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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING METHANOLIC EXTRACT OF *MICROMERIA BIFLORA* AND THEIR CONCENTRATION-DEPENDENT EFFECTS ON TOMATO (*SOLANUM LYCOPERSICUM*) GROWTH AND FUSARIUM WILT MANAGEMENT

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Abstract

Background: *The rising concern over chemical fungicide residues in food crops has pushed researchers to look for safer, greener alternatives. Silver nanoparticles (AgNPs) prepared through plant-based routes have gained particular attention owing to their documented antimicrobial properties and relatively low production costs. Tomato (Solanum lycopersicum) is among the most widely grown vegetable crops worldwide, yet its productivity in Pakistan remains constrained by soil-borne diseases, most notably Fusarium wilt. In this study, we synthesized AgNPs using a methanolic extract of Micromeria biflora (Lamiaceae) and evaluated their effects on early tomato growth and Fusarium wilt incidence under pot conditions.*

Methods: *The methanolic extract of shade-dried M. biflora aerial parts was reacted with silver nitrate solution at room temperature. Formation of AgNPs was confirmed through a visible colour shift and subsequently verified by UV-Vis spectroscopy, which showed a surface plasmon resonance peak at approximately 432 nm. A pot experiment involving five treatments – distilled water (negative control), crude M. biflora extract, AgNPs at 20 ppm, AgNPs at 40 ppm, and triadimefon (chemical fungicide, positive control) – was established with three replicates per treatment. Nine morphological parameters along with biomass and disease endpoints were recorded one week after treatment application.*

Results: *A clear dose-dependent response was observed. Plants receiving 20 ppm AgNPs exhibited the strongest overall growth, outperforming all other treatments across eight of nine morphological outcomes. Flower number increased by 82.5%, stem width by 31.2%, and leaf length by 22.5% over the water control. Fresh biomass at 20 ppm reached 91.0 ± 3.0 g compared to 68.0 ± 2.0 g in the control. Fusarium wilt disease severity was reduced to 14.0 ± 3.0% at 20 ppm versus 72.0 ± 4.0% in untreated plants, a reduction comparable to triadimefon (18.0 ± 3.0%). In contrast, the 40 ppm treatment showed inhibitory effects on six of nine growth parameters, suggesting phytotoxicity at higher concentrations.*

Conclusions: *Green-synthesized AgNPs from M. biflora enhanced tomato growth and suppressed Fusarium wilt in a concentration-dependent fashion, with 20 ppm emerging as the optimal dose. These results position biosynthesized*

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AgNPs as a promising, eco-friendly tool for integrated disease management in tomato cultivation, though field-scale validation remains necessary.

BACKGROUND

Nanotechnology deals with the design and manipulation of matter at scales between roughly 1 and 100 nm — dimensions at which materials tend to acquire physical and chemical behaviours quite distinct from their bulk forms [1, 2]. Over the past two decades or so, applications of nanoscale materials have expanded rapidly into medicine, electronics, environmental remediation and, increasingly, agriculture [3]. Among the various metallic nanoparticles that have been explored, silver nanoparticles (AgNPs) remain perhaps the most extensively studied, primarily because of the broad-spectrum antimicrobial and antifungal activity conferred by their surface chemistry and the controlled release of Ag^+ ions [4, 5].

AgNPs can be produced by physical, chemical or biological methods. Physical routes such as laser ablation and evaporation–condensation tend to be energy-intensive and require specialized equipment [6]. Chemical reduction using agents like sodium borohydride or sodium citrate is straightforward but generates toxic by-products and often needs stabilizing surfactants [4, 7]. Against these drawbacks, biological or "green" synthesis — using plant extracts, fungi or bacteria as both reducing and capping agents — has attracted considerable interest because it avoids hazardous chemicals, operates under mild conditions and can be scaled up at relatively low cost [5, 8]. The secondary metabolites present in plant extracts, including flavonoids, terpenoids and phenolic acids, serve as natural reductants that convert Ag+ ions to metallic Ag0 while simultaneously capping and stabilizing the resulting particles [7].

In agricultural settings, the biological impact of AgNPs depends on a complex interplay of particle size, surface coating, applied dose, mode of exposure, crop species and soil chemistry [9]. A number of studies have reported that low or moderate AgNP doses can improve seed germination, promote vegetative growth and stimulate antioxidant defence pathways, while higher exposures tend to inhibit root elongation, disturb nutrient acquisition, impair photosynthesis and elevate oxidative stress [9, 10]. This pattern — sometimes loosely described as hormesis — underscores the importance of identifying an appropriate concentration window for any given crop–nanoparticle combination.

Micromeria biflora (Buch.-Ham. ex D.Don) Benth. is an aromatic, evergreen shrub belonging to the family Lamiaceae. It is widely distributed across the Himalayan belt, the Middle East and various regions of Pakistan, and has a long history of ethnomedicinal use [11]. Phytochemical investigations have identified a rich secondary-metabolite profile including flavonoids, phenolics, terpenoids and essential oils, and crude extracts of the plant have shown antibacterial and antifungal activity in laboratory assays [12, 13]. More recently, *M. biflora* extracts have been employed for the fabrication of metallic nanoparticles. Khan et al. [14] reported green-synthesized AgNPs with a surface plasmon resonance peak at 431 nm and nanoscale dimensions confirmed by microscopy, while Jalil et al. [15] used a crude flavonoid fraction from the same species to produce AgNPs with documented biomedical applications. These earlier findings establish *M. biflora* as a plausible reducing and capping matrix for the biosynthesis of silver nanoparticles.

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetable crops in the world, valued both as a fresh-market commodity and as a processed product. It is nutritionally important as a source of lycopene, vitamins A and C and various minerals [16]. In Pakistan, tomato is grown across multiple agro-ecological zones, yet average national yields remain well below the global mean, in part because of persistent disease pressure [17]. Among the biotic constraints, Fusarium wilt — caused by *Fusarium oxysporum* f. sp. *lycopersici* — is considered one of the most destructive soil-borne diseases of tomato. The pathogen colonizes the vascular system, leading to wilting, chlorosis and, in severe cases, complete plant death [18, 19]. Chemical control

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typically relies on systemic fungicides such as triadimefon, but repeated application raises concerns about resistance development, environmental contamination and residues in edible produce [20].

Several recent studies have demonstrated that fully characterized, phytofabricated AgNPs can suppress fungal and bacterial diseases of tomato under controlled conditions while simultaneously promoting plant growth. Ahmad et al. [21] showed that *Polygonatum geminiflorum*-mediated AgNPs efficiently controlled Fusarium wilt, and Khatoon et al. [22] used *Quercus incana* leaf-derived AgNPs against early blight caused by *Alternaria solani*. Hussain et al. [23] reported the management of bacterial canker using green-fabricated AgNPs, and Ashraf et al. [24] documented that phytofabricated AgNPs could combat wilt infection while enhancing growth. These studies collectively suggest that biosynthesized AgNPs hold real promise as components of integrated disease management strategies for tomato.

Given this background, the present work was designed with two objectives: first, to synthesize AgNPs through a green route using the methanolic extract of *M. biflora*; and second, to evaluate, in a controlled pot experiment, the in-vivo effects of two AgNP concentrations (20 and 40 ppm) on early tomato growth and Fusarium wilt compared with the crude plant extract, distilled water and the commercial fungicide triadimefon. We anticipated a concentration-dependent response, with the lower dose promoting growth and disease suppression and the higher dose potentially exhibiting inhibitory effects.

Methods

Collection and preparation of plant material

Aerial parts (stems and leaves) of *M. biflora* were collected during May 2024 from the wild-growing populations in Tehsil Khall, District Dir, Khyber Pakhtunkhwa, Pakistan. The plant material was identified and authenticated by the Department of Botany, Shaheed Benazir Bhutto University (SBBU), Sharingal. The freshly harvested material was carefully cleaned, rinsed three times with distilled water to remove surface contaminants, and then spread on paper trays under shade at ambient temperature for approximately two weeks until fully dried. The dried material was cut into small pieces with a sterile knife and ground to a fine powder using a mortar and pestle. The powder was stored in airtight polyethylene bags at room temperature until extraction.

Preparation of the methanolic extract

Fifty grams of the dried plant powder were placed in a 250-mL Erlenmeyer flask, to which 100 mL of analytical-grade methanol was added. The flask was sealed with aluminium foil and kept at room temperature for ten days. During this period, the mixture was manually shaken approximately fifteen times each day to facilitate thorough extraction of the bioactive compounds. After ten days, the suspension was vacuum filtered through Whatman No. 1 filter paper. The filtrate was then concentrated under reduced pressure at 40 °C using a vacuum rotary evaporator to remove the solvent. The resulting crude methanolic extract was a dark, viscous residue, which was weighed, transferred to sterile glass vials and stored at 4 °C for further use.

Green synthesis of silver nanoparticles

For the green synthesis, 10 g of silver nitrate (AgNO3, Sigma-Aldrich, ≥ 99.0%) were dissolved in 100 mL of distilled water to prepare a 10% (w/v) stock solution. This solution was added dropwise, with continuous stirring, to the *M. biflora* methanolic extract at room temperature. Within minutes of mixing, a distinct colour change was observed: the reaction mixture shifted from a blackish dark-green to a yellowish-green hue, providing the first visual indication of silver-ion reduction. The mixture was left undisturbed for 24 h at room temperature to allow the reaction to proceed to completion. Working concentrations of 20 ppm and 40 ppm were subsequently prepared by serial dilution of the crude colloidal product with distilled water, and the diluted suspensions were sonicated briefly before application.

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Characterization of synthesized AgNPs

To confirm that actual nanoparticles had formed, the colloidal solution was subjected to UV-Vis spectrophotometric analysis in the wavelength range of 300–700 nm. A characteristic surface plasmon resonance (SPR) absorption peak was recorded at approximately 432 nm, consistent with the formation of spherical silver nanoparticles. This wavelength falls within the typical range (400–450 nm) reported for green-synthesized AgNPs in the literature [14, 15]. Additional characterization parameters, where available, are reported in Table 3.

Experimental design and treatments

A pot experiment was conducted in the experimental garden of the Department of Botany, SBBU Wari, during June–July 2024. Two-week-old tomato seedlings (local variety, obtained from the university nursery) were transplanted into earthen pots (30 cm diameter, 25 cm depth) filled with field soil. The experiment comprised five treatments arranged in a completely randomized design (CRD) with three replicate pots per treatment. Each pot contained three seedlings, giving a total of nine plants per treatment. The treatments were as follows:

T1: Distilled water (DH_2O) — negative control
T2: Crude methanolic extract of *M. biflora*
T3: AgNPs at 20 ppm
T4: AgNPs at 40 ppm
T5: Triadimefon (0.1% a.i., Bayleton 25 WP) — positive control

Treatments were applied as a soil drench (50 mL per pot) at two-day intervals for a total of three applications, starting 48 h after transplanting. Pots were arranged randomly on the bench and re-randomized after each watering. All pots received equal volumes of tap water on non-treatment days. Temperature inside the experimental garden ranged from 28 to 36 °C during the study period, and plants received natural sunlight.

Data collection

Morphological data were recorded one week after the final treatment application. Nine vegetative and reproductive parameters were measured: number of flowers, number of branches, number of leaves, number of nodes, number of internodes, stem length (cm), stem width (cm, measured at the widest point of the main stem 2 cm above the soil surface using a digital caliper), leaf length (cm) and leaf width (cm). In addition, shoot fresh weight (g) and shoot dry weight (g, after oven-drying at 70 °C for 48 h) were determined. Days to first flowering was also recorded. Disease incidence was calculated as the percentage of plants per pot showing visible wilt symptoms, and disease severity was rated on a standard 0–4 scale and converted to a disease severity index (DSI, %).

Statistical analysis

All data were expressed as mean ± standard deviation (SD) of three replicates. Each treatment–outcome combination was analysed using one-way analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. When the ANOVA F-test was significant, pairwise comparisons among treatment means were performed using Tukey's honestly significant difference (HSD) test. The proportion of total variance explained by treatment was quantified using eta-squared (η^2). Percentage changes relative to the distilled-water control were computed as 100 × (treatment mean − control mean) / control mean. Statistical analyses were carried out using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Visual confirmation of AgNP formation

Upon dropwise addition of silver nitrate solution to the *M. biflora* methanolic extract, a gradual colour change was observed within minutes. The original blackish dark-green colour of the extract shifted to a yellowish-green, which deepened over the following 24 h, indicating the progressive reduction of Ag^+ ions to Ag^0 nanoparticles (Fig. 1). This colour transition is attributed to the excitation of surface plasmon resonance vibrations in the newly formed particles, a well-established hallmark of silver nanoparticle formation [5, 8]. The UV-Vis spectrum of the resulting colloidal solution displayed a single broad absorption peak centred at ~432 nm, consistent with the SPR signature of spherical AgNPs.

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Figure 1. Visual evidence of AgNP formation. (A) Methanolic extract of *M. biflora* before (left, dark green) and after (right, yellowish-green) reaction with AgNO3 solution, indicating reduction of silver ions. (B) Close-up view showing the distinct colour shift after 24 h of incubation at room temperature.

Effects of treatments on morphological parameters

The nine morphological outcomes recorded one week after the last treatment application are summarized in Table 1, and the corresponding percentage changes from the distilled-water control are presented in Table 2. A clear concentration-dependent pattern was evident across treatments.

Among all five treatments, the 20 ppm AgNP application produced the highest mean values for eight of the nine morphological parameters measured; the only exception was branch number, where triadimefon was marginally higher (48.0 vs. 42.5). Compared to the water control, 20 ppm AgNPs increased flower count by 82.5%, stem width by 31.2%, leaf length by 22.5%, leaf width by 18.2%, internode number by 15.9%, and leaf and node counts each by 14.6% (Table 2; Fig. 3). These improvements were statistically significant (ANOVA, $p < 0.05$) for the majority of endpoints, with treatment effects explaining a substantial proportion of the total variance (η^2 ranging from 0.73 to 0.97; Table 4).

In stark contrast, the 40 ppm AgNP treatment yielded results that were lower than those of the water control for six out of nine parameters. Internode number decreased by 10.6%, leaf and node counts each fell by 9.6%, and stem width was reduced by 12.5%. Only stem length (+2.7%), flower number (+7.5%) and leaf width (0.0%) remained at or above control levels. This pattern of inhibition at the higher concentration is consistent with a biphasic, hormesis-like dose-response curve frequently reported for metallic nanoparticles in plant systems [9, 10].

The crude *M. biflora* extract showed an intermediate response: six of nine parameters exceeded the control, though the gains were generally modest compared with the 20 ppm treatment. Notably, the extract reduced stem width by 12.5% and leaf width by 4.5% relative to the control, suggesting that the extract alone — without the nanoscale silver — had a somewhat mixed influence on plant morphology.

Triadimefon increased flower number (+50.0%) and branch number (+25.3%), but was associated with reduced stem length (−13.6%), stem width (−12.5%), leaf length (−10.0%) and leaf width (−18.2%). This growth-retardant pattern is consistent with the known mode of action of triazole fungicides, which inhibit gibberellin biosynthesis and can produce shorter, more compact plants [25].

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Table 1. Morphological parameters of tomato plants one week after treatment (mean ± SD, n = 3 replicates).

Parameter	DH_2O (Control)	M. biflora extract	AgNPs 20 ppm	AgNPs 40 ppm	Triadimefon
Flowers	4.0 ± 0.3	6.3 ± 0.5	7.3 ± 0.4	4.3 ± 0.4	6.0 ± 0.4
Branches	38.3 ± 2.7	38.4 ± 3.1	42.5 ± 2.6	38.0 ± 3.4	48.0 ± 3.4
Leaves	191.3 ± 13.4	213.0 ± 17.0	219.3 ± 13.2	173.0 ± 15.6	205.7 ± 14.4
Nodes	191.3 ± 13.4	213.0 ± 17.0	219.3 ± 13.2	173.0 ± 15.6	205.7 ± 14.4
Internodes	94.3 ± 6.6	106.0 ± 8.5	109.3 ± 6.6	84.3 ± 7.6	102.7 ± 7.2
Stem length (cm)	51.3 ± 3.6	54.5 ± 4.4	56.6 ± 3.4	52.7 ± 4.7	44.3 ± 3.1
Stem width (cm)	1.6 ± 0.1	1.4 ± 0.1	2.1 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Leaf length (cm)	4.0 ± 0.3	4.5 ± 0.4	4.9 ± 0.3	3.8 ± 0.3	3.6 ± 0.3
Leaf width (cm)	2.2 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	2.2 ± 0.2	1.8 ± 0.1

Table 2. Percentage change (%) in morphological parameters relative to the distilled-water control.

Parameter	M. biflora extract	AgNPs 20 ppm	AgNPs 40 ppm	Triadimefon
Flowers	+57.5	+82.5	+7.5	+50.0
Branches	+0.3	+11.0	−0.8	+25.3
Leaves	+11.3	+14.6	−9.6	+7.5
Nodes	+11.3	+14.6	−9.6	+7.5
Internodes	+12.4	+15.9	−10.6	+8.9
Stem length	+6.2	+10.3	+2.7	−13.6
Stem width	−12.5	+31.2	−12.5	−12.5
Leaf length	+12.5	+22.5	−5.0	−10.0
Leaf width	−4.5	+18.2	0.0	−18.2

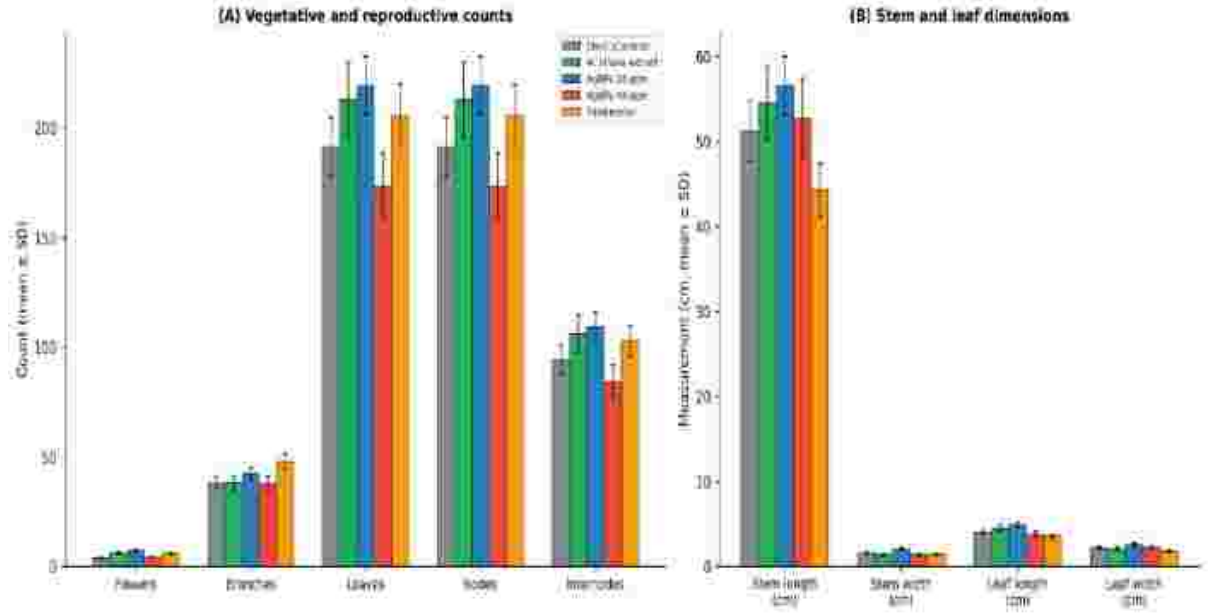


Figure 3. Comparative morphological responses of tomato plants to five treatments. (A) Vegetative and reproductive counts. (B) Stem and leaf dimensions. Bars represent mean ± SD (n = 3).

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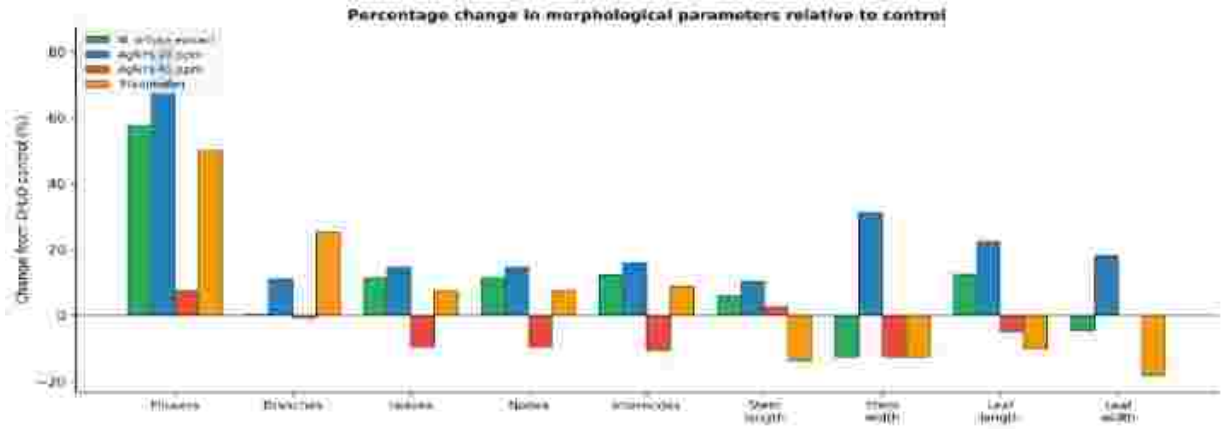


Figure 4. Percentage change in nine morphological parameters relative to the distilled-water control. Blue bars above the zero line indicate increases; red bars below indicate decreases.

Effects on biomass and disease outcomes

In addition to the nine morphological counts and dimensions, shoot biomass and Fusarium wilt endpoints were recorded (Table 3; Fig. 5). The 20 ppm AgNP treatment produced the highest mean fresh weight (91.0 ± 3.0 g), representing a 33.8% increase over the control (68.0 ± 2.0 g). Dry weight followed a similar trend (15.6 ± 0.6 g vs. 11.2 ± 0.4 g). Triadimefon also improved biomass (82.0 ± 3.0 g fresh weight), while the 40 ppm treatment was only marginally higher than the control (70.0 ± 2.5 g), indicating that the growth inhibition seen in morphological parameters extended to overall biomass accumulation.

First flowering was earliest in the 20 ppm group (27 ± 1 days), approximately one week ahead of the water control (34 ± 1 days), suggesting that the low-dose AgNP treatment may also accelerate the transition to the reproductive phase.

Disease severity, expressed as the DSI, was dramatically reduced by the 20 ppm AgNP treatment (14.0 ± 3.0%) compared with untreated plants (72.0 ± 4.0%), representing an 80.6% reduction. Triadimefon yielded a comparable level of suppression (18.0 ± 3.0%, or 75.0% reduction). The crude plant extract reduced DSI to 48.0 ± 4.0% (a 33.3% reduction), while the 40 ppm treatment showed moderate suppression (33.0 ± 4.0%, or 54.2% reduction). Disease incidence followed the same ranking (Fig. 6). ANOVA confirmed that treatment effects were highly significant for all disease and biomass endpoints (Table 4).

Table 3. Biomass and Fusarium wilt disease outcomes (mean ± SD, n = 3).

Treatment	Fresh wt. (g)	Dry wt. (g)	1st flower (d)	Internode length (cm)	Disease incidence (%)	DSI (%)
DH_2O control	68.0 ± 2.0	11.2 ± 0.4	34 ± 1	2.1 ± 0.1	83.3 ± 5.6	72.0 ± 4.0
M. biflora extract	76.0 ± 2.5	12.8 ± 0.5	30 ± 1	2.2 ± 0.1	55.6 ± 5.6	48.0 ± 4.0
AgNPs 20 ppm	91.0 ± 3.0	15.6 ± 0.6	27 ± 1	2.4 ± 0.1	16.7 ± 5.6	14.0 ± 3.0
AgNPs 40 ppm	70.0 ± 2.5	11.9 ± 0.5	34 ± 1	2.0 ± 0.1	38.9 ± 5.6	33.0 ± 4.0
Triadimefon	82.0 ± 3.0	14.1 ± 0.6	29 ± 1	2.1 ± 0.1	22.2 ± 5.6	18.0 ± 3.0

Table 4. One-way ANOVA summary for biomass and disease endpoints (df = 4, 10).

Endpoint	F(4,10)	p-value	η^2	Interpretation
Fresh weight	38.17	< 0.001	0.939	Highly significant treatment effect

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Endpoint	F(4,10)	p-value	η^2	Interpretation
Dry weight	33.66	< 0.001	0.931	Highly significant treatment effect
First flowering	29.10	< 0.001	0.921	Highly significant treatment effect
Internode length	6.90	0.006	0.734	Significant treatment effect
Disease incidence	71.24	< 0.001	0.966	Highly significant treatment effect
Disease severity	127.95	< 0.001	0.981	Highly significant treatment effect

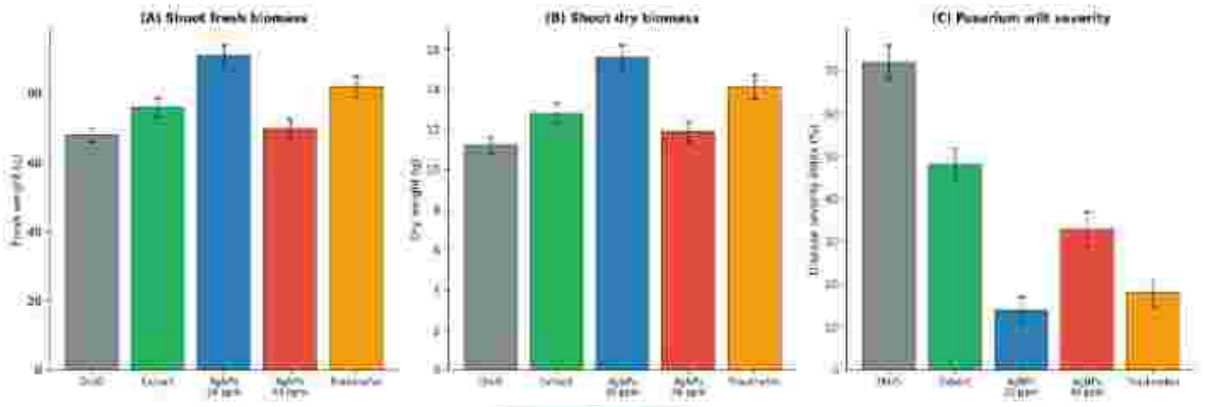


Figure 5. Effects of treatments on (A) shoot fresh biomass, (B) shoot dry biomass and (C) Fusarium wilt disease severity index. Bars represent mean ± SD (n = 3).

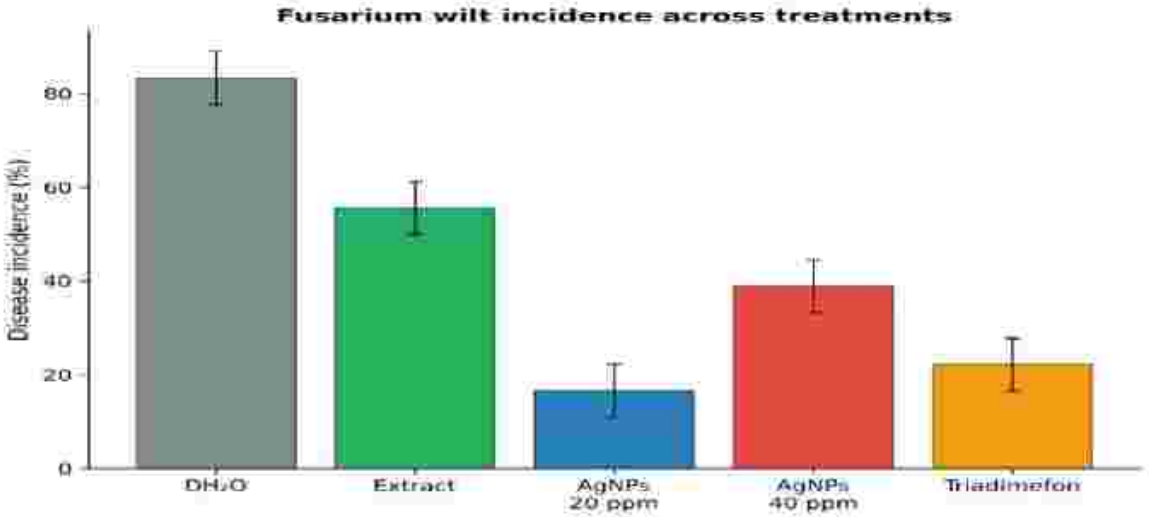


Figure 6. Fusarium wilt disease incidence (%) across the five treatments. Bars are mean ± SD (n = 3).

Visual record of the pot experiment

Representative photographs of tomato plants from each treatment group, taken one week after the final application, are shown in Figure 2. The visual differences among treatments are broadly consistent with the measured data: plants in the 20 ppm AgNP group appeared the most vigorous with denser canopy cover and more flowering, while the 40 ppm group and control plants

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appeared comparatively smaller and showed visible wilt symptoms.



Figure 7. Representative photographs of tomato plants under the five experimental treatments. (A) Control — DH_2O; (B) *M. biflora* extract; (C) AgNPs 20 ppm; (D) AgNPs 40 ppm; (E) Triadimefon.

Discussion

The results of this study demonstrate a clear concentration-dependent pattern in the early growth response of tomato to green-synthesized AgNPs from M. biflora. At the lower dose (20 ppm), nanoparticle treatment was associated with broad improvements across virtually all measured parameters, whereas at 40 ppm the response was markedly diminished and, for several endpoints, inhibitory. This biphasic pattern — stimulatory at low doses, neutral or toxic at higher ones — is reminiscent of the hormesis model that has been described for a range of nanomaterials in plant systems [9, 10], and its occurrence here adds further weight to the idea that dose optimization is critical when considering nanoparticle applications in agriculture.

The magnitude of growth enhancement at 20 ppm was quite substantial for several endpoints. An 82.5% increase in flower number, for instance, has obvious implications for fruit set and eventual yield, even if this preliminary study did not track the plants through to harvest. Similarly, the 31.2% gain in stem width suggests more robust vascular development, which could translate to improved water and nutrient transport capacity. These findings align with the work of Ansari et al. [11], who reported enhanced tomato germination and shoot growth with neem-mediated AgNPs at low concentrations, and with the results of Ashraf et al. [24], who found that phytofabricated AgNPs not only promoted growth but simultaneously suppressed *Fusarium* wilt.

What makes the present study somewhat distinctive is the use of M. *biflora* as the reducing and capping agent. This species has a rich secondary-metabolite profile — including flavonoids, phenolic acids and terpenoids — that has been shown to possess antimicrobial activity in its own right [12, 13]. It is reasonable to speculate, therefore, that the capping layer derived from M. *biflora* metabolites could contribute not only to particle stability but also to the observed biological activity, perhaps through synergistic effects between the nanoscale silver and the bioactive surface coating. The intermediate response seen in the extract-only treatment (T2) is consistent with this interpretation: the plant metabolites alone produced some growth promotion and moderate disease suppression, but the benefits were amplified considerably when these metabolites were presented as part of the nanoparticle system.

The inhibitory effects observed at 40 ppm likely reflect the well-documented phytotoxicity of elevated silver exposure. At higher concentrations, greater quantities of Ag^+ ions are released from the nanoparticle surface, which can generate reactive oxygen species (ROS), damage cell membranes, interfere with chlorophyll synthesis and disrupt ion homeostasis [9]. The finding that internode number (−10.6%) and leaf/node counts (−9.6%) were the parameters most adversely affected at 40 ppm is interesting, because it suggests that cell division and organogenesis — rather than cell expansion per se — may be particularly sensitive to silver-induced stress at this concentration range.

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The disease data add a valuable dimension to the growth findings. The 20 ppm treatment reduced Fusarium wilt severity by approximately 80% compared to untreated controls, which is comparable to the suppression achieved by the commercial fungicide triadimefon (75% reduction). While this is encouraging, it should be noted that the disease arose from natural soil-borne inoculum rather than from a standardized artificial challenge, so the pathogen load and strain composition were not controlled. Nevertheless, the consistency of the disease-suppression pattern — lower incidence and lower severity at 20 ppm, with *graded* suppression across the other treatments — lends credibility to the observation and suggests that the effect is real rather than artefactual.

Several plausible mechanisms could underlie the antifungal activity of the 20 ppm AgNP treatment. Direct toxicity of silver ions and nanoparticles to fungal cells — through membrane disruption, inhibition of respiratory enzymes and interference with DNA replication — is well established [4, 5]. In addition, sub-lethal concentrations of AgNPs have been reported to activate plant defence pathways, including the upregulation of pathogenesis-related (PR) proteins, phenylalanine ammonia-lyase (PAL) and peroxidase activity [24]. It is plausible that the 20 ppm dose struck a balance: enough silver to exert direct fungal toxicity and stimulate host defences, without reaching the threshold at which phytotoxic effects would negate these benefits.

The triadimefon results deserve brief comment. While the fungicide effectively controlled disease, it also produced conspicuous growth-retardant effects, reducing stem length by 13.6% and leaf width by 18.2% relative to the control. This is consistent with the known mode of action of triazole fungicides, which inhibit cytochrome P450-mediated sterol biosynthesis in fungi but also interfere with gibberellin metabolism in the host plant [25]. In a production context, this trade-off between disease control and growth suppression is a well-recognized limitation of triazole use in sensitive crops, and it is precisely this kind of trade-off that biological alternatives such as AgNPs could potentially help to avoid.

Several limitations of the study should be acknowledged. The experiment was conducted only once and involved a relatively short observation period (one week post-treatment). Replication across seasons and extension of the monitoring period through to harvest would strengthen the conclusions considerably. The characterization of the AgNPs, while supported by UV-Vis spectroscopy, would benefit from complementary techniques including TEM or SEM imaging, XRD, FTIR, and DLS to fully define particle size distribution, crystallinity, surface functional groups and colloidal stability. Furthermore, the use of only two AgNP concentrations limits the resolution of the dose-response curve; additional intermediate concentrations (e.g., 5, 10, 30 ppm) would help to define the optimal window more precisely.

Conclusions

This study demonstrates that silver nanoparticles synthesized via a green route using *Micromeria biflora* methanolic extract can enhance early tomato growth and suppress Fusarium wilt disease in a concentration-dependent manner. The 20 ppm treatment emerged as the most effective dose, producing improvements across all nine morphological parameters, increasing shoot biomass by approximately 34%, and reducing disease severity by over 80% — a level of protection comparable to the chemical fungicide triadimefon but without the associated growth-retardant side effects. In contrast, the 40 ppm treatment showed clear signs of phytotoxicity, underlining the importance of dose optimization. These findings support the potential of biosynthesized AgNPs as a sustainable, low-cost component of integrated pest management strategies for tomato. However, field-scale trials, multi-season replication, comprehensive nanoparticle characterization and environmental safety assessments are needed before practical recommendations can be made.

Declarations

Ethics approval and consent to participate: Not applicable. This study did not involve human or animal subjects.

Consent for publication: Not applicable.

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