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RESEARCH ARTICLE

Bioconversion of Black Soldier Fly (*Hermetia illucens* Linn.) Pupal Cases to Chitosan: Assessing the Insecticidal and Phytotonic Effects on Tomato (*Solanum lycopersicum* L.)

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ABSTRACT

The growing demand for sustainable agricultural inputs has intensified interest in insect-derived biomaterials as eco-friendly alternatives to synthetic agrochemicals. This study extracted chitosan from the pupal cases of the Black Soldier Fly (BSF), *Hermetia illucens* Linn., in a sequential chemical deproteinization-demineralization-deacetylation process, and evaluated its insecticidal and phytotonic effects on tomato (*Solanum lycopersicum* L.). From 20 g of dried pupal case material, chitin yields of 10-12 per cent and chitosan yields of 6-10 per cent of the initial dry weight were obtained, respectively, corresponding to a final chitosan yield of 2.8 g (14%). Foliar application of graded chitosan concentrations (1-5%) was evaluated in Completely Randomized Design against five economically important tomato pests: whitefly (*Bemisia tabaci* Gennadius), aphid (*Aphis gossypii* Glover), thrips (*Frankliniella schultzei* Trybom), leafhopper (*Amrasca devastans* Distant), and pinworm (*Tuta absoluta* Meyrick) - across three successive spray cycles, with observations recorded at 3, 7, and 15 Days After Spraying (DAS) and pooled as consolidated spray means for analysis. Pest populations declined progressively with increasing chitosan concentration and successive sprays. The 5 per cent chitosan treatment (T5) was most effective, reducing whitefly, thrips and leafhopper populations to 4.20, 1.30 and 1.30 no./ leaf, as against 15.20, 11.33 and 10.13 no./leaf in the untreated control, respectively, aphid incidence was 2.03 per cent in T5 as against 13.23 per cent in control, and pinworm damage to 3.50 per cent in T5 as against 15.20 per cent in control after the third spray. Concurrently, T5 recorded the highest plant height (47.0 cm), the more number of leaves (10/plant) and flowers (48/plant), as against 32.5 cm, 5 and 24 flowers/plant, respectively, in the untreated control. These findings establish BSF pupal-case chitosan as a dual-function, eco-friendly biopesticide and plant growth promoter with strong potential for integration into sustainable tomato production systems.

Keywords: Black Soldier Fly; *Hermetia illucens*; *Chitosan; Biopesticide; Tomato; Phytotonic Effect; Sustainable Agriculture*

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INTRODUCTION

Modern agricultural systems face continuous pressure from diverse insect pest complexes that cause substantial economic yield losses in high-value horticultural crops such as tomato (*Solanum lycopersicum* L.). Sucking pests, including whiteflies (*Bemisia tabaci* Gennadius), aphids (*Aphis gossypii* Glover), thrips (*Frankliniella schultzei* Trybom), and leafhoppers (*Amrasca devastans* Distant), together with internal borers such as the tomato pinworm (*Tuta absoluta* Meyrick), severely impair plant vitality, act as vectors for phytopathogenic viruses, and diminish market quality. Conventional reliance on synthetic chemical insecticides to manage this pest complex has triggered adverse environmental accumulation, the development of pest resistance, and ecological disruption of natural enemy populations, driving a transition toward bio-based, biodegradable pest control agents that align with global sustainability goals.

Chitosan, a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units, is widely recognized for its antimicrobial, elicitor, and insecticidal properties. It has traditionally been sourced from marine crustacean shell waste; however, supply variability, allergenicity concerns, and seasonal dependence on marine harvests have driven interest in alternative, terrestrial chitin-chitosan sources. The Black Soldier Fly (*Hermetia illucens* Linn.) is a premier agent of organic-waste bioconversion that accumulates significant chitin reserves in its larval and pupal exoskeleton. Valorizing the pupal case, an otherwise discarded by-product of BSF-based waste-processing enterprises, into high-value chitosan offers a circular bioeconomy pathway that simultaneously supports organic waste management and sustainable crop protection.

Although crustacean-derived chitosan has been extensively studied as a biopesticide and plant biostimulant, comparatively little work has evaluated the dual insecticidal and phytotonic performance of BSF pupal-case chitosan under dose-responsive, controlled pot-culture conditions on a major solanaceous crop. The present study was therefore undertaken with the objectives of (i) extracting and processing chitosan from BSF pupal cases through a sequential chemical extraction method, and (ii) evaluating its insecticidal efficacy against a representative multi-pest complex of tomato and its concurrent phytotonic (growth-promoting) effect, using standardized biometrical evaluation protocols across three successive spray cycles.

MATERIALS AND METHODS

Experimental Site and Duration

The pot culture experiment was conducted at the Department of Entomology, Dr. M.S. Swaminathan Agricultural College and Research Institute, Eachangkottai, Thanjavur, Tamil Nadu. The experimental material consisted of the tomato variety CO 3. Seeds were sown and raised in portrays in protected conditions, and healthy seedlings were transplanted into pots at the four-leaf stage (age of 4–5 weeks). The experiment was laid out in Completely Randomized Design (CRD) with six treatments and four replications. Each treatment consisted of four pots, a total of 24 experimental pots. Chitosan was extracted from Black Soldier Fly pupal cases and evaluated its insecticidal and phytotonic effects on tomato.

Chitosan Extraction from BSF Pupal Case

Dried pupal cases of Black Soldier Fly (20 g) were used as the starting material for chitosan extraction. Chitosan was extracted using the chemical method, following the procedure of Battampara *et al*. (2020), originally developed for silkworm pupae and adapted here for BSF pupal case. The extraction proceeded through three sequential steps.

Deproteinization

Dried, defatted pupal case material (20 g) was treated with 10 per cent NaOH at a solid/liquid ratio of 1:10 (g/mL). The reaction was carried out under agitation at 80 °C for 3 h. The resulting solid was filtered and washed repeatedly with distilled water until a neutral pH was achieved, and then dried in an oven at 70 °C (Figure 1).

Demineralization

The deproteinized pupal case material was subjected to demineralization using 7 per cent HCl at a material-to-liquid ratio of 1:10 (g/mL), followed by agitation at 250 rpm for 24 h at room temperature. The material was subsequently filtered, washed with distilled water until neutral pH, and dried at 60 °C to obtain chitin (Figure 2).

Deacetylation

The extracted chitin was subjected to deacetylation using 55 per cent NaOH at



Figure 1. Sequential steps involved in the deproteinization of dried and defatted Black Soldier Fly (*Hermetia illucens*) pupal case

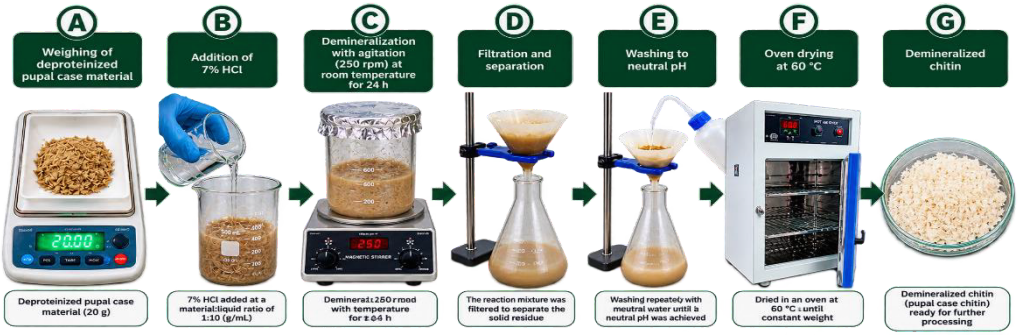


Figure 2. Sequential steps involved in the demineralization of deproteinized Black Soldier Fly (Hermetia illucens) pupal case

a solid-to-liquid ratio of 1:12 (g/mL). The reaction mixture was maintained at 90–95 °C for 24 h under continuous agitation at 250 rpm. After treatment, the resulting material was filtered and repeatedly washed with distilled water until neutral pH was achieved. The washed material was subsequently dried in an oven at 70 °C until constant weight, yielding chitosan as a light brown to brown, dry, flaky and coarse powder, with a degree of deacetylation of approximately 80 per cent (Figure 3).

The insecticidal potential of BSF-derived chitosan was evaluated on tomato in Completely Randomized Design (CRD) comprising six treatments (T0-T5) and four replications. The treatments were T0, water spray (control); T1, (1%) chitosan; T2, (2%) chitosan; T3, (3%) chitosan; T4, (4%) chitosan; T5, (5%) chitosan. Chitosan was dissolved in 1 per cent acetic acid and the solutions were applied as foliar sprays for three successive spray cycles at various crop phonological stages, vegetative stage, flowering and fruit initiation

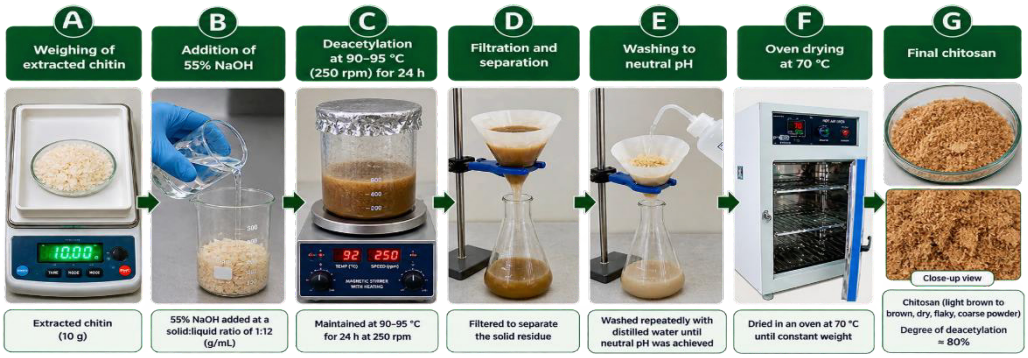


Figure 3. Sequential steps involved in the deacetylation of extracted chitin to obtain chitosan

stage . Populations of *B.tabaci, A. gossypii*, *F. schultzei, A. devastans*, and *T. absoluta* were recorded before treatment (pretreatment) and at 3, 7, and 15 days after spraying (DAS) following each of the three spray cycles. The data recorded at 3, 7, and 15 DAS within each spray cycle were pooled into a single consolidated mean observation for the first, second, and third spray and means were subjected to statistical analysis and are reported below. Whitefly, thrips and leafhopper were counted per leaf; aphid infestation was recorded as per cent incidence and pinworm damage as per cent fruit damage. The tomato plants were subjected to foliar application of chitosan at 1, 2, 3, 4 and 5 per cent concentrations, along with an untreated control (T0), as illustrated in Figure 4.

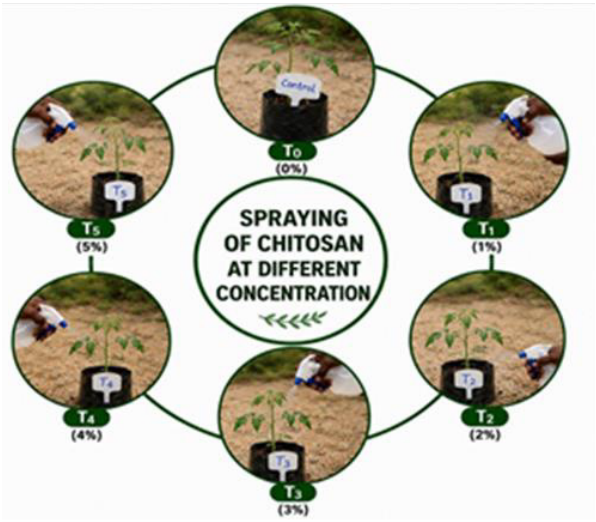


Figure 4. Foliar application of chitosan at different concentrations in pot-culture condition

Phytotonic Evaluation

Phytotonic parameters *viz*, plant height (cm), number of leaves per plant, number of flowers per plant, were recorded at the appropriate phenological stages for each treatment.

Statistical Analysis

All recorded data were subjected to Analysis of Variance (ANOVA) appropriate for a Completely Randomized Design. Critical Differences (CD at p = 0.05), Standard Error of Difference (SE(d)) were calculated to determine the statistical significance among treatment means. The data on insect count were transformed using square-root transformation and damage (aphid incidence and pinworm damage) was transformed using arc sine transformation.

RESULTS AND DISCUSSION

Chitosan Extraction Yield

The chemical extraction method successfully yielded chitosan from BSF pupal case biomass. From an initial 20 g of dried pupal case material, chitin yield was 10-12 per cent of dry weight (3.3 g), and the final chitosan yield after deacetylation was 6-10 per cent of the initial dry weight (2.8 g), corresponding to an overall recovery of (14%). The extracted chitosan was light brown to brown in colour with a dry, flaky, coarse appearance, confirmed successful demineralization, deproteinization, and deacetylation of the starting material. These findings are consistent with those of Soetemans *et al*. (2020), demonstrated that BSF pupal exuviae are a promising alternative source of chitin, with physicochemical properties comparable to those of conventional crustacean-derived chitin. Similarly, Bhavsar *et al.* (2021) reported that BSF-derived chitosan exhibited good purity and functional properties, rendering it suitable for both textile and agricultural applications, while Witono *et al.* (2024) and Yuan (2024) confirmed that sequential demineralization-deproteinization-deacetylation protocols, with attention to alkali concentration, temperature, and reaction time, are critical determinants of chitosan yield and quality from BSF biomass. Mohan *et al.* (2024) and Debbarma *et al.* (2026) reported that the use of BSF pupal exoskeletons as a renewable, insect-derived source of agriculturally relevant chitosan.

Insecticidal Efficacy on Major Tomato Pests

Foliar application of BSF-derived chitosan demonstrated significant, dose-dependent suppression of all five target pests relative to the untreated control (T0), in which pest populations progressively over successive spray cycles. Across all pests, the 5 per cent chitosan treatment (T5) consistently recorded the lowest population densities by the third spray, followed in order by T4, T3, T2, and T1 (Table 1- 5).

Across all five pest species, the order of insecticidal effectiveness was consistently T5 (5%) > T4 (4%) > T3 (3%) > T2 (2%) > T1 (1%) > T0 (control), confirming a clear dose-dependent relationship between chitosan concentration and pest suppression. Chitosan does not act as a conventional chemical insecticide; rather, it suppresses insect

Table 1. Efficacy of BSF chitosan on *Bemisia tabaci* population

Treatment	Concentration (%)	Pretreatment	Whitefly (no./ leaf)		
			First spray (mean)*	Second spray (mean)*	Third spray (mean)*
T0	Control (water spray)	12.3 (3.58)	12.93 (3.66)^{d}	14.00 (3.81)^{d}	15.20 (3.96)^{d}
T1	1	10.1 (3.26)	9.20 (3.11)^{c}	7.87 (2.89)^{c}	6.77 (2.70)^{c}
T2	2	9.9 (3.22)	8.80 (3.05)^{c}	7.50 (2.83)^{c}	6.40 (2.63)^{c}
T3	3	8.7 (3.03)	7.73 (2.87)^{b}	6.37 (2.62)^{b}	5.20 (2.39)^{b}
T4	4	8.1 (2.93)	7.30 (2.79)^{ab}	5.57 (2.46)^{a}	4.40 (2.21)^{a}
T5	5	7.7 (2.86)	6.80 (2.70)^{a}	5.40 (2.43)^{a}	4.20 (2.17)^{a}
SE(d)			0.31	0.25	0.19
CD (p=0.05)			0.65	0.53	0.40

*Mean of observations recorded at 3, 7 and 15 Days After Spray (DAS).
Figures in parentheses are square-root transformed values.
In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05).

Table 2. Efficacy of BSF chitosan on *Aphis gossypii per cent incidence*

Treatment	Concentration (%)	Pretreatment	Aphid (% incidence)		
			First spray (mean)*	Second spray (mean)*	Third spray (mean)*
T0	Control (water spray)	10.8 (19.19)	11.77 (20.06)^{f}	12.50 (20.70)^{f}	13.23 (21.33)^{f}
T1	1	10.9 (19.28)	8.67 (17.12)^{e}	6.67 (14.97)^{e}	5.47 (13.53)^{e}
T2	2	10.7 (19.09)	7.27 (15.64)^{d}	5.10 (13.05)^{d}	4.33 (12.01)^{d}
T3	3	10.8 (19.19)	6.00 (14.18)^{c}	4.00 (11.54)^{c}	3.47 (10.74)^{c}
T4	4	10.9 (19.28)	4.80 (12.66)^{b}	3.00 (9.97)^{b}	2.77 (9.58)^{b}
T5	5	10.8 (19.19)	3.73 (11.14)^{a}	2.30 (8.72)^{a}	2.03 (8.19)^{a}
SE(d)			0.29	0.22	0.18
CD (p=0.05)			0.61	0.46	0.38

*Mean of observations recorded at 3, 7 and 15 Days After Spray (DAS).
Figures in parentheses are arc sine transformed values.
In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05).

Table 3. Efficacy of BSF chitosan on *Frankliniella schultzei* population

Treatment	Concentration (%)	Pretreatment	Thrips (no./leaf)		
			First spray (mean)*	Second spray (mean)*	Third spray (mean)*
T0	Control (water spray)	8.9 (3.07)	9.43 (3.15)^{f}	10.33 (3.29)^{f}	11.33 (3.44)^{f}
T1	1	8.8 (3.05)	7.00 (2.74)^{e}	5.43 (2.44)^{e}	4.30 (2.19)^{e}
T2	2	8.8 (3.05)	5.87 (2.52)^{d}	4.17 (2.16)^{d}	3.30 (1.95)^{d}
T3	3	8.9 (3.07)	4.97 (2.34)^{c}	3.30 (1.95)^{c}	2.50 (1.73)^{c}
T4	4	8.8 (3.05)	4.10 (2.14)^{b}	2.47 (1.72)^{b}	1.80 (1.52)^{b}
T5	5	8.9 (3.07)	3.33 (1.96)^{a}	1.80 (1.52)^{a}	1.30 (1.34)^{a}
SE(d)			0.26	0.20	0.15
CD (p=0.05)			0.55	0.42	0.32

*Mean of observations recorded at 3, 7 and 15 Days After Spray (DAS).
Figures in parentheses are square-root transformed values.
In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05).

Table 4. Efficacy of BSF chitosan on *Amrasca devastans* population

Treatment	Concentration (%)	Pretreatment	Leafhopper (no./leaf)		
			First spray (mean)*	Second spray (mean)*	Third spray (mean)*
T0	Control (water spray)	7.6 (2.85)	8.13 (2.94)^{f}	9.13 (3.10)^{f}	10.13 (3.26)^{f}
T1	1	7.5 (2.83)	6.20 (2.59)^{e}	4.83 (2.31)^{e}	4.00 (2.12)^{e}
T2	2	7.6 (2.85)	5.20 (2.39)^{d}	3.80 (2.07)^{d}	3.03 (1.88)^{d}
T3	3	7.5 (2.83)	4.43 (2.22)^{c}	3.03 (1.88)^{c}	2.40 (1.70)^{c}
T4	4	7.5 (2.83)	3.77 (2.07)^{b}	2.40 (1.70)^{b}	1.80 (1.52)^{b}
T5	5	7.6 (2.85)	3.03 (1.88)^{a}	1.83 (1.53)^{a}	1.30 (1.34)^{a}
SE(d)			0.24	0.18	0.14
CD (p=0.05)			0.51	0.38	0.30

*Mean of observations recorded at 3, 7 and 15 Days After Spray (DAS).
Figures in parentheses are square-root transformed values.
In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05).

Table 5. Efficacy of BSF chitosan on *Tuta absoluta* per cent fruit damage

Treatment	Concentration (%)	Pretreatment	Tomato pinworm (% damage)		
			First spray (mean)*	Second spray (mean)*	Third spray (mean)*
T0	Control (water spray)	12.3 (20.53)	12.93 (21.07)^{f}	14.00 (21.97)^{f}	15.20 (22.95)^{f}
T1	1	12.2 (20.44)	10.70 (19.09)^{e}	8.93 (17.39)^{e}	7.27 (15.64)^{e}
T2	2	12.4 (20.62)	9.83 (18.27)^{d}	7.80 (16.22)^{d}	6.33 (14.57)^{d}
T3	3	12.3 (20.53)	8.70 (17.15)^{c}	6.50 (14.77)^{c}	5.00 (12.92)^{c}
T4	4	12.4 (20.62)	7.67 (16.08)^{b}	5.40 (13.44)^{b}	4.10 (11.68)^{b}
T5	5	12.3 (20.53)	6.83 (15.15)^{a}	4.70 (12.52)^{a}	3.50 (10.78)^{a}
SE(d)			0.32	0.25	0.19
CD (p=0.05)			0.67	0.53	0.40

*Mean of observations recorded at 3, 7 and 15 Days After Spray (DAS).

Figures in parentheses are arc sine transformed values.

In a column, means followed by the same letter(s) are not significantly different by LSD (P=0.05).

infestation by forming a protective film on the leaf surface, exhibiting antifeedant and repellent properties, disrupting insect physiology, and inducing plant defence responses. Similar observations were reported by Badawy and Rabea (2009), found that chitosan possesses insecticidal and growth-regulating properties against several agricultural insect pests. El Hadrami *et al*. (2010) reported that chitosan activates plant defence mechanisms and enhances resistance against insects and pathogens, while Malerba and Cerana (2016) concluded that chitosan functions as a natural biopolymer capable of inducing systemic resistance and reducing pest incidence while remaining environmentally safe. The trends in pest population across the pretreatment observation and the three spray cycles are shown in Figure 5.

Phytotonic Performance

Foliar applications of BSF-derived chitosan exerted a pronounced positive influence on plant development and growth vigour. Growth parameters improved progressively with increasing chitosan concentration, with T5 (5%) recorded the highest values across all measured indices (Table 6).

The 5 per cent chitosan treatment (T5) recorded the maximum plant height (47.0 cm), the highest number of leaves (10/plant) and flowers (48/plant), over the control. The untreated control (T0) recorded the lowest growth confirming that 5 per cent chitosan is the optimum concentration for promoting tomato growth and improving productivityin the experimental conditions. These findings align with Pichyangkura and Chadchawan (2015), concluded that chitosan functions as an effective plant growth promoter by stimulating physiological and biochemical processes that improve plant growth and flowering. The treatment-wise growth responses, are represented in Figure 6.

CONCLUSION

This study demonstrates the successful valorization of *Hermetia illucens* pupal cases into functional chitosan, obtained at 14 per cent recovery via a sequential deproteinization-demineralization-deacetylation process, possessing dual efficacy for sustainable tomato cultivation. The extracted chitosan exhibited potent biopesticidal properties against major tomato pests, including *B. tabaci*, *A. gossypii*, *F. schultzei*, *A. devastans* and *T. absoluta*. The 5 per cent concentration (T5) recorded the greatest population reduction by the third spray cycle, bringing whitefly, thrips and leafhopper to 4.20, 1.30 and 1.30 no./leaf, as against 15.20, 11.33 and

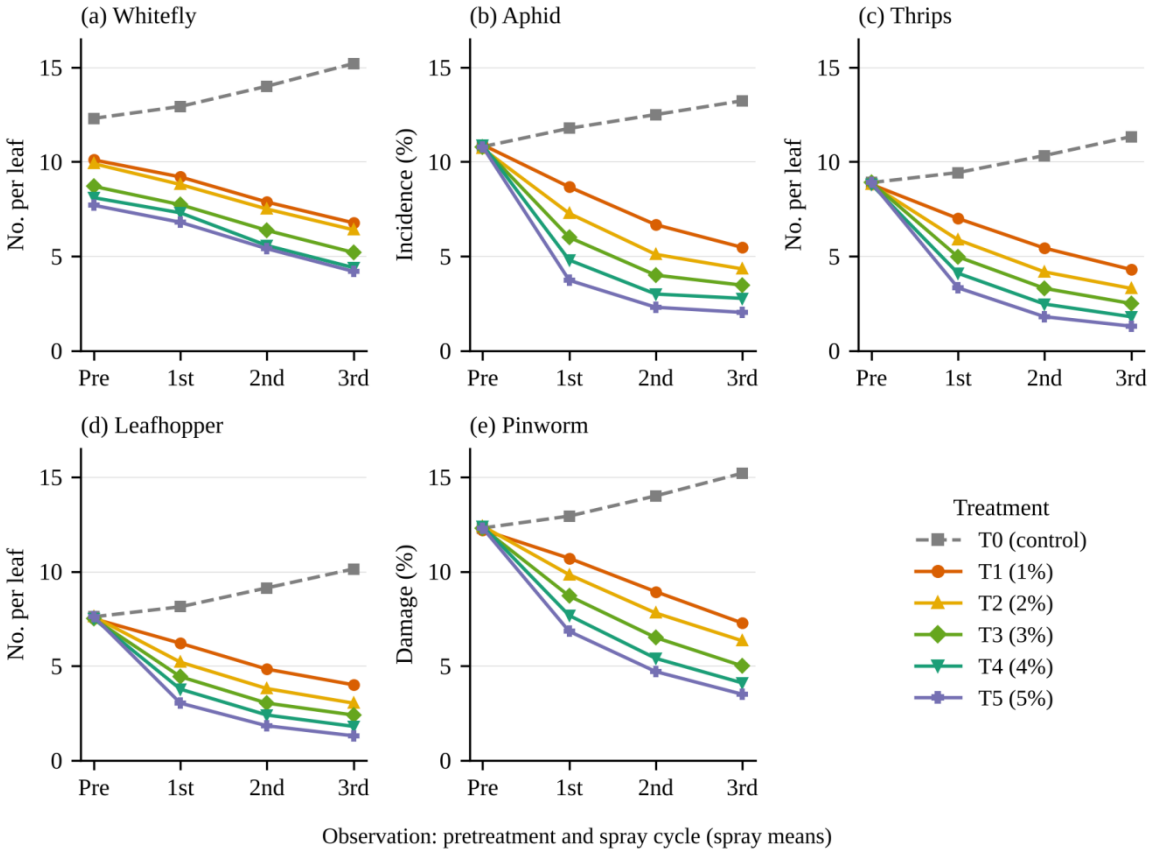


Figure 5. Population of (a) whitefly, (c) thrips and (d) leafhopper, (b) per cent incidence of aphid and (e) per cent damage by pinworm on tomato pre treatment and after the first, second and third sprays of BSF chitosan (T_1–T_5) compared with the untreated control (T_0)

Table 6. Phytotonic activity and agronomic performance of tomato plants treated with BSF chitosan

Treatment	Concentration (%)	Plant height (cm)	No. of leaves / plant	No. of flowers / plant
T0	Control (water spray)	32.5^{c}	5^{e}	24^{f}
T1	1	33.4^{bc}	6^{d}	28^{e}
T2	2	34.2^{bc}	6^{d}	33^{d}
T3	3	35.1^{b}	8^{b}	38^{c}
T4	4	46.0^{a}	7^{c}	43^{b}
T5	5	47.0^{a}	10^{a}	48^{a}
SE(d)		1.12	0.34	1.05
CD (p=0.05)		2.36	0.72	2.21

Means followed by the same letter(s) in a column are not significantly different by LSD (P=0.05).

10.13 no./leaf in the untreated control, respectively, aphid incidence to 2.03 per cent as against 13.23 per cent, and pinworm damage to 3.50 per cent as against 15.20 per cent. Foliar application of BSF-derived chitosan exerted a pronounced phytotonic effect, significantly improving vegetative growth parameters relative to the untreated

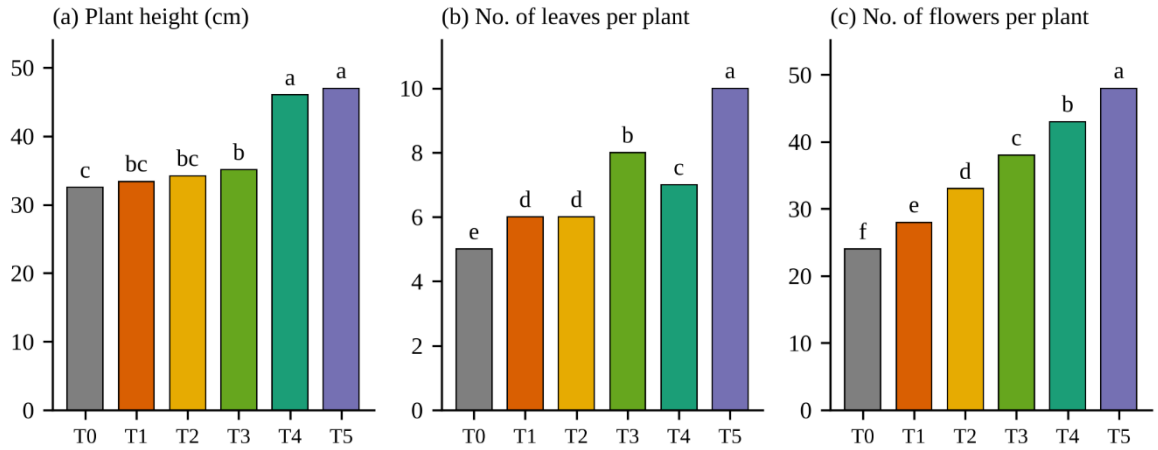


Figure 6. Effect of foliar BSF chitosan on (a) plant height, (b) number of leaves and (c) number of flowers per tomato plant.

control, with T5 recording a plant height of 47.0 cm, 10 leaves/plant and 48 flowers/plant, as against 32.5 cm, 5 leaves/plant and 24 flowers/plant, respectively, in the control. A limitation of the present pot-culture study was its short duration and controlled setting; field-scale validation across seasons and agro-climatic zones is required before commercial recommendation. Collectively, these findings indicate that BSF pupal-case chitosan serves as an eco-friendly, highly effective natural input for integrated pest management and plant growth promotion in sustainable tomato production systems, warranting further field-scale validation and formulation development for commercial application.

Ethics Statement

This study did not involve human participants or vertebrate animals. The research was conducted solely on plant materials; therefore, ethical approval was not required.

Originality and Plagiarism

The authors confirm that this manuscript is an original work and has not been published, in whole or in part, elsewhere. All sources of information have been appropriately cited, and the manuscript is free from plagiarism.

Consent for Publication

All authors have read and approved the final version of the manuscript and consent to its publication in the journal.

Competing Interests

The authors declare that they have no competing financial or personal interests that could have influenced the work reported in this paper.

Data Availability

The data supporting the findings of this study are included within the manuscript. Additional data are available from the corresponding author upon reasonable request.

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