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RESEARCH ARTICLE

Assessment of bio-nanoemulsion seed priming on sucking pest management and vigor enhancement in cotton

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Abstract

Cotton (*Gossypium* spp. L.) is the most important natural fibre crop. Various sucking pests, including aphids, jassids, whiteflies and thrips, often constrain its productivity. Seed treatment is recognized as a vital strategy for managing these pests, thereby reducing the need for repeated foliar insecticide applications. The azadirachtin nano emulsion (NE) was prepared using azadirachtin (1 % w/v), 1 % chitosan (50 % v/v) and 5 % (v/v) neem oil as key components. In this study, we evaluated the effect of an azadirachtin-based polymer NE (NE 1 %) for its effects on seed germination and early-season pest suppression under both laboratory and field conditions. The optimised NE exhibited a mean droplet size of 159.4 nm and a low polydispersity index (PDI = 0.189), indicating good stability and uniformity. Laboratory assays revealed that application of NE at 10 mL kg^{-1} significantly enhanced germination (72 %) ($p < 0.003$) and vigor index (2434.47) ($p < 0.002$) outperforming thiamethoxam and water priming. In contrast, under field trials, thiamethoxam 25 % water dispersible granule (WG) (7 g kg^{-1}) (a synthetic insecticide) was more effective in suppressing aphids, leafhoppers, whiteflies and thrips, maintaining near-zero aphid populations for 5 weeks. Application of NE at 5, 7 and 10 mL kg^{-1} showed a dose-dependent improvement and closely matched thiamethoxam in action at 10 mL kg^{-1} by reducing whitefly and thrips populations. This is the first report of using azadirachtin NE as a seed treatment in cotton. Although synthetic treatments ensured longer persistence, the azadirachtin nanoformulation offers a promising, eco-friendly, sustainable option for Integrated Pest Management (IPM).

Keywords: azadirachtin; nanotechnology; seed treatment; sucking pest; sustainable agriculture; thiamethoxam

Introduction

Cotton (*Gossypium* spp. L.) is the most important natural fibre crop, serving as a cornerstone of the textile industry and supporting the livelihoods of millions of farmers across tropical and subtropical regions. The crop provides economically valuable by-products such as cottonseed oil and protein-rich seed cake in addition to fibre, further enhancing its significance (1, 2). Cotton is cultivated in over 80 countries because of its adaptability to diverse agro-climatic conditions. The major producers of cotton include China, India, United States, Brazil and Pakistan. Notably, India accounts for more than one-quarter of the global cotton output. However, national productivity remains below potential and is largely constrained by biotic and abiotic stresses, particularly insect/pest pressure, soil limitations and climatic variability (3, 4). Among biotic constraints, early-season sucking pests such as aphids (*Aphis gossypii*), jassids (*Amrasca biguttula biguttula*), whiteflies (*Bemisia tabaci*) and thrips (*Thrips tabaci*) cause significant damage to cotton during the seedling stage. These pests interfere with photosynthesis, suppress plant growth and degrade fibre quality. In addition, whiteflies and aphids serve as vectors for viral pathogens, while jassids and thrips induce leaf curling, necrosis, silvering and poor seedling establishment (5, 6). Therefore, effective management of these sucking insects is critical to prevent huge yield losses. Early intervention is essential to prevent severe crop damage. One of the sustainable methods includes seed treatment, which protects seedlings from initial pest infestations and reduces the need for multiple foliar insecticide applications (7). Neonicotinoids are a major class of systemic insecticides that act on nicotinic acetylcholine receptor (nAChR) in insects, particularly thiamethoxam 25 % WG (7 g kg^{-1} seed) seed treatment insecticide is recommended by the Central Insecticides Board and Registration Committee (CIBRC), Government of India, due to their systemic action and long-lasting protection. However, growing environmental concerns, such as persistence and toxicity against non-target organisms, as well as regulatory frameworks, have driven interest in safer alternatives (1, 3, 5).

Many natural, eco-friendly insecticides have been explored to get rid of various pests (8). Plant-based insecticide azadirachtin (principal bioactive compound) obtained from *Azadirachta indica* offers a sustainable alternative due to its broad-spectrum activity, like interference with chemoreceptors causing antifeedant behavior and repellency action, disruption of endocrine regulation by inhibiting hormone release and alteration of ecdysteroid and juvenile hormone levels. These effects collectively lead to growth inhibition, abnormal moulting and reproductive suppression in insects (9, 10) and low mammalian toxicity. Rapid degradation under sunlight, high temperature and alkaline conditions limits their persistence and field efficacy (11-15). To address this issue, encapsulation of azadirachtin in biocompatible polymers like chitosan has been tried. It has emerged as a promising strategy to enhance stability, provide controlled release and improve uptake efficiency. The plant growth-promoting and defence-inducing effects of chitosan make it a suitable material for seed treatment applications (16). Chitosan polymer used to encapsulate oil-in-water NEs, acts as a stabiliser at higher concentration due to electrostatic repulsion between droplets and demonstrated significant efficacy as a green pest management tool against sucking pests (17, 18). Researchers are currently exploring nanotechnology as a promising and innovative approach for advancing agricultural practices (19-23). Advances in nanotechnology have facilitated the development of NE formulations for seed treatment with superior dispersion, penetration ability, increased bioavailability, thus achieving higher efficacy at lower doses and reduced environmental risk (24-27). Azadirachtin shows systemic movement through both xylem and phloem, enabling effective control of phloem-feeding pests (28, 29).

Studies related to the efficacy of azadirachtin-based NE seed treatments for the management of early-season sucking pests in cotton are limited. Therefore, the objective of the present study is to evaluate the performance of azadirachtin NE as a seed treatment agent on cotton against early season sucking pests with an emphasis on sustainable pest management and reduced environmental impact.

Materials and Methods

Chemicals

Azadirachtin technical powder (20 % purity) was procured from Ecobiocides and Botanicals Pvt. Ltd. (Tamil Nadu, India). Chitosan was obtained from Himedia Laboratories Pvt. Ltd. (Maharashtra, India). The non-ionic surfactant Tween 80 was sourced from Molychem (Mumbai, Maharashtra, India). All the chemicals and solvents used were of analytical grade and purchased from certified local suppliers.

Preparation of azadirachtin NE

The NE was formulated using azadirachtin (1 % w/v), neem oil (5 % v/v), Tween 80 (as surfactant) (10 % v/v), distilled water (35 % v/v) and 1 % chitosan solution (50 % v/v). Initially, azadirachtin and neem oil were mixed with Tween 80 under magnetic stirring to form the oil phase. This mixture was slowly added to an aqueous phase containing distilled water and 1 % chitosan solution to form a coarse emulsion. The resultant emulsion was further subjected to ultrasonication using a probe sonicator operating at 20 kHz in pulse mode for 15 min to reduce droplet size and obtain a nano-range emulsion. The final concentration of the prepared NE was 10000 ppm (1 %). The final NE was stored in amber bottles at 4 °C until further use.

Characterization of NE

Droplet size and polydispersity index

The mean droplet size and polydispersity index (PDI) were determined using dynamic light scattering (DLS) with a Malvern Zetasizer (Malvern Panalytical Ltd., UK). Samples were diluted to 10 % with deionized water to prevent multiple scattering and measurements were taken in triplicate. Data were recorded as Z-average diameter (nm) and PDI. A PDI < 0.25 was considered indicative of narrow size distribution and homogeneity.

Phase-contrast microscopy

Droplet morphology was examined under a phase contrast microscope (Model: Euromex iScope Microscope, The Netherlands) at 1000× magnification. A drop of the sample was mounted on a clean glass slide, covered with a coverslip and observed under oil immersion. Photomicrographs were taken using a digital camera attached to the microscope for documentation (30).

Seed treatment and germination studies

Cotton seeds (variety Co 17) were delinted and treated with NE at the concentration of 5, 7 and 10 mL kg^{-1} of seed, based on the recommended chemical seed treatment rates of 7 g kg^{-1} for thiamethoxam 25 % WG, corresponding doses of 7 mL kg^{-1} and its higher dose of 10 mL kg^{-1} and its lower dose of 5 mL kg^{-1} of the NE formulation were selected for seed treatment to assess its comparative efficacy. Thiamethoxam treatment was given to seeds at a concentration of 25 % WG at 7 g kg^{-1}, acted as a positive control. The distilled water-treated seeds (water priming) acted as the negative control. Treated seeds were shade -dried for 2 hr before further evaluation.

Germination assay

Seed germination was carried out using the roll towel method under laboratory conditions (31). The experiment followed a completely randomized design (CRD) with 4 replications of 25 seeds each. Seed quality parameters such as germination percentage (32), root length, shoot length, seedling fresh and dry weight (g per 10 seedlings) and vigor index [germination % × seedling dry weight (g)] were recorded on the 12th day (33).

Field evaluation against sucking pests

A field experiment was conducted during the *Kharif* 2025 season at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore (11.0122° N, 76.9354° E) and the soil type was red loamy. The study was laid out in a randomized block design (RBD comprising five treatments with 4 replications. Observations on the incidence of sucking pests were initiated from seedling emergence and continued up to 8 weeks after sowing. For each experimental plot, 5 plants were randomly selected and the population of major sucking pests, including aphids, leafhoppers, thrips and whiteflies, was recorded. Pest assessment was performed on 3 leaves per plant, representing the top, middle and bottom of the canopy. Field pest population data were recorded at weekly intervals and subjected to statistical analysis to evaluate treatment effects over time.

Statistical analysis

All experimental data were analyzed using R software (version 3.6.0). Seed germination data were subjected to analysis of variance (ANOVA) under a CRD. The field data on pest incidence were analysed using RBD. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at a 5 % level of significance wherever applicable. For percentage data, a proper transformation (such as arcsine transformation) was applied before analysis and significance was determined at $p < 0.05$.

Results and Discussion

Particle size analysis and phase contrast microscopy

The NE was prepared using Tween 80, a non-ionic surfactant with a high hydrophilic-lipophilic balance (HLB) value of 13.5 that stabilizes droplets efficiently. Non-ionic surfactants are generally less toxic and favor the formation of stable NEs due to their uncharged head groups. Ultrasonication significantly reduced droplet size compared to non-sonicated samples (34). This reduction is due to the intense cavitation forces that disrupt and homogenize droplets during sonication (35, 36). The formulation exhibited a pH of 4.73 (acidic) due to the glacial acetic acid used to dissolve the chitosan and a viscosity of 15.4 Pa·s. DLS analysis of the azadirachtin-polymer NE showed a mean droplet size of 159.4 nm with a narrow PDI of 0.189, indicating uniform distribution (Fig. 1). The monomodal size distribution and an intercept value of 0.945 suggested high signal quality and the overall grading was marked as "good." The droplets below 200 nm with PDI values below 0.25 are considered ideal for good NE stability as per the previously known criteria (37). Small droplet sizes also impart greater resistance to creaming and sedimentation (38), while low PDI values (< 0.25) favor long-term physical stability (39). Phase contrast microscopy supported the DLS findings and showed spherical, uniformly dispersed droplets with clear boundaries and no visible aggregation (Fig. 1). The droplets displayed a core-shell structure, implying effective encapsulation of azadirachtin within the chitosan-surfactant matrix. Such core-shell morphologies are known to enhance stability and allow controlled release of bioactive compounds (40).

Seed germination bioassay

The results of the germination assay show that there is a statistically significant difference in the germination percent ($p < 0.003$) and vigor index (VI) ($p < 0.002$). The shoot length, root length and seedling length exhibited a non-significant difference between the treatment means at $p < 0.05$. The best showed that seeds treated with 10 mL kg^{-1} NE showed high germination (about 72 %) and the highest VI (2434.47). The treatment at 7 mL kg^{-1} NE also showed significant improvement (64 % germination, VI = 2133.69). In

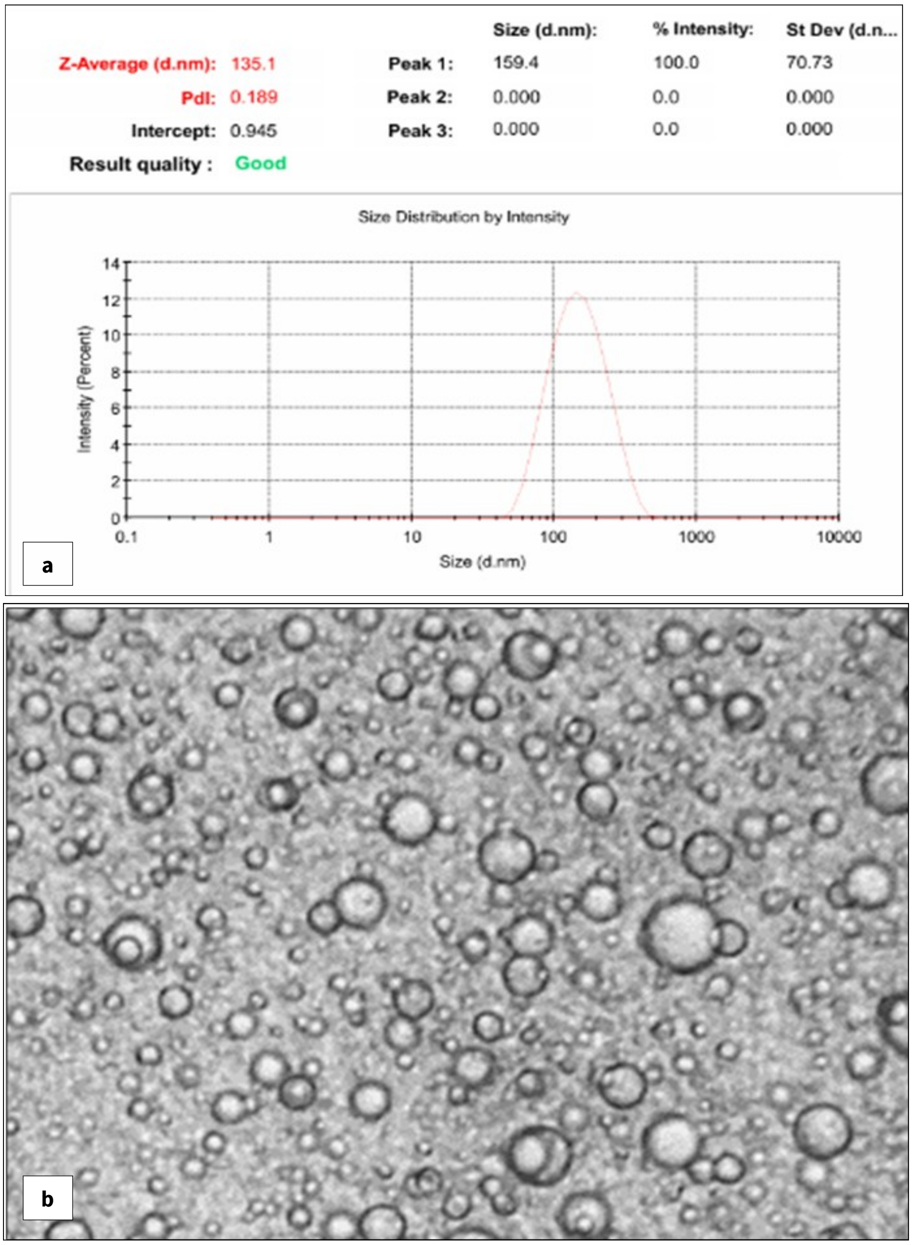


Fig. 1. (a) Droplet size (nm) and polydispersity index (b) droplet morphology of bio-nanoemulsion.

contrast, thiamethoxam-treated seeds showed lower germination (56 %) and VI (1718.02). The untreated control recorded a VI of 1752.92. The results indicate a clear dose-dependent improvement with increasing concentration of NE (Fig. 2). The enhanced germination and seedling vigor observed with NE treatment can be attributed to the nanoscale droplet size, which improves uniform coating, water uptake and penetration of azadirachtin into the seed coat, thereby enhancing bioavailability. Additionally, chitosan, used as a stabilizer in the formulation, is known to possess plant growth-promoting properties, including stimulation of seed metabolism, activation of defense-related enzymes and improved nutrient uptake, which likely contributed to increased seedling vigor (41, 42). Similar enhancements in seedling vigor due to neem-based botanicals have been reported in cotton and other crops (43). The neem leaf powder at 10 g kg^{-1} improved cotton seedling vigor and length (44). A germination of 72.50 % was also observed in cotton seeds primed with curry leaf extract (45). Seed treatments using *A. indica* and *Boswellia dalzielii* extracts have also shown high yield, better germination and high VIs (46). The present results suggest that nanoencapsulation may improve the availability and uptake of azadirachtin, making the NE formulation act as a dual-benefit seed treatment that is a sustainable option along with a possible pest deterrent effect.

Field evaluation of sucking pests

In field trials, thiamethoxam (7 g kg^{-1}) consistently performed best against all major sucking pests in cotton. It maintained 0 aphid infestation for the first 5 weeks and recorded the lowest populations for leafhoppers, whiteflies and thrips by week 8. NE treatments also significantly reduced pest populations relative to control, with a clear dose-response pattern. The NE at 10 mL kg^{-1} performed better than 7 mL kg^{-1} and 5 mL kg^{-1} for all 4 pests, though still slightly inferior to thiamethoxam for each pest category (Fig. 3 & 4). The untreated control had the highest infestation levels across all pests. This superior control by thiamethoxam aligns with previous findings on neonicotinoid seed treatments. A study reported an excellent early-season control of sucking pests in cotton with thiamethoxam (6), while previous researchers' observed prolonged suppression of leafhoppers and aphids using imidacloprid-treated seeds (3). Neonicotinoids offer rapid systemic uptake and long-lasting protection (47-49), explaining the strong performance as noted in our study. However, the novelty of this research lies in the use of azadirachtin NE as a seed treatment. Though neem-based foliar sprays are widely studied, seed-based delivery via NE could enhance the systemic availability of azadirachtin (50). The NE at 10 mL kg^{-1} approached thiamethoxam's performance for whiteflies and thrips, suggesting scope for optimization and formulation improvement. This highlights its potential as a greener alternative for integrated pest management in cotton.

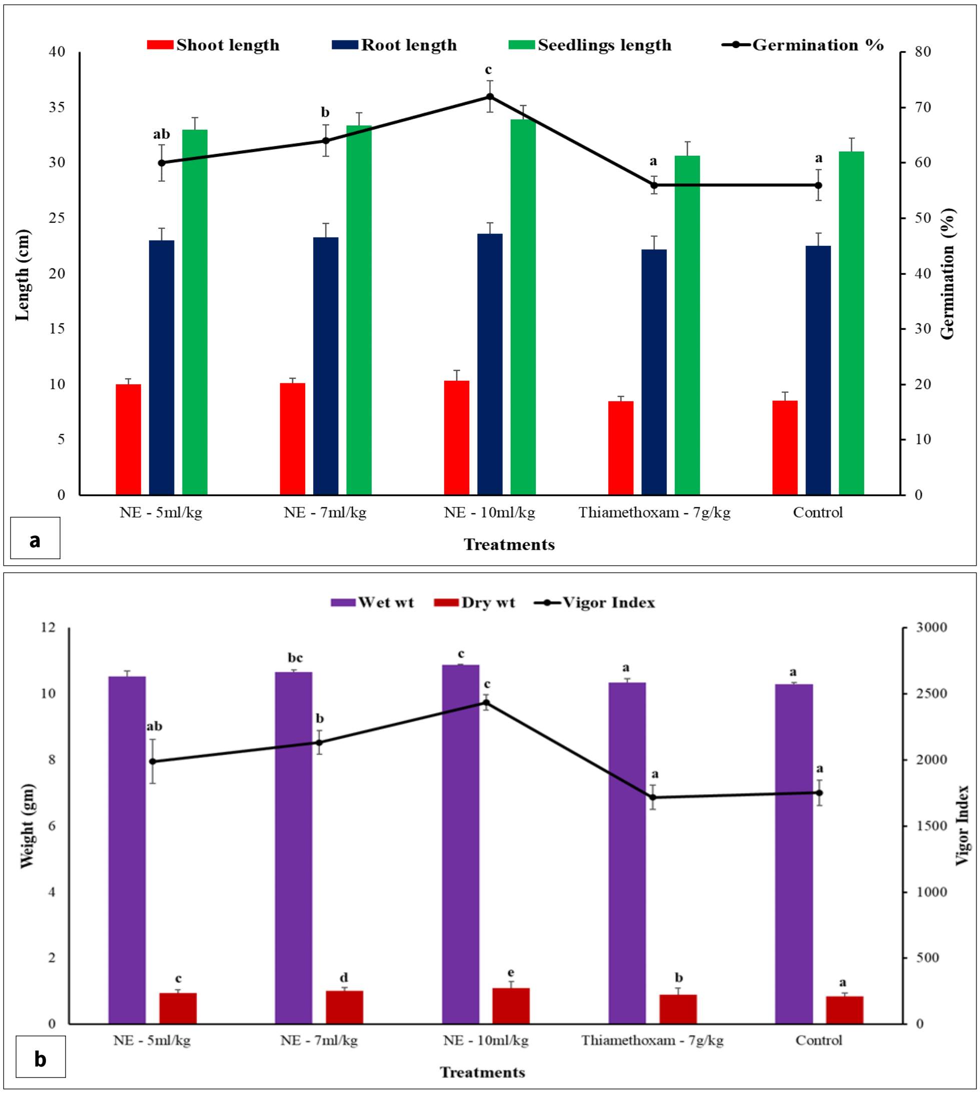
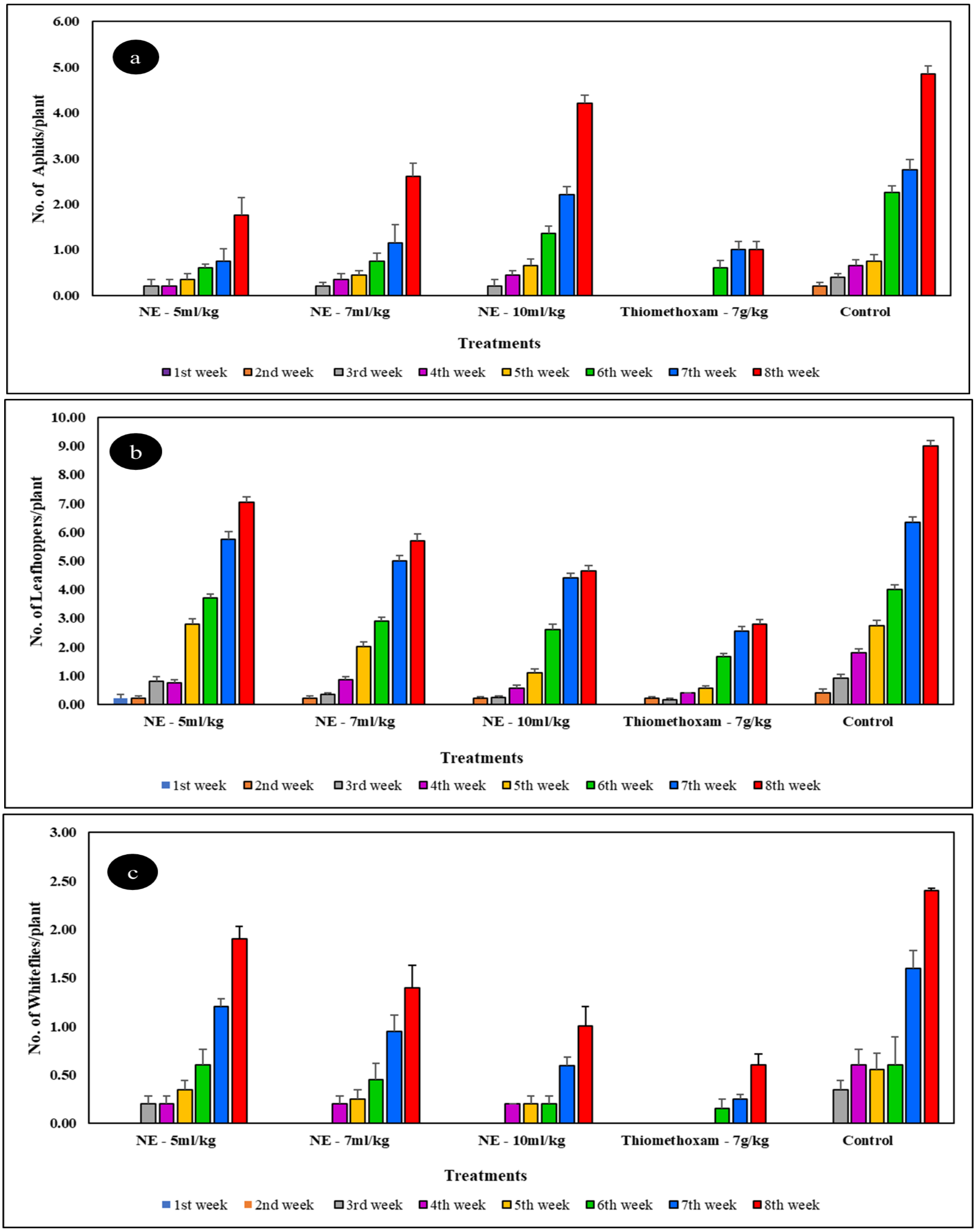


Fig. 2. Effect of bio-nanoemulsion and thiamethoxam on agro-morphological characters of cotton seeds (a) shoot, root, seedling length, germination % (b) wet, dry weight, vigor index.

Fig. 3. Field evaluation of cotton seed treatment with bio-nanoemulsion and thiamethoxam against early sucking pests in cotton.



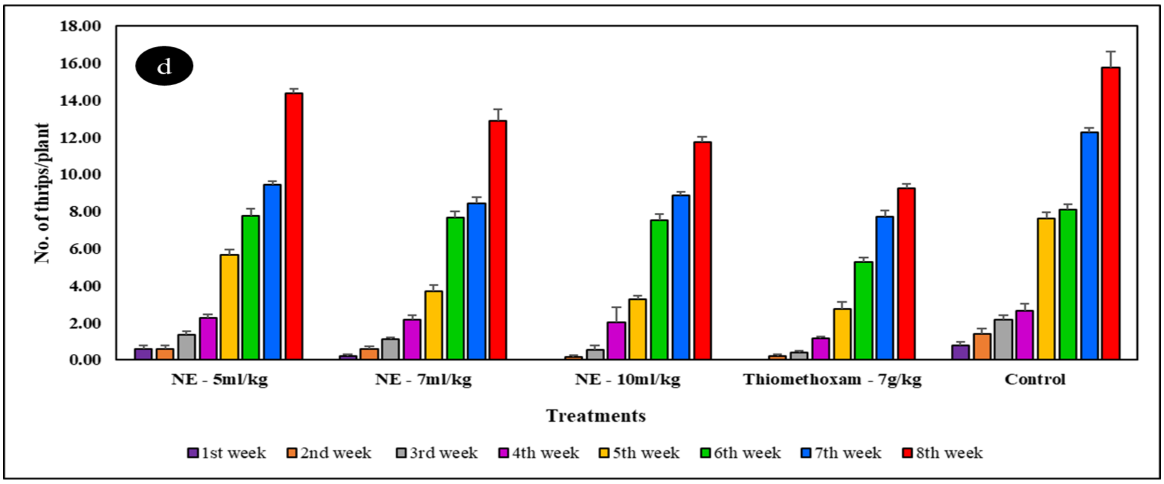


Fig. 4. Effect of bio-nanoemulsion and thiamethoxam on cotton pests (a) aphids, (b) leafhoppers, (c) whiteflies, (d) thrips.

Conclusion

The present investigation provides the first evidence of azadirachtin incorporated in a polymer-based NE formulation applied as a seed treatment for managing early-season sucking pests in cotton. The azadirachtin NE exhibited a droplet size of 159.4 nm with a low PDI of 0.189, ensuring high stability. At 10 mL kg^{-1}, it significantly enhanced seed germination and seedling vigor in cotton. Thiamethoxam showed strong performance under field conditions; however, the NE performed best after the insecticide treatment, which may be attributed to its nanoscale droplet size that enhances surface coverage, uniform dispersion and better penetration into seed tissues. Chitosan, used as an encapsulation agent, enhanced the stability of the formulation and supported insecticidal activity. Azadirachtin-based NE seed treatments at 10 mL kg^{-1} that act against sucking pests, offer a novel and eco-friendly approach that can be effectively integrated into IPM to reduce reliance on synthetic insecticides and meet the needs of organic and sustainable farming systems. Further studies should assess the long-term field performance, storage stability and compatibility of the NE with other biocontrol agents under diverse agroclimatic conditions.

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Authors' contributions

VM was responsible for data curation, drafting the original manuscript and compiling the research work. SSS, DS and IP contributed to the original draft and assisted with data analysis, while PI supported data curation and validation. KM contributed to the conceptualization, project administration and manuscript preparation. PJN and MK were involved in reviewing and editing the manuscript. SRP carried out the investigation, SA[1] contributed to methodology development and SA[2] provided resources. SM and RK were responsible for resources and validation [SA[1] stands for Suganthi A and SA[2] for Subramanian A].

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflicts of interest to declare.

Ethical issues: None

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