

Article

Dose-Reduction Potential of Spinosad via Combination with Green-Synthesized Silver Nanoparticles for Thrips Control

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Abstract

The green synthesis of nanoparticles offers a solution to control pesticide-resistant pests while minimizing environmental and health risks. *Thrips tabaci* is an injurious pest that attacks garlic crops and spreads the Iris yellow spot virus. The present research was performed to evaluate the synergistic effects of silver nanoparticles (AgNPs) synthesized by *Teucrium polium* with Spinosad against *T. tabaci* and assess their impact on garlic photosynthetic pigments. The characterization of the prepared nanoparticles was carried out by SEM, XRD, and Malvern zeta sizer. Antimicrobial activity was assessed using microdilution. Photosynthetic pigments were measured with a spectrophotometer after treating garlic cloves with four different concentrations of AgNPs and Spinosad mixture along with positive control (Spinosad) and negative control (tap water). Toxic bioassays were conducted under laboratory, greenhouse, and open field conditions. The results indicate all treatments, except for the 100% AgNPs, resulted in 100% second instar larvae and adult mortality after 72 h in the laboratory. In greenhouse conditions, the 50% Spinosad–50% AgNPs achieved 93.85% larvae mortality, and the 75% Spinosad–25% AgNPs achieved 100% adult mortality after a week. In open field conditions, the combination 50% Spinosad–50% AgNPs showed high efficacy, resulting in 65.97% mortality of larvae and 73.06% mortality of adults after 72 h. This study reveals that AgNPs have active pesticide properties against *T. tabaci* with minimal environmental and health risks.

Keywords: silver nanoparticles; garlic; green synthesis; *Thrips tabaci*; *Teucrium polium*; insecticidal



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1. Introduction

The world population is proposed to reach 9.8 billion by 2050, dictating a need for agricultural innovations to meet growing food demand. Pesticides play an important role in decreasing agricultural losses from pests; however, despite these benefits, they pose high environmental and human health risks [1].

Thrips tabaci Lindeman (*Thysanoptera*, *Thripidae*) is a major pest that affects garlic and onion crops, feeding on leaves and producing silvery patches that develop into white

blotches, leading to leaf curling and drying, which can result in smaller or absent seeds [2–4]. Extensive infestation exposes plants to fungal diseases and bacterial centre rot, as well as Iris yellow spot virus (IYSV), aggravating crop loss [3,5,6]. Although pesticides are used for controlling *T. tabaci*, excessive application has led to pesticide resistance, the destruction of beneficial insects and fauna, environmental pollution, food contamination, and increased costs. Sustainable alternatives are urgently required to manage *T. tabaci* infestations effectively [3,6,7].

Nanotechnology has innovated various fields, including biotechnology, agriculture, engineering, and analytical chemistry [8]. It includes the production and investigation of nanoparticles of at least one-dimensional material ranging from 1 to 100 nanometres that show unique properties compared to their bulk equivalent [9–11]. Nanoparticles are of significant importance because they exhibit exceptional and interesting characteristics due to their extremely small particle sizes, large surface area-to-volume ratio, and unique behaviour [12–14].

Nanoparticles are progressively more used in agriculture to improve crop productivity, crop protection, and soil tilth. Many studies highlight the benefits of zinc, gold, silver, silicon, copper, manganese, iron, magnesium, and titanium nanoparticles on plant production and growth by acting as nutrient carriers, soil remediation agents, and pest control [15–17]. Among these, silver nanoparticles (AgNPs) demonstrate strong inhibitory and bactericidal effects [18].

Nanoparticles are synthesized utilizing biosynthetic, chemical and physical methods, categorized into top-down and bottom-up approaches [12,19–21]. In the top-down approach, bulk materials are reduced to nanoscale particles via techniques like attrition milling grinding, and ball milling [21,22]; however, it often results in surface flaws affecting the physical properties of the nanoparticles [23]. Conversely, the bottom-up approach assembles nanoparticles from simpler molecules utilizing chemical and biological means via atoms that grow into nanoscale particles [20,23,24]. However, chemical methods face restrictions due to chemical toxicity, high costs, and environmental and human health risks. These challenges have steered the development of greener, eco-friendly synthesis methods [20,21,25].

The green synthesis approach is a safe, eco-friendly, and biocompatible path for producing nanoparticles through “green chemistry” using microorganisms (e.g., bacteria, fungi, etc.) and plant extracts [9,26]. Green intermediates from these organisms contain polyphenols and proteins, which act as reducing and stabilizing agents to replace chemical reagents to reduce metal ions [27]. Plant-extract-based green synthesis is gaining attractiveness due to its uncomplicatedness, quickness, cost-effectiveness, and environmental benefits. Plants can accumulate heavy metals in their various parts [20], and are instantly available [21]. Additionally, plant extracts are rich in active biomolecules such as proteins, phenolic acids, polyphenols, terpenoids, alkaloids, and sugars [28].

Teucrium polium L., known as “Jaada” in Jordan, a wild-growing herb, is found in the Middle East and the Mediterranean region. It is a traditional and folk medicine plant that has been used for many years as an antifebrile and an antiparasitic to combat stomach and intestinal problems. Moreover, it has anti-inflammatory, antioxidant, and anti-diabetic properties [29,30]. *T. polium* was chosen for this study because of the presence of rich bioactive compounds that help in the green synthesis of AgNPs and exhibit antimicrobial and pesticidal activity.

The current study aims to employ a biological approach using *T. polium* extract to synthesize AgNPs, characterize their properties, and evaluate their antibacterial activity. It also assesses the effectiveness and repellent efficacy of AgNPs against *T. tabaci*, focusing on

potential synergistic effects with the insecticide Spinosad. In addition, it aims to evaluate the effect of AgNPs on the photosynthetic pigments of the leaves of garlic plants.

2. Materials and Methods

2.1. Preparation of Plant Extract

Teucrium polium plants were collected from the land in Al-Karak, Jordan; to verify the plant material's authenticity as the target species, a formal botanical identification was carried out by one of the authors (Issam M. Qrunfleh). The fresh leaves and stems of the plants were washed and air-dried. Soxhlet extractor (Sigma-Aldrich, St. Louis, MO, USA) was used for extraction following the authors of [31] with minor modifications. Crushed plant material (15.10 g) was placed in a thimble, and 350 mL of ethanol was heated to 60 °C. After extraction, 50 mL of the extract was distilled off at 40 °C using a rotary evaporator (BÜCHI Labortechnik, Flawil, Switzerland). The solid residue was weighed, and the extract's concentration was 4.8 mg/mL. The analyses of the *T. polium* extract followed the authors of [32] using Shimadzu QP2020 GC-MS (Shimadzu Corporation, Kyoto, Japan). An amount of 8 µL of the extract was injected with helium as the carrier gas. Volatile components were separated using a DB5 MS fused silica column. Compounds were identified by comparing retention indices and mass spectra with ADAMS-2007 and NIST 2017 libraries.

2.2. Green Synthesis of Silver Nanoparticles

The synthesis of AgNPs was done following the procedure of the authors of [32], with minor modifications. One litre of silver nitrate solution (0.05 N) was heated to 80 °C, and 50 mL of *T. polium* extract was added dropwise under stirring using a magnetic hot plate stirrer (Jenway, Stone, UK). The mixture turned yellow, indicating nanoparticle formation. After 30 min, 5 mL of concentrated ammonium hydroxide was added to adjust the pH to 9, turning the colour dark brown. The reaction proceeded for 24 h in the dark. The suspension was then centrifuged (Thermo Legend D-37520 RT Refrigerated Centrifuge, Thermo Scientific, Waltham, MA, USA) at 10,000 rpm for 15 min. The AgNPs were washed with distilled water, resuspended and stored in a cool and dark place.

2.3. Characterization of Silver Nanoparticles

The morphology and size of green-synthesized AgNPs were evaluated using a Scanning Electron Microscope (Inspect F50-FEI Company, Hillsboro, OR, USA). The samples were coated with platinum using an Emitech 550X sputter coater (Quorum Technologies Ltd., Laughton, UK). The AgNPs configuration was determined via X-ray diffraction (XRD) (Rigaku Miniflex 6, Tokyo, Japan), using Cu. K α . radiation source ($\lambda = 1.540562 \text{ \AA}$) at 15 mA and 40 kV radiation. A 250 mL AgNP suspension was evaporated at 85 °C in a Memmert UM100 oven (Mettler GmbH, Schwabach, Germany), with half the powder calcined at 250 °C. Particle size distribution and zeta potential were measured using photon correlation spectroscopy at 25 °C, with a Malvern Zeta-sizer Nano ZS[®] (Malvern Panalytical, Malvern, UK). The sample was diluted 50 times with distilled water. It was added in low-volume disposable cuvettes [33].

2.4. Antimicrobial Activity of Silver Nanoparticles

The antimicrobial activity of *T. polium* extract and green-synthesized AgNPs was determined using a microdilution assay for evaluating the minimum inhibitory concentration. The test targeted Gram-positive bacteria (*Staphylococcus aureus* (ATCC[®] 29213[™]) and *S. aureus* (ATCC[™] BAA-1026[™])), and Gram-negative bacteria (*Pseudomonas paraeruginosa* (ATCC[®] 9027[™])), following the procedures of the authors of [34,35] with modifications.

The extract and AgNP solutions were serially diluted (2-fold) in Mueller–Hinton broth (0.7 mg/mL to 0.0105 mg/mL). The eleventh well served as the positive control, containing a bacterial culture at a 0.5 McFarland standard in Mueller–Hinton broth to confirm uninhibited growth. The twelfth well acted as the negative control, consisting only of sterile Mueller–Hinton broth to ensure the medium was sterile and to verify the absence of environmental contamination. After 24 h of incubation at 37 °C, the plates were scanned with an ELISA reader (BioTek Instruments, Winooski, VT, USA) at 570 nm, and the MIC was defined as the lowest concentration inhibiting detectable growth.

2.5. Determination of Photosynthetic Pigments Content

To study the impact of AgNPs on chlorophyll content, garlic cloves were soaked in AgNP concentrations (0.73, 0.55, 0.36, and 0.18 mg/mL) for three days until the roots arose. The cloves were planted in polyethylene pots filled with soil (6.5 pH) and potting mix (Mikskaar, Tartu, Estonia, 2:1 ratio) in an open space. After a month, fresh leaves were gathered, washed, and 50 mg samples were extracted in 25 mL of 80% acetone with a crusher and pestle, then centrifuged at 4 °C (4000 rpm for 10 min). The supernatant was diluted 20 times with acetone (80%). Each treatment was replicated three times. Chlorophyll a, b, and carotenoid content were determined using a spectrophotometer (WPA, Cambridge, UK) at wavelengths of 470, 645, and 663 nm. The chlorophyll content was calculated using the following Equations (1)–(3) [36]:

$$\text{Chlorophyll } a = 12.25A_{663.2} - 2.79A_{646.8} \quad (1)$$

$$\text{Chlorophyll } b = 21.5A_{646.8} - 5.1A_{663.2} \quad (2)$$

$$C \times +c (\text{carotenoid}) = (1000A_{470} - 1.82Ca - 85.02Cb)/198 \quad (3)$$

where Ca, Cb, and $C \times +c$ represent chlorophyll a, b, and total carotenoid concentrations, respectively, and A indicates optical density (absorbance) at the specified wavelength.

2.6. Evaluation of Silver Nanoparticles Against *Thrips tabaci*

2.6.1. Vegetative Material

Garlic plants were used as the host plant for *T. tabaci*. Cloves were planted in polyethylene pots in a peat moss–perlite substrate (2:1 ratio) under greenhouse conditions (25 ± 2 °C, RH of $60 \pm 10\%$ [37]) at Al-Balqa Applied University, Jordan. The plants were watered as needed.

2.6.2. Insect Rearing

T. tabaci were collected from infested garlic plants and subsequently reared on garlic plants in $60 \times 60 \times 60$ cm pathogen- and parasite-free cages under greenhouse conditions at Al-Balqa Applied University, Jordan. Adults were released on the plants for oviposition and reproduction, targeting the adult and 2nd instar larvae stages as the most damaging to the plant.

2.6.3. Preparation of Treatments

Six treatments were prepared: AgNPs suspension alone, and three different combinations of the pesticide Spinosad (Spintor[®], Corteva Agriscience, Wilmington, DE, USA) with AgNPs in varying proportions (see Table 1). Tap water served as the negative control since it was used to prepare the treatments as a solvent, and the pesticide alone served as the positive control.

Table 1. Treatments for *Thrips tabaci* control.

Treatment	Ratios	AgNPs (mg/mL)	Spinosad (mL/L)
1	100% Tap water	-	-
2	100% AgNPs	0.73	-
3	75% Spinosad–25% AgNPs	0.18	0.3
4	50% Spinosad–50% AgNPs	0.36	0.2
5	25% Spinosad–75% AgNPs	0.55	0.1
6	100% Spinosad	-	0.4

2.6.4. Experiments Under Laboratory Conditions

The experiment was conducted in the laboratory of the Plant Production and Protection Department at Al-Balqa Applied University, under 25 ± 2 °C and $60 \pm 5\%$ relative humidity. Garlic leaf cuts (5 cm) were placed in 9 cm Petri dishes with moistened cotton, along with five adults per leaf cut (25 adults per dish). The larvae were placed carefully in separate dishes (11 larvae per dish). Treatments were applied using a Potter Spray Tower (Burkard Scientific Ltd., Uxbridge, UK). The experiment used a completely randomized design (CRD) with three replicates per treatment.

2.6.5. Experiments Under Greenhouse Conditions

The experiment was conducted in a greenhouse at Al-Balqa Applied University, Jordan, under approximately 25–30 °C. Adults oviposited on garlic plants, then were removed with a homemade aspirator. The eggs were tracked from hatching to the 2nd larval instar. A number of pots were isolated immediately, while others were left until the larvae matured into adults. The treatments were applied manually using a spray. The experiment used a complete randomized design (CRD) with four replicates per treatment.

2.6.6. Experiments Under Open Field Conditions

This experiment was conducted under natural infestation conditions of *T. tabaci* in a garlic field in the Al-Yadudah region of Amman. A 15 m² area was divided into six plots (each 3 m² with two rows) and designed using a randomized complete block design (RCBD) with three replicates per treatment (three plants each). The treatments were applied manually using a spray.

2.6.7. Mortality Evaluation

Mortality was evaluated at 24 h, 72 h, and one week in the greenhouse, laboratory, and field experiments. A dissecting microscope (FUP-09-3C, Faithful instrument Co., Ningbo, China) was used in the laboratory, while a magnifying lens was used for greenhouse and field experiments. Surviving and dead larvae and adults were counted on the selected plants; individual *T. tabaci* were considered dead if they shrank or changed colour [38].

2.6.8. Repellent Assay

Following a modified procedure according to the authors of [39], a 9 cm diameter filter paper was cut in half. One mL of each concentration was applied to one half using a dropper, while the other half received 1 mL of water as a control. The halves were air-dried, taped together, and placed in 9 cm Petri dishes. Ten *T. tabaci* adults were released on each disc, and the dishes were covered. Each treatment was replicated three times. Insects

were counted on each strip every 7, 12, 24, and 48 h. Treatments from Table 1 were used. Repellency percentage was calculated using Formula (4) from [40]:

$$\%Repellency = (Nc - 5) \times 20\% \quad (4)$$

where Nc = repellency record.

2.7. Statistical Analysis

All data were analyzed using SAS 9.3 (2012) software. The mortality data were corrected using Abbott's Formula (5) [41] and then analyzed using ANOVA.

$$Corrected mortality = \frac{M \text{ in treatment} - M \text{ in control}}{100 - M \text{ in control}} \times 100\% \quad (5)$$

Mean values were compared using Tukey's HSD test at a 5% significance level. ANOVA was conducted using a completely randomized design (CRD) for the greenhouse bioassays and a randomized complete block design (RCBD) for the field bioassays. Tukey's test was used for mean separation with $p < 0.05$. Repellency data were calculated as percentages, with arcsine transformed for ANOVA, and compared using one-way ANOVA. The chlorophyll content was analyzed with three replicates per treatment. The results are presented as mean $\pm$ SE, with significance set at $p \leq 0.05$.

3. Results and Discussion

3.1. GC-MS Analysis

The components of *T. polium* extract were identified by comparing pure sample retention times (RT), and calculating linear hydrocarbon retention indices. Mass spectrometry libraries were also used. Gas chromatography and mass spectrum analysis of the ethanolic extract yielded a chromatogram with 28 peaks (Figure 1); Table 2 shows the compounds found in the extract.

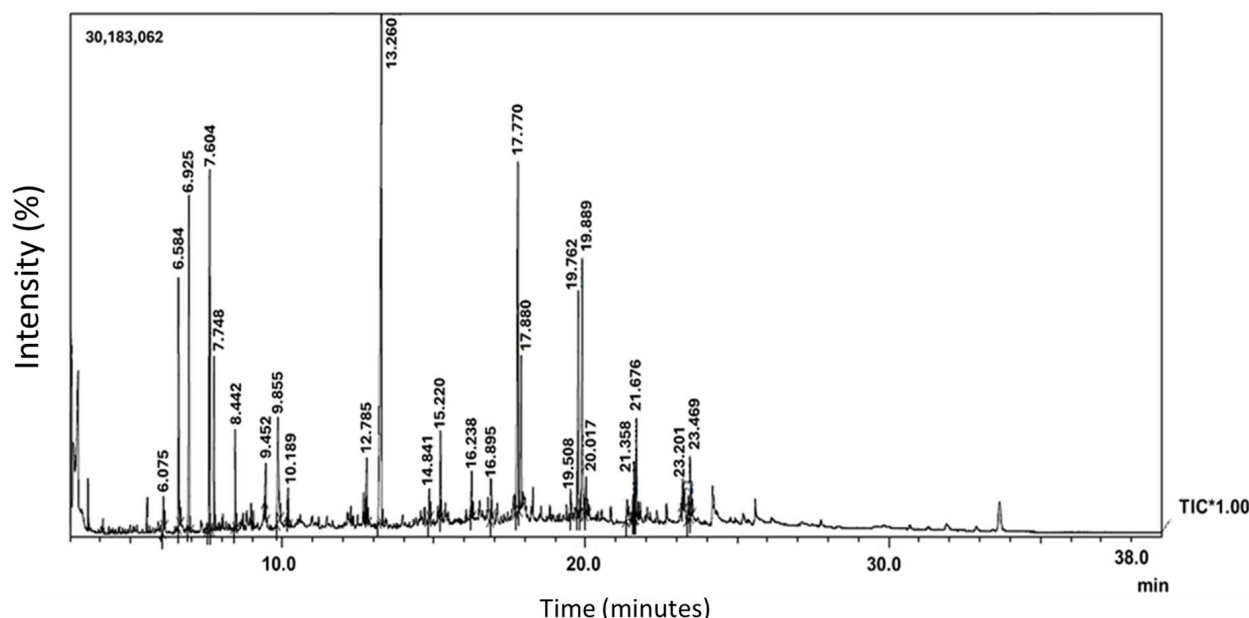


Figure 1. Chromatogram of GC-MS of ethanolic extract of *Teucrium polium*. TIC*1.00: Stands for Total Ion Chromatogram scaling factor (*1.00).

Table 2. Identified components of ethanolic leaf extract of *Teucrium polium* using GC-MS.

Peak	R. Time	Area	Area %	Name
1	6.075	2,598,484	0.63	2,5,5-Trimethyl-3,6-heptadien-2-ol
2	6.584	20,150,684	4.88	(3S)-3-Ethenyl-2,5-dimethylhex-4-en-2-ol
3	6.925	33,361,447	8.08	2,6-Octadiene, 2,4-dimethyl-
4	7.604	38,525,069	9.33	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-
5	7.748	13,306,634	3.22	Thujone
6	8.442	7,171,992	1.74	(S)-3,3,6-Trimethylhepta-1,5-dien-4-yl acetate
7	9.452	4,954,982	1.20	2-Nonynoic Acid cis-3-Hexen-1-yl Ester
8	9.855	16,349,744	3.96	1,7,7-Trimethylbicyclo[2.2.1]heptane-2,3-diol
9	10.189	3,139,353	0.76	(1S,3S,5S)-1-Isopropyl-4-methylenebicyclo[3.1.0]hexan-3-yl acetate
10	12.785	6,761,349	1.64	cis-.beta.-Farnesene
11	13.260	83,017,851	20.10	Beta-Sesquiphellandrene
12	14.841	2,842,566	0.69	Beta-eudesmol
13	15.220	7,739,250	1.87	2-Cyclohexen-1-one, 4-(1,5-dimethyl-4-hexenyl)-
14	16.238	4,010,101	0.97	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol
15	16.895	3,724,328	0.90	Mansonone E
16	17.770	52,181,738	12.64	Pellitorin
17	17.880	15,152,725	3.67	4a-But-3-enyl-2-t-butyl-tetrahydrocyclopenta[1,3]dioxin-4-one
18	19.508	3,936,771	0.95	Levallorphan, trimethylsilyl ether
19	19.762	24,659,108	5.97	Achilleamide
20	19.889	30,512,866	7.39	Cyclohexanamine, N-cycloheptylidene-
21	20.017	4,679,084	1.13	Hexadecanamide
22	21.358	3,755,533	0.91	N-Isobutyl-(2E,4E,8Z,10Z)-dodecatetraenamide
23	21.558	2,590,864	0.63	4,8,12,16-Tetramethylheptadecan-4-olide
24	21.616	5,806,536	1.41	Palmitoleamide
25	21.676	11,048,969	2.68	Butane, 2-(2,2-dichloro-1,3-dimethylcyclopropyl)
26	23.201	2,593,495	0.63	Piperidine, 1-(1-oxo-2,4-hexadienyl)-
27	23.383	2,797,534	0.68	(2E,4E)-1-(Piperidin-1-yl)tetradeca-2,4-dien-1-one
28	23.469	5,620,557	1.36	Tricyclo[3.3.0.0(2,8)]octane-3,7-dione, 4-methyl-4-(propan-2-on-1-yl)-

Among the compounds found in the sample, the most common ones (those with the highest Area %) are as follows:

- The most prevalent ingredient in the extract is beta-sesquiphellandrene (20.10%).
- Pellitorin (12.64%): An important alkamide that contains nitrogen. Umbellulone is a common name for bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- (9.33%).
- The volatile unsaturated hydrocarbon 2,6-Octadiene, 2,4-dimethyl- (8.08%).
- Cyclohexanamine, N-cycloheptylidene: A derivative of cyclic amines (7.39%).
- Achilleamide (5.97%): The sample also contained this noteworthy alkamide. Santolina alcohol is another name for (3S)-3-Ethenyl-2, 5-dimethylhex-4-en-2-ol (4.88%).

3.2. Synthesis of Silver Nanoparticles

The green synthesis of AgNPs using *T. polium* extract (at 80 °C and pH 9) proved that the extract could reduce the silver cation (+1) to zero. The colour change to a dark brown was a sign of AgNP creation (Figure 2) [32]. The initial Gas Chromatography–Mass Spectrometry (GC/MS) analysis of *T. polium* ethanolic extract identified several bioactive

compounds (Table 2). The synthesis results revealed that the extract contained terpenoids (e.g., β -Sesquiphellandrene), amides (e.g., Hexadecanamide), alcohols (e.g., 3,5,9-Trimethyl-deca-2,4,8-trien-1-ol), and ketones (e.g., Mansonone E), which are common in plant extracts and could serve as reducing agents while also capping and stabilizing nanoparticles by preventing aggregation [42]. These findings align with prior studies on the role of *T. polium* in synthesizing AgNPs. The authors of [43] synthesized stable AgNPs because of the capping and reducing nature of the phytoconstituents in *T. polium*.

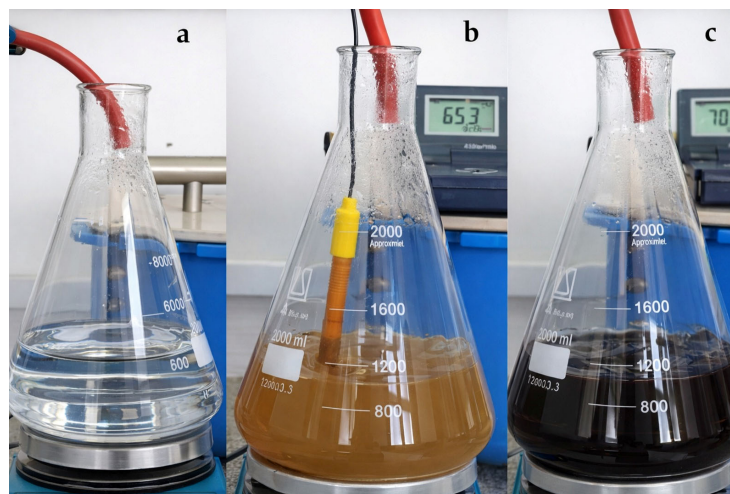


Figure 2. Synthesis of AgNPs using *Teucrium polium* extract and its identification by the colour change. (a) AgNO₃ solution. (b) After addition of plant extract. (c) After addition of NH₃OH.

3.3. Characterization of Silver Nanoparticles

3.3.1. Scanning Electron Microscope (SEM)

The SEM images confirmed that the particle size of the green-synthesized AgNPs using plant extract is within the nanoscale, as the average particle size is about 33.2 nm (Figure 3). According to the SEM images the particles' shape is spherical with smooth edges. They are also presented as single particles or agglomerated.

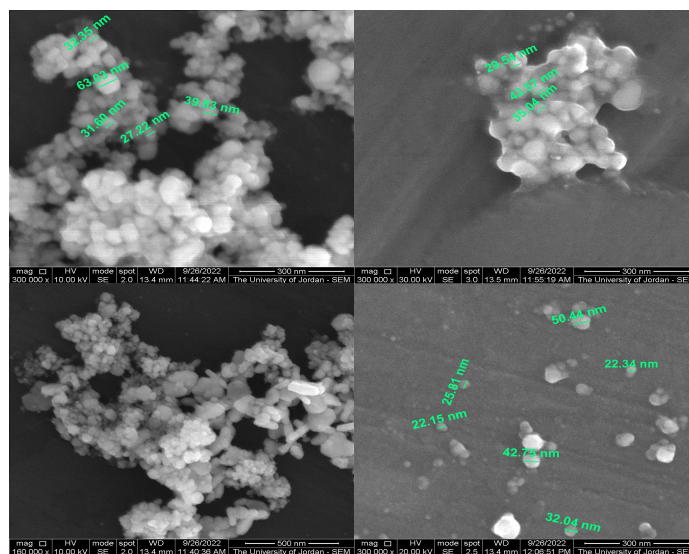


Figure 3. Green synthesis of AgNPs; size and shape observed by SEM at 10, 20 and 30 kV and 160,000 $\times$ and 300,000 $\times$ magnification.

3.3.2. Particle Size Distribution and Zeta Potential

The hydrodynamic diameter and the surface charge of free and agglomerated AgNPs formed from utilizing plant extract were determined with a zeta sizer. The dynamic light scattering (DLS) method was used to determine the dimensional properties of the generated particles. DLS results found a Z-Average of 270.2 nm (Table 3). In addition, the Z-Average indicated that the AgNPs were spherical and monomodal because of the single peak (Figure 4a). The Polydispersity index (PDI) value of 0.343 indicates the nanoparticles were monodisperse [44]. PDI values greater than 0.7 indicate that the sample displayed a wide size range due to the grouping of AgNPs into clusters [45]. The difference in average particle sizes determined by DLS zeta sizer and SEM may result from DLS assessing a hydrodynamic rather than a physical size [46]. The electrostatic stabilization of the AgNPs was estimated by measuring their zeta potential. The zeta sizer measurements show that the AgNPs synthesized using ethanolic plant extract were stable due to their negative charge -23.5 mV (Figure 4b). Negative values also confirmed the high surface energy and particle repulsion [44].

Table 3. Size distribution, zeta potential and PDI of AgNPs prepared using *T. polium* extract.

Sample	Hydrodynamic Diameter (DLS) (nm) (Mean $\pm$ SD)	Zeta Potential (mV) (Mean $\pm$ SE)	Polydispersity Index (PDI)
AgNPs	270.2 $\pm$ 28.04	-23.5 ± 8.04	0.343

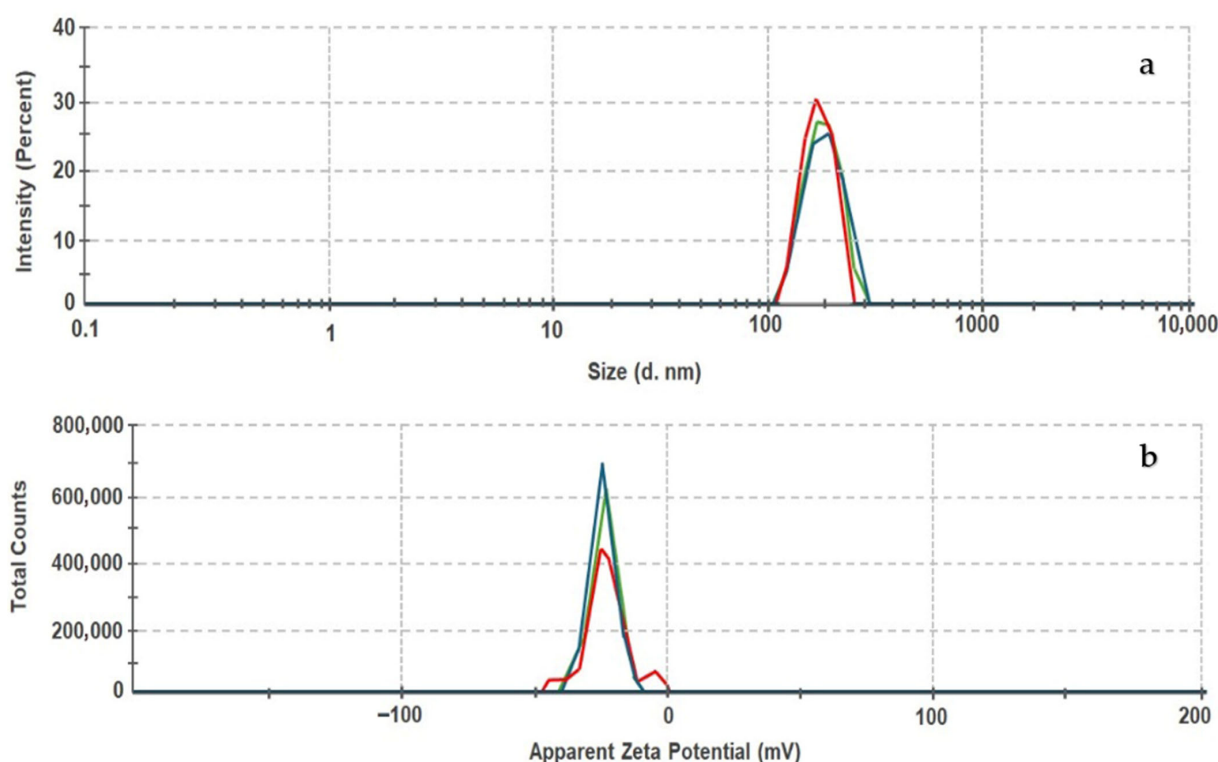


Figure 4. (a) Hydrodynamic diameter and (b) surface charge of AgNPs prepared using *Teucrium polium* extract as measured by zeta sizers. Green, red, and blue curves represent triplicates independent measurements of the same sample.

SEM analysis revealed that the silver nanoparticles' average physical diameter was 33.2 nm. DLS measurements, however, showed a much greater hydrodynamic diameter of 270 nm. The development of a hydration layer surrounding the particles and the partial

agglomeration of the nanoparticles within the suspension is a frequent occurrence in metal-based nanofluids and are the main causes of this increase.

3.3.3. X-Ray Diffraction (XRD) Analysis

The X-ray diffraction (XRD) technique can be helpful for determining crystal structure and size. It is also used for estimating the atomic arrangement and lattice parameters [47]. Figure 5 displays the typical X-ray diffraction patterns of green-synthesized AgNPs using *T. polium* before and after calcination, revealing that the metal content of the nanoparticle powder is pure silver.

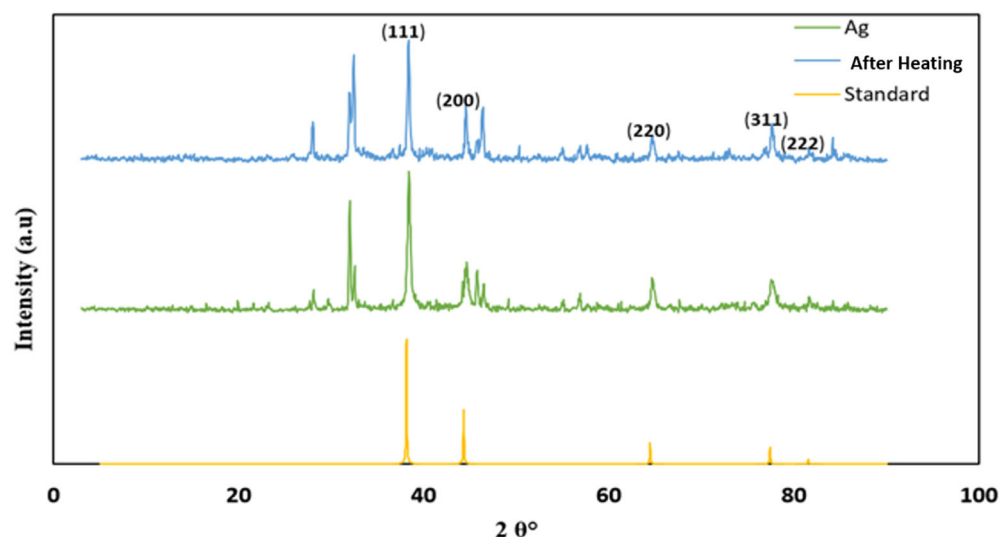


Figure 5. X-ray diffraction showing peaks for powder AgNPs synthesized by *Teucrium polium* extract. (Ag): synthesized AgNPs using plant extract at 85 °C; (After Heating): calcined synthesized AgNPs at 250 °C; (Standard): data file of silver (ICDD/JCP).

The spectra of crude AgNPs showed diffraction peaks at 2θ values of 38.4, 44.65, 64.75, 77.5, and 82.1 corresponding to the Miller indices (111), (200), (220), (311), and (222) lattice planes (Figure 5-Ag). After calcination, diffraction peaks at 2θ with values of 38.35, 44.55, 64.7, 77.55, and 82 were observed, matching the planes of (111), (200), (220), (311), and (222) (Figure 5-after heating). These peaks were compared with the powder diffraction data files of known compounds (ICDD/JCPDS, PDF 217 Nos. 04-0783 and 84-0713), matched with the face-centred cubic (fcc) structure of silver, and conformed the crystalline nature of the nanoparticles. A slight shift in the peaks after calcination suggested that the plant extract's organic components had been destroyed. As already established, any change in the interlayer of the lattice spacing caused by organic modification or polymer interpolation leads to a change in the peak's position, broadness, and intensity in XRD spectra [48]. Plant extracts reduce silver ions (Ag^+) to metallic silver, but the reaction frequently produces a mixture of metallic and oxide forms, according to research on microwave-assisted and leaf-extract-mediated green synthesis [49,50]. XRD peak identification: According to Korkmaz and Karadağ [49] standard peaks for metallic silver usually show up at 2θ values of roughly 38°, 44°, 64°, and 77°. Nevertheless, the (111), (200), (220), and (311) planes of pure Ag_2O nanoparticles were associated with additional peaks at values like 32.06°, 40.03°, 46.36°, and 65.03° [51].

3.3.4. Antibacterial Activity

The antibacterial activity of green-synthesized AgNPs from *T. polium* extract was tested against *Staphylococcus aureus* (29213), *Staphylococcus aureus* (BAA-1026), and *Pseudomonas*

paraeruginosa, (Table 4). The minimum inhibitory concentration (MIC) was determined using the broth dilution method. The AgNPs' antibacterial activity showed that increasing the concentration of AgNPs reduces the MIC value of bacterial strains in parallel. The antibacterial effects of *T. polium* extract were tested separately. The results revealed no inhibition signal of this extract on both *S. aureus*, so bacteria growth occurred in all wells. For *P. paraeruginosa*, the antibacterial effect of the extract was insignificant, with a MIC value of 3.2505 mg/mL. The AgNPs had significant antibacterial activity against *S. aureus* (29213), *S. aureus* (BAA-1026), and *P. paraeruginosa*, with MIC values of 9.3, 6.4, and 5 µg/mL, respectively. The increased antibacterial activity could be attributed to the quantity of silver. According to previous research, AgNPs release silver ions that accumulate in the bacterial cell membrane and then react with thiol groups and proteins to produce free radicals, which damage the membrane and slowly kill bacteria [52]. The lack of significant differences in inhibition between Gram-positive and Gram-negative bacteria could be explained by the wide range of antibacterial properties of the AgNPs mechanism. The ability of AgNPs to penetrate the bacterial cell and release silver ions leads to oxidative stress, enzymatic inhibition, DNA damage, and disrupting essential cellular processes, leading to the death of the bacterial cells, regardless of the differences in their cell envelope structures [53–55].

Table 4. MIC of bacteria for AgNPs synthesized from *T. polium* extract.

Bacteria	AgNPs MIC (µg/mL)	Extract MIC (µg/mL)
<i>S. aureus</i> (ATCC® 29213™)	9.3	-
<i>S. aureus</i> (ATCC™ BAA-1026™)	6.4	-
<i>P. paraeruginosa</i> (ATCC® 9027™)	5	3250.5

3.4. Photosynthetic Pigments

Plants with high levels of photosynthetic pigments, such as chlorophyll and carotenoids, are beneficial because they have an increased ability to absorb light energy, which improves growth. They also improve the efficiency of converting light energy into chemical energy, thereby accumulating more biomass and better overall plant health [56,57]. The results revealed and evaluated the impact of AgNPs on photosynthetic pigments in garlic leaves. Garlic plants were treated with various concentrations of AgNPs, resulting in different photosynthetic pigment readings. In control plants, chlorophyll-a, chlorophyll-b, carotenoids, and total photosynthetic pigments averaged 6.58, 4.31, 1.79, and 12.68 mg/mL, respectively. At the highest AgNP concentration (0.73 mg/mL), photosynthetic pigment content increased to 15.49, 17.36, 4.03, and 36.88 mg/mL. Lower AgNP concentrations led to reduced photosynthetic pigment content as shown in Table 5. This result is in harmony with Sharma's results [58]. They issued that the application of high AgNP concentrations on *Brassica* plants enhanced chlorophyll content. At 100 ppm AgNP treatment, the chlorophyll-a and chlorophyll-b content increased by 40% and 25%, respectively. Lei et al. discovered that nano-TiO₂ improved photosynthesis and growth in spinach [59]. While our results showed an increase in the amount of photosynthetic pigment with the increase in the AgNP amount, other research, such as Verma et al. [36], found less chlorophyll in *Phaseolus vulgaris* treated with higher AgNP levels. The difference could be due to the plant species or the plant's tolerance to AgNPs. Thus, the amount of nanoparticles should be tailored to each plant species [57,60]. The likely explanation is that AgNPs, known for their antibacterial properties, reduce pathogenic microbes on garlic cloves, leading to healthier plants with increased chlorophyll production [61]. In addition, AgNPs may improve nutrient uptake by altering the root shape, ensuring the bioavailability of nutrients needed for total chlorophyll production [62].

Table 5. Effect of different concentrations of green-synthesized AgNPs on photosynthetic pigments of garlic plants.

AgNPs Concentration (mg/mL)	Chlorophyll a (mg/mL) (Mean $\pm$ SE)	Chlorophyll b (mg/mL) (Mean $\pm$ SE)	Carotenoids (mg/mL) (Mean $\pm$ SE)	Total Pigments (mg/mL)
Control	6.58 ^e $\pm$ 0.0067	4.31 ^d $\pm$ 0.069	1.79 ^e $\pm$ 0.026	12.68
0.73	15.49 ^a $\pm$ 0.019	17.36 ^a $\pm$ 0.025	4.03 ^a $\pm$ 0.026	36.88
0.55	9.25 ^c $\pm$ 0.037	6.86 ^c $\pm$ 0.049	2.85 ^c $\pm$ 0.003	18.96
0.36	12.1 ^b $\pm$ 0.067	11.38 ^b $\pm$ 0.111	3.63 ^b $\pm$ 0.018	27.11
0.18	7.32 ^d $\pm$ 0.041	4.09 ^d $\pm$ 0.167	2.29 ^d $\pm$ 0.062	13.7

Treatment means having the same letters are not statistically significant from one another. All means were compared at a significance level (α) of 0.05.

3.5. Evaluation of Silver Nanoparticles Against *Thrips tabaci*

3.5.1. Laboratory Conditions

This section provides the findings of the laboratory experiment, focusing on the efficacy of AgNPs mixed with the pesticide Spinosad against *T. tabaci* at various concentrations. The evaluated mixtures were 0.18–0.3, 0.36–0.2, 0.55–0.1 and 0.73–0.4 (AgNPs mg/mL–Spinosad mL/L). At 24 h in second instar larvae, all treatments showed varied mortality rates, with AgNPs alone exhibiting the lowest (81.8%) and 50%Sp-50%AgNPs showing the highest (95.8%). ANOVA showed no significant difference at 24 h ($df = 4$, $F = 2.99$, $Pr = 0.0730$), but significant differences at 72 h ($df = 4$, $F = 745.36$, $Pr < 0.0001$) and 168 h ($df = 4$, $F = 3.96$, $Pr = 0.0353$) (Figure 6A). In the adult stage, all treatments except AgNPs alone resulted in 100% mortality, with 50%Sp-50%AgNPs, 25%Sp-75%AgNPs, and Spinosad demonstrating the most effectiveness. AgNPs had the lowest mortality rate (31.9%). ANOVA analysis showed significant differences at 72 h ($df = 4$, $F = 142.23$, $Pr < 0.0001$) but not at 168 h ($df = 4$, $F = 41.67$, $Pr = 0.4516$) (Figure 6B). The efficacy of treatments under laboratory conditions varied according to the concentration and composition of the nanoparticles and pesticide used. Longer exposure times generally resulted in higher mortality rates.

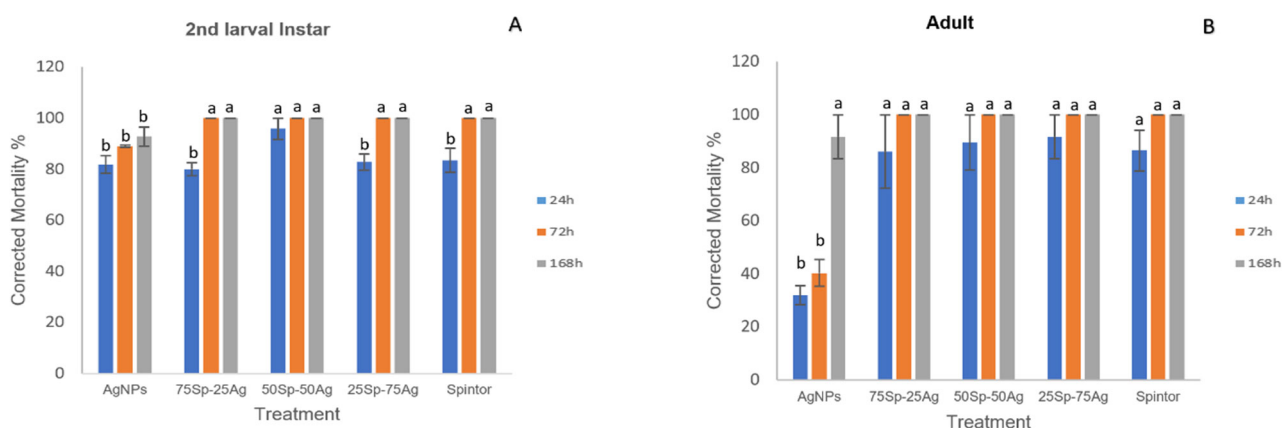


Figure 6. Corrected mortality (% $\pm$ SE) of second instar larvae (A) and adults (B) of *Thrips tabaci* after 24, 72, and 168 h, when treated with 100% AgNPs, 75Sp-25Ag = 75% Spinosad–25% AgNPs, 50Sp-50Ag = 50% Spinosad–50% AgNPs, 25Sp-75Ag = 25% Spinosad–75% AgNPs, and 100% Spinosad in laboratory conditions. Different letters above the bars indicate statistical significance ($p < 0.05$).

3.5.2. Greenhouse Conditions

The pesticidal activity of AgNPs combined with Spinosad against second instar larvae and adult *T. tabaci* in greenhouse conditions was tested at various concentrations:

0.18–0.3, 0.36–0.2, 0.55–0.1 mg/mL (AgNPs)–mL/L (Spinosad), and single applications of 0.73 mg/mL AgNPs and 0.4 mL/L Spinosad. All treatments resulted in considerable mortality in second instar larvae after 24 and 72 h. AgNPs alone had the lowest mortality (43.165% at 24 h, 52.138% at 72 h), whereas 75% Spinosad–25% AgNPs had the highest (87.03% at 24 h, 88.763% at 72 h). ANOVA revealed significant differences between treatments throughout these times ($df = 4$, $F = 27.66$, $Pr < 0.0001$; $df = 4$, $F = 22.4$, $Pr < 0.0001$). After a week, AgNPs alone differed considerably from other treatments, but there was no significant difference between the combinations and Spinosad alone (Figure 7A). After 24 h, all treatments in adults exhibited some level of mortality, with AgNPs alone at 47.895% and Spinosad at 90.455%. Significant differences were observed between AgNPs and other treatments ($df = 4$, $F = 10.35$, $Pr = 0.0003$). After 72 h, no significant differences were found ($df = 4$, $F = 1.25$, $Pr = 0.3314$), although mortality from AgNPs increased to 82.883%. After 168 h, no significant differences were seen ($df = 4$, $F = 1.6$, $Pr = 0.2262$), with Spinosad and 75% Spinosad–25% AgNPs obtaining 100% mortality (Figure 7B).

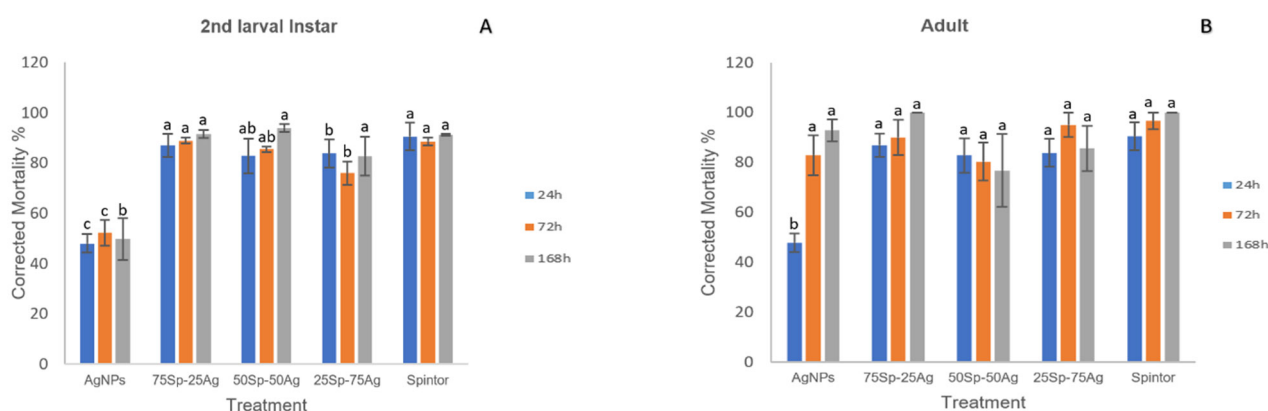


Figure 7. Corrected mortality (% $\pm$ SE) of second instar larvae (A) and adults (B) of *Thrips tabaci* after 24, 72, and 168 h, when treated with 100% AgNPs, 75Sp-25Ag = 75% Spinosad–25% AgNPs, 50Sp-50Ag = 50% Spinosad–50% AgNPs, 25Sp-75Ag = 25% Spinosad–75% AgNPs, and 100% Spinosad in greenhouse conditions. Different letters above the bars indicate statistical significance ($p < 0.05$).

3.5.3. Open Field Conditions

Figure 8 represents the corrected mortality (% $\pm$ SE) of second instar larvae and adults of *Thrips tabaci* after 24, 72, and 168 h. The toxicity of AgNPs and Spinosad was evaluated with combinations of 0.18–0.3, 0.36–0.2, and 0.55–0.1 (AgNPs mg/mL–Spinosad mL/L) and single applications (0.73 mg/mL AgNPs, 0.4 mL/L Spinosad). Manual spraying resulted in considerable mortality for *T. tabaci* second instar larvae and adults in open fields. After 24 h, AgNPs alone had a low mortality rate of 29.72%, whereas 50%Sp-50%AgNPs and Spinosad showed higher rates (48.56% and 47.04%, respectively). No significant differences were observed (ANOVA: $df = 6$, $F = 2.03$, $Pr = 0.1746$). At 72 h, 50%Sp-50%AgNPs and Spinosad maintained higher mortality than AgNPs alone. At 168 h, AgNPs alone resulted in substantially lower mortality ($df = 6$, $F = 2.43$, $Pr = 0.122$). In adults, no significant differences were seen after 24 h ($df = 6$, $F = 1.84$, $Pr = 0.2067$). AgNPs (49.71%), 75%Sp-25%AgNPs (48.99%), and 50%Sp-50%AgNPs (50.53%) showed comparable mortality rates, however 25%Sp-75%AgNPs had lower rates (38.83%). Spinosad had the greatest percentage (55.9%). At 72 h, 50%Sp-50%AgNPs and Spinosad exhibited greater mortality rates ($df = 6$, $F = 3.42$, $Pr = 0.0559$), whereas 25%Sp-75%AgNPs had the lowest. No significant differences were seen at 168 h ($df = 6$, $F = 1.9$, $Pr = 0.1967$). Overall, 50%Sp-50%AgNPs and Spinosad had the highest mortality.

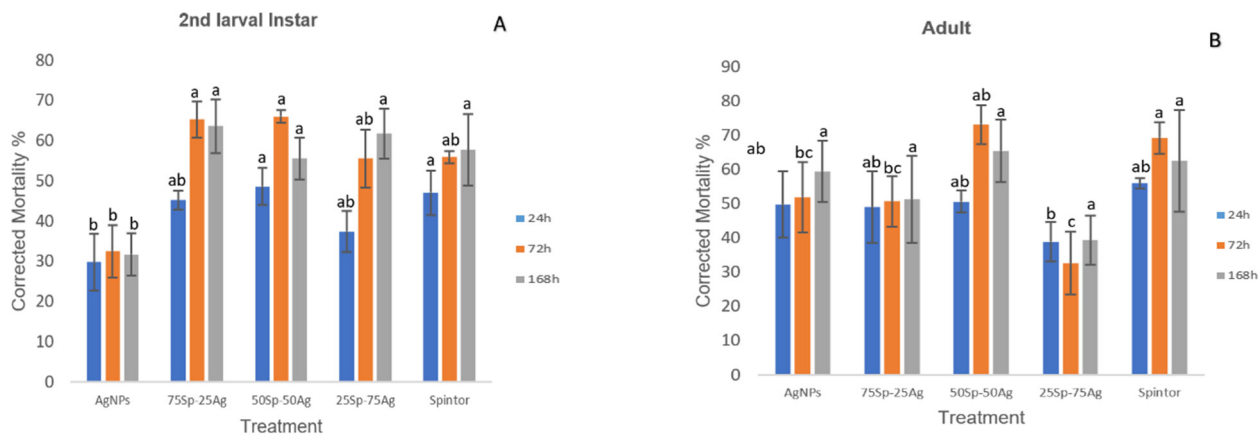


Figure 8. Corrected mortality (% $\pm$ SE) of second instar larvae (A) and adults (B) of *Thrips tabaci* after 24, 72, and 168 h, when treated with 100% AgNPs, 75Sp-25Ag = 75% Spinosad–25% AgNPs, 50Sp-50Ag = 50% Spinosad–50% AgNPs, 25Sp-75Ag = 25% Spinosad–75% AgNPs, and 100% Spinosad in open field conditions. Different letters above the bars indicate statistical significance ($p < 0.05$).

3.5.4. Repellent Assay

The repellent activity of green-synthesized AgNPs, Spinosad, and combinations of AgNPs and Spinosad were tested against *T. tabaci*. The repellent activity data are shown in Table 6. After seven hours of exposure, low repellency activity (6.67%, 13.33%, 6.67%, and 20% repulsion) was detected for 100% AgNPs, 75% AgNP–25% Spinosad, 50% AgNP–50% Spinosad, and 25% AgNP–75% Spinosad. Spinosad alone exhibited moderate repellency (53.33%). At the 12th hour, repellency records had increased sufficiently for all treatments. After 24 and 48 h of monitoring, repellency levels increased slightly. The repellency increased over time and with higher Spinosad concentration in the combinations. All treatments show more than 50% repellency after 24 h. Only 25% AgNP–75% Spinosad exhibits 100% repellency after 48 h. The values were not statistically significant at 0.05 level.

Table 6. Repellent activity of *Thrips tabaci* against AgNPs, Spinosad, and different concentration of their combinations.

Treatment	#	Percent Repulsion (PR): $PR = (Nc - 5) \times 20\%$				Chi Square	p-Value
		7 h	12 h	24 h	48 h		
Spinosad (Spintor)	30	53.33	73.33	86.67	93.33	75.551	0.00
100% AgNPs	30	6.67	53.33	60	66.67	45.329	0.00
75%AgNP-25%Sp	30	13.33	53.33	86.67	86.67	80.903	0.00
50%AgNP-50%Sp	30	6.67	46.67	86.67	93.3	102.163	0.00
25%AgNP-75%Sp	30	–20	20	53.33	100	166.222	0.00

Nc: repellency record, #: number of adults added to each Petri dish.

T. tabaci, which impact onions and garlic, are commonly managed with synthetic pesticides, but long-term use causes resistance and pesticide residues on food. Furthermore, they can be costly [63]. Concerns about excessive chemical use have prompted farmers to seek eco-friendly alternatives, such as nanotechnology. Nanoparticles have several advantages, including lower toxicity, improved stability, and increased effectiveness through enhanced absorption [64]. The results revealed the pesticidal potential of green-synthesized AgNPs mixed with a commercial pesticide against *T. tabaci* at various (AgNP–Spinosad) mixing ratios, with a focus on enhancing efficacy with lower pesticide doses. Possible explanations are: AgNPs kill pests by penetrating their skin and binding with proteins,

sulphur, and phosphorus, ultimately leading to hypoxia [65–68]. The presence of Ag⁺ ions disrupts the cell surface, interferes with metabolism, and induces oxidative stress, resulting in cell death [69,70]. Many physical and chemical factors could affect the biological activity of AgNPs, including particle size, surface properties, and the presence of bioactive phytochemicals in *T. polium* extract, which act as reducing and stabilizing agents. It has been established that smaller-sized AgNPs have a more pronounced biological activity due to their larger surface area and better contact with biological membranes [71]. The phytochemicals on the surface of AgNPs may contribute to their overall toxicity and stability [72].

At a maximum concentration of 0.73 mg/mL, AgNPs were effective against *T. tabaci* larvae and adults. Combining AgNPs with Spinosad increased mortality, with all combinations achieving 100% mortality by the third day in laboratory conditions. AgNPs alone caused 88.99% mortality in larvae and 40.28% in adults, while Spinosad alone showed 100% efficacy. In greenhouse conditions, most treatments maintained high mortality by day 7, except for AgNPs alone and some combinations, which showed reduced efficacy. In open fields, mortality was high by day three but declined over a week due to the uncontrolled environmental conditions. While Spinosad is low in toxicity, higher concentrations may harm non-target organisms. According to many studies, the quick decomposition of Spinosad did not influence the pest's reproductive factors [73,74]. Also, certain pests developed resistance to it [75].

The findings show that combining AgNPs and Spinosad proves highly effective at controlling *T. tabaci* under controlled laboratory conditions. However, the effectiveness of AgNPs alone appears to decrease over time, especially in more unpredictable environments like greenhouses and open fields. This shows that, while AgNPs are beneficial, their persistence and stability may be impaired outside controlled environments. To overcome this disadvantage, future formulations of AgNPs could incorporate coating or capping agents, carriers such as polymer matrices or silica, or slow-release formulations to enhance stability, prevent agglomeration, and prolong antibacterial activity under various external conditions [71,76]. Spinosad, on the other hand, consistently obtained high mortality, proving its superior efficacy. However, because of the possible toxicity to non-target organisms and the development of pest resistance, it should be used with caution. Combining AgNPs with Spinosad may address these difficulties by lowering the needed Spinosad concentration, lessening the risk to non-target species and potentially delaying resistance development. While some AgNPs and Spinosad combinations exhibited mortality rates similar to the application of the Spinosad alone, in this study, the minimum lethal concentration of Spinosad alone was not identified. Thus, the definitive synergistic effect between AgNPs and Spinosad cannot be confirmed. Further studies, where various Spinosad concentrations are used, would be required to establish its possible interactions with AgNPs.

The findings of the authors of [40] are also relevant to current research since the authors observed and compared the insecticidal efficacy of nanoparticles with commercial insecticide malathion against *Tribolium castaneum*, with the results indicating that after nine days, the combination of malathion and AgNPs shows 100% mortality at the highest concentration. At a 50 mg concentration, AgNPs show 75% mortality, while malathion showed 95% mortality. This notion was further corroborated by the authors of [77,78], who found the pesticide action of synthesized AgNPs utilizing *Euphorbia prostrata*, achieving total mortality (100%) after seven days against *Sitophilus oryzae*. The study demonstrates that green-synthesized AgNPs efficiently control *T. tabaci* larvae and adults after prolonged exposure. Combining AgNPs with pesticides provides a more efficient and cost-effective way to manage pesticide-resistant pests.

The authors of [40] investigated the repellent activity of synthetic AgNPs, malathion, and a combination of the two against *Tribolium castaneum*. The results revealed that the

peak repellency was observed after two hours of exposure. Repellence decreases over time. They reported that the mixture of AgNPs and Malathion had 100% repellence at greater concentrations through the experiment. Moreover, in all cases at high concentrations showed more than 50% repellence.

The Dose-Reduction Potential between green-synthesized AgNPs and Spinosad reflects a significant advancement in nano-enabled pesticides, optimizing both ecological safety and pest mortality. Our recent work results align with recent studies demonstrating the potent insecticidal efficacy of nano-silver against the potato tuber moth, *Phthorimaea operculella* and the two spotted spider mite, *Tetranychus urticae* [79,80]. By using botanical extracts, this tactic ensures high hemocompatibility and reduced environmental negative impacts, mirroring the safety profiles of optimized nanoparticles' systems [81,82]. Ultimately, this integrated strategy provides a sustainable mechanism for thrips control [83], preserving the integrity of the garlic–soil system.

4. Conclusions

The results of this study show that the synthesized silver nanoparticles (AgNPs) are round and have strong antibacterial properties. These AgNPs were found to improve photosynthetic pigments when used on garlic plants, which suggests that they have a positive effect on physiology. In addition to promoting plant growth, the produced AgNPs demonstrated substantial pesticidal efficacy against *T. tabaci*, proving especially effective against insect populations that have developed resistance to commercial products. A significant finding of this research is the synergistic potential of combining AgNPs with the insecticide Spinosad (Spintor), which signifies a promising strategy for Integrated Pest Management (IPM).

The AgNPs-Spinosad combination was able to kill as many insects as the full-strength insecticide, even when the concentration of the pesticide was lower. This decrease indicates a feasible approach for diminishing chemical dependence and alleviating related environmental risks. These combinations look very promising, but more research is needed to figure out exactly how AgNPs and Spinosad interact. Future research must concentrate on establishing LC50 values and delineating particular synergistic coefficients to thoroughly optimize these reduced-dose formulations as a global solution for sustainable pest resistance management.

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Abbreviations

The following abbreviations are used in this manuscript:

AgNPs	Silver nanoparticles
DLS	Dynamic light scattering
ELISA	Enzyme-linked immunosorbent assay
GC-MS	Gas chromatography–mass spectrometry
IYSV	Iris yellow spot virus
MIC	Minimum inhibitory concentration
PDI	Polydispersity index
SEM	Scanning Electron Microscope
XRD	X-ray diffraction

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