1.05± 0.05 (1.02)^{a}	1.16± 0.17 (1.23)^{ab}	3.33± 0.47 (10.51)^{d}	20.00± 0.00 (26.55)^{d}	30.00± 0.81 (33.19)^{c}	36.66± 0.57 (37.26)^{c}
T3 – Colloidal chitosan - 8000 ppm	0.11± 0.01 (0.33)^{a}	0.37± 0.09 (0.61)^{b}	0.94± 0.10 (0.97)^{a}	0.49± 0.18 (0.70)^{a}	0.92± 0.25 (0.96)^{a}	0.65± 0.13 (0.81)^{a}	13.33± 0.47 (21.40)^{c}	43.33± 0.57 (41.15)^{c}	50.00± 0.00 (50.74)^{b}	53.33± 0.57 (46.91)^{b}
T4 – Colloidal chitosan - 10000 ppm	0.10± 0.01 (0.32)^{a}	0.10± 0.04 (0.32)^{a}	Dead	Dead	Dead	Dead	26.66± 0.47 (31.07)^{b}	66.66± 0.57 (54.71)^{b}	83.33± 0.47 (65.87)^{a}	100.00± 0.00 (90.00)^{a}
T5 – Acetic acid 1%	0.10± 0.01 (0.32)^{a}	1.18± 0.01 (1.08)^{d}	2.03± 0.01 (1.43)^{c}	4.05± 0.06 (2.01)^{d}	5.58± 0.10 (2.36)c	5.28± 1.08 (2.30)^{bc}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (0.66)^{e}
T6 –Tween80 0.05%	0.10± 0.01 (0.32)^{a}	1.18± 0.03 (1.08)^{d}	2.07± 0.07 (1.44)^{c}	4.06± 0.04 (2.01)^{d}	5.69± 0.11 (2.38)^{c}	3.63± 3.16 (1.90)^{b}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}
T7 – Azadirachtin 1 EC @ 2 ml/l	0.10± 0.00 (0.32)^{a}	0.06± 0.06 (0.25)^{a}	Dead	Dead	Dead	Dead	40.00± 0.81 (39.23)^{a}	76.66± 1.15 (61.09)^{a}	90.00± 0.00 (71.53)^{a}	100.00± 0.00 (90.00)^{a}
T8 – Untreated check	0.11± 0.01 (0.33)^{a}	1.19± 0.02 (1.09)^{d}	2.08± 0.03 (1.44)^{c}	4.27± 0.05 (2.07)^{e}	5.66± 0.14 (2.87)^{c}	5.97± 0.06 (2.44)^{c}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}	0.00± 0.00 (4.05)^{d}	0.00± 0.00 (4.05)^{e}
SEd	0.006	0.044	0.038	0.087	0.106	0.97	0.40	0.37	0.36	0.30

*Mean values of three replications as $\bar{x}$± SD; +,#Figures in parentheses square root transformed values +(√x+0.5); @,$Figures in parentheses arc sine transformed values @ (x+0.5); mean followed by same letter not significantly different from each other, DMRT ($p \leq 0.05$); SEd: Standard error of difference.

normal ecdysis process was affected, with symptoms of inhibition of feeding and weight gain, and the larvae were very small compared with the controls (Rabea et al., 2006). Colloidal chitosan 7% caused 85.38 % antifeedant activity against the first instar larva of *S. frugiperda,* further it inhibited the larval growth in a time-dependent manner from the first day of feeding (Moorthy et al*.,* 2021). Chitosan mixture (chitosan with secondary metabolites of *B. bassiana*) showed growth inhibition of 80.68% at 3000 ppm concentration against third instar larva of *S. littoralis* (Abdullah and Sucker, 2021). Thus, the present study observed that colloidal chitosan possesses the toxic and growth inhibition effect on *P. xylostella* larva upon chronic feeding. Hence, it could be explored further as an ecofriendly biomolecule.

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