



Comparative efficacy of silver nanoparticles from agro-industrial waste of cumin, anise, and banana peel against *Fusarium oxysporum* on common bean (*Phaseolus vulgaris*)

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Received: 27 October 2025 / Accepted: 11 April 2026 / Published online: 7 May 2026
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Abstract *Fusarium oxysporum*, the causal agent of root rot and vascular wilt in common bean (*Phaseolus vulgaris*), represents a substantial risk to legume cultivation. The current study offers a comparative examination of the antifungal efficacy of agro-industrial wastes from cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), and banana peel (*Musa paradisiaca*), and their green-synthesized silver nanoparticles (AgNPs), against *F. oxysporum* infecting

P. vulgaris. The biosynthesized AgNPs were found to be spherical with tiny sizes ranging from 20, 25, and 35 nm for cumin-AgNPs, anise-AgNPs, and banana peel-AgNPs, respectively. The agro-industrial waste extracts revealed a diverse range of bioactive compounds that served as reducing agents. *In vitro* antifungal activity revealed a dose-dependent suppression of *F. oxysporum* mycelial proliferation. The half-maximal effective concentrations (EC₅₀) for the crude extracts ranged from 125 to 200 µg/mL. AgNPs showed markedly improved EC₅₀ values, ranging from 62.5 µg/mL in cumin-AgNPs to 100 µg/mL in

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10658-026-03231-4>.

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banana-AgNPs. *In vivo* greenhouse experiments on infected *P. vulgaris* demonstrated that all treatments significantly decreased disease severity. Soil drench application of agro-industrial waste extracts reduced disease severity to 59.64% (cumin waste), 66.52% (anise waste), and 74.86% (banana peel), compared with 85.14% in the infected control. The AgNPs fabricated from agro-industrial wastes offered enhanced protection, decreased disease severity to 36.17% (cumin-AgNPs), 43.30% (anise-AgNPs), and 51.52% (banana peel-AgNPs). The applications significantly alleviated the physiological and oxidative stress caused by *F. oxysporum*. The cumin-AgNPs treatment significantly restored chlorophyll *a* and chlorophyll *b* content to 2.45 and 0.92 mg/g, respectively, comparable to the healthy control (2.50 and 0.95 mg/g) and much higher than the infected control (1.10 and 0.40 mg/g). Moreover, it triggered a defense response, markedly enhancing the activities of antioxidant enzymes, including peroxidase and polyphenol oxidase, in the treated plant compared with the infected control. The current findings clearly indicate that AgNPs synthesized from agro-industrial wastes could serve as effective, sustainable nanobiopesticides, surpassing the use of a single agro-industrial waste for the integrated management of *Fusarium* root rot and vascular wilt in common beans.

Keywords Biological control · Biopesticides · Disease suppression · Root rot management · Soil-borne plant pathogens

Introduction

The common bean (*Phaseolus vulgaris*) is particularly susceptible to soil-borne pathogens, including *Fusarium oxysporum*, which causes root rot and vascular wilt. *F. oxysporum* infections impair plant

health by altering vascular function and metabolism, ultimately resulting in yield losses that adversely affect socio-economic systems dependent on bean cultivation (Irvin et al., 2025).

Traditional disease control methods rely on synthetic fungicides. Concerns about environmental impact and the emergence of resistant fungal strains are increasingly limiting their long-term effectiveness (Fenta & Mekonnen, 2024).

These issues have driven interest in developing sustainable solutions that lower synthetic pesticide use and enhance plant resilience. In this context, biogenic synthesis of metal nanoparticles (NPs) from plant resources has emerged as a promising link between nanotechnology and green chemistry. Plant wastes, including seeds, peels, and stalk residues, usually discarded in agricultural processes, contain rich phytochemicals, polyphenols, flavonoids, alkaloids, and terpenes that act as reducing and capping agents, allowing eco-friendly methods for NPs production (Nyandoro et al., 2025).

Silver nanoparticles (AgNPs) exhibit notable antimicrobial activity. Their nanoscale size enables them to penetrate and interact with fungal membranes, damaging cellular structures, generating reactive oxygen species (ROS), and disrupting fungal enzyme functions (Jian et al., 2022). Furthermore, the phytochemicals coating these NPs, derived from plant extracts, can act synergistically to enhance antimicrobial activity, reduce NPs toxicity, and improve targeting accuracy.

Previous studies have shown how AgNPs combat various fungal pathogens in both *in vitro* and *in vivo* environments. For example, Ansari et al. (2023) used neem (*Azadirachta indica*) leaf extract to produce AgNPs that inhibited *Alternaria solani* (early blight) by 70–100% at 50 ppm while boosting defense enzymes. Abdelaziz et al. (2023) also synthesized AgNPs from *Ziziphus spina-christi* leaves, attaining 75% protection against *F. oxysporum* wilt and improving immunity. Meanwhile, Ashraf et al. (2025) demonstrated *Punica protacta*-based AgNPs reducing *Fusarium* wilt severity by up to 34.5% in field trials, alongside improved yield and defense gene expression.

Plant waste-derived AgNPs significantly enhance the activity of enzymatic antioxidants, including peroxidase (POD) and polyphenol oxidase (PPO), thereby helping detoxify ROS and reduce

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cellular oxidative damage (Asad et al., 2025). However, detailed studies on how plant waste-derived AgNPs affect host physiological and defense responses are still limited. In addition, species-specific differences in NPs effectiveness and plant responses necessitate more comprehensive comparative studies. Tackling these issues through research will help develop optimized, species-specific nano-products for sustainable agriculture.

The objective of the present study is to compare the effectiveness of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs to highlight the added value of nanotechnology in plant protection, through the following: (i) to synthesize and characterize AgNPs from agro-industrial wastes [cumin waste extract (CWE), anise waste extract (AWE), and banana peel extract (BPE)]; and evaluate their physicochemical properties; (ii) to assess the biological effectiveness of these materials (raw extracts and their AgNPs) by evaluating their antifungal activity against *F. oxysporum* and their ability to mitigate oxidative stress in common bean plants; and (iii) to evaluate and compare their impact on plant performance under biotic stress, including effects on photosynthetic pigments, biochemical constituents, and growth parameters. These aims help fill significant knowledge gaps by examining how plant waste-based nanomaterials can promote effective strategies for plant disease management. This integrated approach aligns with the growing global focus on sustainable intensification of agriculture, reducing reliance on chemical pesticides, and boosting resilience through innovative biotechnological solutions.

Materials and methods

Preparation of agro-industrial waste (cumin, anise, and banana peel)

Agro-industrial wastes of cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), and banana (*Musa paradisiaca*) peels were collected, thoroughly washed under running water to remove dirt and debris, and air-dried under shade at ambient temperature for 7 days to preserve thermolabile compounds.

The dried plant materials were ground to a fine powder using a mechanical grinder. For extraction, 50 g of each powder was soaked in 500 mL of 70% ethanol and stirred continuously at room temperature for 72 h. The extracts were then filtered through Whatman number 1 filter paper (Whatman, Maidstone, UK), and the filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Wang et al., 2018). The concentrated extracts were stored at 4°C for subsequent NPs synthesis and bioactivity assays, as recommended by Wang et al. (2018).

The green synthesis and characterization of AgNPs derived from CWE, AWE, and BPE can be found in the Supplementary Material file. In addition, the liquid chromatography-mass spectrometry (LC–MS) analysis of active chemicals found in the agro-industrial waste and the associated AgNPs is detailed in the Supplementary Material.

Release of active compounds from AgNPs fabricated from agro-industrial waste (cumin, anise, and banana peel)

Freeze-dried AgNPs (10 mg) were suspended in 20 mL phosphate-buffered saline (PBS, pH 7.4) and incubated at 37°C with agitation. Aliquots (1 mL) were collected at 0.5, 1, 2, 4, 8, 12, and 24 h, centrifuged at 15,000×g for 10 min, and the supernatants were analyzed via UV–Vis spectrophotometry (Shimadzu UV-1800, Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan) and LC–MS to quantify the released bioactive compounds (Weng et al., 2020).

Biological activity assays of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs

Samples of crude extracts (CWE, AWE, and BPE) and their corresponding green-synthesized AgNPs (CW-AgNPs, AW-AgNPs, BP-AgNPs) were each dissolved or suspended in 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) to obtain stock concentrations. These stocks were then serially diluted with distilled water to obtain final working concentrations of 25, 50, 100, and 200 µg/mL for antioxidant and antifungal activity assays.

Antioxidant assays of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Each sample (1 mL) at the desired test concentration was mixed with 1 mL of freshly prepared 0.1 mM DPPH solution in methanol. The mixture was vortexed briefly and incubated in the dark at room temperature for 30 min to minimize photo-degradation of the radical. After incubation, the absorbance was measured at 517 nm using a UV–vis spectrophotometer (Shimadzu Corporation). Methanol and DMSO blanks were included, and ascorbic acid was used as a positive control (Gecer, 2021).

The percentage of DPPH scavenging activity was calculated using the formula:

$$\%inhibition = [(A_{control} - A_{sample}) / A_{control}] \times 100\%$$

where $A_{control}$ is the absorbance of the control (DPPH + solvent) and A_{sample} is that of the test sample.

The half-maximal inhibitory concentration (IC_{50}) values were obtained by plotting the inhibition percentage against the log concentration and determining the concentration required to scavenge 50% of the DPPH radicals (Flieger et al., 2021).

Antifungal activity of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs

Agar well diffusion method To evaluate antifungal efficacy, potato dextrose agar (PDA) plates from Lab M Limited, Lancashire, United Kingdom, were inoculated with 100 μ L of a standardized fungal spore suspension (1×10^6 spores/mL) for each target pathogen: *F. oxysporum*, *Colletotrichum lindemuthianum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. After solidification, wells (8 mm diameter) were punched into the agar using a sterile cork borer.

Each well was loaded with 50 μ L of sample solution at the prepared concentrations (25, 50, 100, 200 μ g/mL). Control wells received the solvent only. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. The diameters of the inhibition zones around each well were measured in millimeters, representing the antifungal potency.

Broth microdilution assay for minimum inhibitory concentrations (MIC) determination MICs were determined following the CLSI M38-A2 guidelines for filamentous fungi (Espinel-Ingroff et al., 2011). Briefly, two-fold serial dilutions of each sample in Sabouraud dextrose broth (Lab M Limited) were prepared in 96-well microtiter plates. Each well received 100 μ L of sample at the correct concentration and 100 μ L of fungal spore suspension (final inoculum $0.5\text{--}2.5 \times 10^4$ CFU/mL).

Plates were incubated at 28°C for 48–72 h. MIC was defined as the lowest concentration of the test sample at which no visible fungal growth was observed. Each assay included positive (fungal-only) and negative (broth-only) controls, and all tests were performed in triplicate. The corresponding half-maximal effective concentrations (EC_{50}) were subsequently estimated from dose–response curves generated from the microdilution data using nonlinear regression (four-parameter logistic model) in GraphPad Prism.

Experimental design and treatments

Common bean (*P. vulgaris*) seeds were surface-sterilized by immersion in 2% sodium hypochlorite for 3 min, then thoroughly rinsed with sterile distilled water. After drying, the seeds were sown in 2-L plastic pots filled with autoclaved loamy sand. The pots were maintained in a greenhouse at $25 \pm 2^\circ\text{C}$ and 65% relative humidity, with a 16:8 h (day: night) photoperiod.

A total of eight drench treatment groups were established: (T1) healthy control (non-infected control, untreated); (T2) *F. oxysporum*-infected control (inoculated, untreated); (T3) inoculated with *F. oxysporum* + CWE; (T4) inoculated with *F. oxysporum* + AWE; (T5) inoculated with *F. oxysporum* + BPE; (T6) inoculated with *F. oxysporum* + CW-AgNPs; (T7) inoculated with *F. oxysporum* + AW-AgNPs; and (T8) inoculated with *F. oxysporum* + BP-AgNPs.

Freshly prepared fungal spore suspensions of *F. oxysporum* (1×10^6 spores/mL) were applied as a drench (10 mL per pot) at the seedling stage (7 days after sowing) to all treatments except the healthy control. At the same time, the corresponding treatments were administered as soil drench (for extracts/AgNPs: 50 mg/kg soil for each). All treatments and control groups consisted of at least three biological replicates (pots), each containing five plants. A randomized complete block design (RCBD) was strictly followed, with treatments

randomly allocated across the greenhouse benches to minimize location bias and ensure statistical rigor.

Assessment of disease severity under greenhouse conditions

Thirty days post-inoculation, plants were visually examined for symptoms of *Fusarium* wilt (leaf yellowing, wilting, vascular browning, necrosis, and plant collapse). Disease severity was rated using categorical scale (0–5), where: 0=no visible symptoms, 1=slight yellowing or wilting on <20% of leaves, 2=moderate chlorosis/wilting on 20–40% of leaves, 3=severe wilting on 40–60% of leaves, 4=plant collapse with >60% tissue affected, and 5=dead plant.

Disease severity percentage (DS%) for each pot was calculated using the formula (Mayee & Datar, 1986): There is no spacing in the equation, please correct it.

$$DS\% = \left(\frac{\sum (\text{rating} \times \text{number of plants per category})}{\text{total plants} \times \text{maximum rating}} \right) \times 100$$

Protection efficacy (PE%) was then computed as: There is no spacing in the equation, please correct it.

$$PE\% = \frac{DS\%_{\text{infected control}} - DS\%_{\text{treatment}}}{DS\%_{\text{infected control}}} \times 100$$

The rationale for selecting this specific time point in the present study is to ensure that all plants were exposed to the pathogen for the same duration. It allows the disease to progress from initial infection to observable symptoms, while remaining early enough to capture differences in resistance or treatment efficacy before complete plant collapse. In addition, the 0–5 scale provides sufficient categories to distinguish subtle differences between treatments (e.g., slight yellowing versus moderate wilting). This was also confirmed by other studies using a different scale (Kumar et al., 2022). All assessments were performed independently by two trained observers blinded to the treatments.

Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on *P. vulgaris* growth in the presence of *F. oxysporum*

Thirty days post-inoculation, plants were carefully uprooted, and growth parameters were recorded to assess the impact of treatments on *P. vulgaris*

growth. The height of the main stem from the soil surface to the apical meristem was measured with a tape measure and expressed in cm. To measure total leaf area, fully expanded leaves from each plant were collected and measured with a portable leaf area meter. The total leaf area per plant was calculated and expressed in cm².

All mature pods from each plant were manually counted at harvest, and the average number of pods per plant was calculated for each treatment. From each plant, ten representative pods were randomly selected, opened, and the number of seeds per pod was counted.

The length of the main root (from the root crown to the root tip) was measured using a ruler and expressed in cm. Above-ground plant parts (stems and leaves) were separated, placed in paper bags, and oven-dried at 70°C for 72 h until constant weight. Dried samples were weighed using an analytical balance, and shoot biomass was expressed in g per plant.

Roots were also carefully washed under running tap water to remove soil particles, placed in paper bags, and oven-dried at 70°C for 72 h. Dried roots were weighed, and root biomass was expressed in g per plant.

Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on antioxidant enzymes

Fresh leaf samples (0.5 g) were homogenized in phosphate buffer (pH 7), centrifuged, and the supernatants were used. POD activity was measured spectrophotometrically by recording the oxidation rate of guaiacol at 470 nm. PPO activity was assayed by measuring catechol oxidation at 495 nm (Haque et al., 2014).

Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on photosynthetic pigment content

Contents of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined in the leaves at harvest. A Shimadzu UV-2101/3101 PC scanning spectrophotometer (Shimadzu Corporation) was used to measure the

optical densities of chlorophyll *a* and chlorophyll *b* at 645 and 663 nm, respectively (Holden, 1965). Carotenoids were estimated at 470 nm (Davies, 1965).

Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on biochemical constituents of common bean

Carbohydrate quantification was performed by the phenol–sulfuric acid method as described by DuBois et al. (1956). Briefly, 0.5 mL of sample extract was mixed with 0.5 mL of 5% phenol solution in a glass tube, followed by rapid addition of 2.5 mL concentrated sulfuric acid. The mixture was vortexed, incubated at room temperature for 10 min, and then placed in a 30°C water bath for an additional 20 min. The resulting yellow-orange color was measured spectrophotometrically at 490 nm.

Glucose standard was used for calibration, and results were expressed as mg of glucose equivalent per g of fresh weight (FW). Total protein content was assayed using the Bradford method (Bradford, 1976). Samples (100 µL extract) were mixed with 5 mL Bradford reagent and vortexed. After 5 min incubation at room temperature, the absorbance was measured at 595 nm using a spectrophotometer. Bovine serum albumin (BSA) was used to generate the standard curve, and protein levels were reported as mg BSA equivalent per g FW.

Lipids were extracted using the Soxhlet method according to Floch et al. (1975). Approximately 1 g of dried ground bean tissue was placed in a thimble and extracted with 100 mL of petroleum ether for 6 h in a Soxhlet apparatus. After extraction, the solvent was removed by evaporation, and the residual lipids were collected and weighed directly to determine the gravimetric lipid content (mg/g dry weight).

Total phenolic content (TPC) was quantified by the Folin-Ciocalteu colorimetric assay (Singleton et al., 1999). In each assay, 0.5 mL plant extract was mixed with 2.5 mL Folin-Ciocalteu reagent (10% v/v) and 2.0 mL of 7.5% sodium carbonate. The mixture was incubated for 30 min at 40°C, and the absorbance was measured at 765 nm. Gallic acid was used as a reference standard, and results were expressed as mg gallic acid equivalents (GAE) per g FW.

Statistical analysis

Data were analyzed using ANOVA followed by Tukey's post hoc test at $P < 0.05$ for significance. All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard error (SE).

Results

Extraction yield of agro-industrial waste (cumin, anise, and banana peel)

The extraction yields from the three plant waste materials varied considerably. CWE exhibited the highest extraction yield (12.8 g extract/100 g dry waste), followed by AWE (9.4 g extract/100 g dry waste), while BPE showed the lowest yield (6.7 g extract/100 g dry waste).

These differences can be attributed to the varying solubility and availability of extractable bioactive compounds in each plant matrix.

The comprehensive characterization of green-synthesized AgNPs derived from CWE, AWE, and BPE is presented in Table S1 and Fig. S1. In addition, the active compound profiles of the examined agro-industrial waste and their AgNPs, as determined by LC–MS are presented in Table S2.

Biological activity of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs

Antioxidant activity

Fig. S2 presents a comparative analysis of antioxidant potency between plant waste-derived extracts and their corresponding biogenic AgNPs formulations, assessed by DPPH radical-scavenging activity.

The data demonstrated that NPs synthesis substantially enhances radical-scavenging capacity across all formulations, with improvements inversely proportional to the source extract's antioxidant baseline.

Across all three types, NPs formulation yielded statistically significant reductions in IC₅₀ values, with CW-AgNPs demonstrating the most substantial efficacy enhancement, a 19.3% improvement in antioxidant potency. The IC₅₀ decreased from 35.2 µg/mL in the crude CWE to 28.4 µg/mL following AgNPs synthesis. AW-AgNPs exhibited moderate

efficacy enhancement with a 6.7% improvement in IC_{50} reduction (Fig. S2).

The NPs formulation decreased the IC_{50} from 52.1 $\mu\text{g/mL}$ (AWE) to 48.6 $\mu\text{g/mL}$ (AW-AgNPs). On the other hand, BP-AgNPs displayed the most modest enhancement at 3.9% improvement, with IC_{50} values decreasing from 81.7 $\mu\text{g/mL}$ (BPE) to 78.5 $\mu\text{g/mL}$ (BP-AgNPs) (Fig. S2).

Antifungal activity

As shown in Table S3, the antifungal activity of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs was evaluated against four phytopathogenic fungi (*F. oxysporum*, *C. lindemuthianum*, *R. solani*, and *S. sclerotiorum*) across four concentrations (25–200 $\mu\text{g/mL}$).

All treatments exhibited dose-dependent inhibitory effects, with inhibition zones increasing in proportion to concentration. CW-AgNPs demonstrated the strongest antifungal performance, with inhibition zones against *F. oxysporum* increasing from 12.5 mm (25 $\mu\text{g/mL}$) to 37.4 mm (200 $\mu\text{g/mL}$), yielding an EC_{50} of approximately 62.5 $\mu\text{g/mL}$ (Table S2). The parent crude extract (CWE) achieved an EC_{50} of ~125 $\mu\text{g/mL}$, confirming a two-fold improvement through NPs synthesis. AW-AgNPs exhibited intermediate efficacy with inhibition zones rising from 9.8 mm to 30.5 mm (EC_{50} ~75 $\mu\text{g/mL}$), while BP-AgNPs displayed the weakest performance (EC_{50} ~100 $\mu\text{g/mL}$) (Table S3).

The MIC values further corroborate the enhanced antifungal efficacy of NPs formulations. CW-AgNPs and AW-AgNPs exhibited the lowest MIC (25 $\mu\text{g/mL}$) against all tested fungi, representing a twofold improvement over their parent extracts (CWE and AWE, MIC=50 $\mu\text{g/mL}$) and a fourfold improvement over BPE (MIC=100 $\mu\text{g/mL}$). BP-AgNPs showed moderate activity (MIC=50 $\mu\text{g/mL}$), still demonstrating a two-fold enhancement over their crude extract counterpart (Table S3).

Across all formulations, NPs synthesis consistently delivered a two-fold improvement in both EC_{50} and MIC values relative to parent extracts, confirming enhanced bioavailability and standardized antifungal efficacy (Table S3). CW-AgNPs emerge as the optimal candidate for antifungal applications requiring superior potency and dose–response characteristics.

Release of active compounds from agro-industrial waste (cumin, anise, and banana peel) and their AgNPs

Table 1 presents the cumulative release profile of bioactive compounds from CW-AgNPs, AW-AgNPs, and BP-AgNPs over 24 h, demonstrating how release kinetics directly influence antioxidant and antifungal efficacy. CW-AgNPs demonstrated the highest compound release rates, initiating at 15.2% (30 min) and reaching 89.7% by 24 h. AW-AgNPs released compounds approximately 11.4% lower on average than CW-AgNPs, progressing from 12.5% (30 min) to 85.2% (24 h). BP-AgNPs exhibited the slowest release kinetics, averaging 15.3% lower than CW-AgNPs across all time points, with initial release of 11.8% (30 min) and final release of 82.4% (24 h). The performance differential diminished over the 24-h period, with the greatest disparity at 30 min and convergence toward 24 h.

Quantitative analysis revealed strong negative correlations between cumulative compound release and biological activities. DPPH radical scavenging activity showed R^2 values of 0.9925 (CW-AgNPs), 0.9859 (AW-AgNPs), and 0.9087 (BP-AgNPs), with corresponding IC_{50} values of 28.4, 41.2, and 78.5 $\mu\text{g/mL}$ (Fig. 1).

Antifungal activity against *F. oxysporum* exhibited R^2 values of 0.9328 (CW-AgNPs), 0.9667 (AW-AgNPs),

Table 1 Cumulative release profile of silver nanoparticles synthesized from agro-industrial waste (cumin, anise, and banana peel) over 24 h

Time Point (h)	Active compounds release (%)		
	CW-AgNPs	AW-AgNPs	BP-AgNPs
0.5	15.2 ± 0.13 a	12.5 ± 0.16 b	11.8 ± 0.12 c
1.0	28.6 ± 0.27 a	23.8 ± 2.1 b	22.4 ± 2.0 c
2.0	45.3 ± 0.20 a	38.5 ± 0.17 b	36.9 ± 0.26 c
4.0	58.7 ± 0.49 a	52.1 ± 0.33 b	49.8 ± 0.31 c
8.0	74.2 ± 0.49 a	68.4 ± 0.27 b	65.3 ± 0.52 c
12.0	83.5 ± 0.02 a	78.6 ± 0.19 b	75.8 ± 0.21 c
24.0	89.7 ± 0.42 a	85.2 ± 0.10 b	82.4 ± 0.29 c

Values are presented as the means ± SE of three independent replicates. Within each row, values with the same letter are not significantly different ($P > 0.05$) according to Tukey's post hoc test

CWE cumin waste extract, AWE anise waste extract, BPE banana peel extract, AgNPs silver nanoparticles

and 0.9087 (BP-AgNPs), with EC_{50} values of approximately 62.5, 75, and 100 $\mu\text{g/mL}$, respectively (Fig. 1).

In vivo greenhouse experiment

Assessment of disease severity under greenhouse conditions

Table 2 compares the disease management efficacy of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs in controlling *F. oxysporum*-induced root rot and vascular wilt in *P. vulgaris*.

Infected control plants exhibited severe disease progression with 85.14% disease severity and no protection. Crude extracts provided moderate disease suppression: CWE achieved 59.64% disease severity with 30.0% protection efficacy; AWE showed 66.52% severity with 21.9% protection; and BPE exhibited 74.86% severity with 12.1% protection (Table 2).

AgNPs formulations substantially improved disease control across all treatments. CW-AgNPs achieved

outstanding efficacy with only 36.17% disease severity and 57.5% protection in disease compared to infected controls and a 91.7% improvement in protection relative to crude CWE.

AW-AgNPs demonstrated 43.30% severity and 49.1% protection, representing a 56.7% reduction in severity and a 124% improvement over the AWE crude extract. BP-AgNPs exhibited 51.52% severity and 39.5% protection, achieving a 49.5% reduction in severity and a 226% improvement relative to the crude extract of BPE (Table 2).

Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on *P. vulgaris* growth in the presence of *F. oxysporum*

Table 3 assesses the impact of the tested agro-industrial waste and their AgNPs on the development of *P. vulgaris* under various treatments in the presence of *F. oxysporum*.

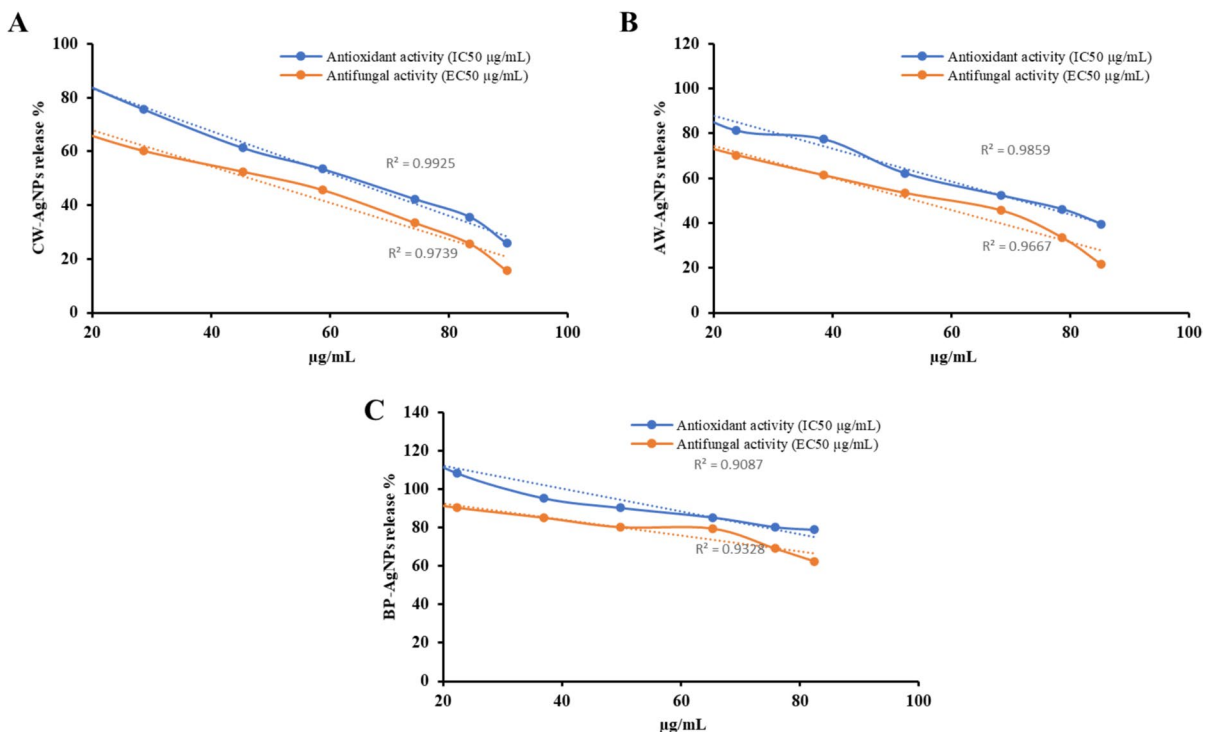


Fig. 1 Correlation between the released compounds from AgNPs fabricated from agro-industrial waste (cumin, anise, and banana peel) during 24 h and their antioxidant and antifungal activities. Release percentage (%) of (A) CW-AgNPs; (B)

AW-AgNPs; and (C) BP-AgNPs. CWE, cumin waste extract; AWE, anise waste extract; BPE, banana peel extract; AgNPs, silver nanoparticles; IC_{50} , half-maximal inhibitory concentration; and EC_{50} , half-maximal effective concentrations

Table 2 Comparative effects of agro-industrial waste (cumin, anise, and banana peel) and their silver nanoparticles on disease severity and protection efficacy against *Fusarium oxysporum* in common bean (*Phaseolus vulgaris*)

Treatments	Disease severity (%)	Protection efficacy (%)
Healthy control (no <i>F. oxysporum</i>)	0.0 <i>a</i>	100
Infected with <i>F. oxysporum</i>	85.14 ± 0.89 <i>b</i>	0.00
CWE + <i>F. oxysporum</i>	59.64 ± 0.93 <i>c</i>	30.0
AWE + <i>F. oxysporum</i>	66.52 ± 0.21 <i>d</i>	21.9
BPE + <i>F. oxysporum</i>	74.86 ± 0.67 <i>e</i>	12.1
CW-AgNPs + <i>F. oxysporum</i>	36.17 ± 0.20 <i>f</i>	57.5
AW-AgNPs + <i>F. oxysporum</i>	43.30 ± 0.46 <i>g</i>	49.1
BP-AgNPs + <i>F. oxysporum</i>	51.52 ± 1.02 <i>h</i>	39.5

Values are presented as the means ± SE of three independent replicates. Within each column, values with the same letter are not significantly different ($P > 0.05$) according to Tukey's post hoc test

CWE cumin waste extract, AWE anise waste extract, BPE banana peel extract, AgNPs silver nanoparticles

Healthy control plants demonstrated optimal growth parameters, where infected control plants with *F. oxysporum* showed severe growth suppression across all parameters (Table 3). Crude extract treatments provided moderate recovery. CWE

achieved partial restoration: plant height 64.2 cm (90% of healthy control), leaf area 39.2 cm² (90% of healthy control), 8.1 pods per plant, 3.3 seeds per pod, root length 24.6 cm (87% of healthy control), shoot biomass 12.5 g (88% of healthy control), and

Table 3 Effect of agro-industrial waste (cumin, anise, and banana peel) and their silver nanoparticles on common bean (*Phaseolus vulgaris*) growth under different treatments in the

presence of *Fusarium oxysporum* Please make the first column (Treatments) all in one line please similar to table 2 and table 4)

Treatments	Plant height (cm)	Leaf area (cm ²)	Number of pods/plants	Number of seeds/pods	Root length (cm)	Shoot biomass (g)	Root biomass (g)
Healthy control (no <i>F. oxysporum</i>)	71.2 ± 0.48 <i>a</i>	43.6 ± 0.16 <i>a</i>	9.6 ± 0.12 <i>a</i>	3.7 ± 0.03 <i>a</i>	28.1 ± 0.37 <i>a</i>	14.2 ± 0.10 <i>a</i>	2.5 ± 0.03 <i>a</i>
Infected with <i>F. oxysporum</i>	43.5 ± 0.62 <i>g</i>	29.4 ± 0.25 <i>g</i>	4.1 ± 0.10 <i>g</i>	2.1 ± 0.02 <i>g</i>	15.3 ± 0.32 <i>g</i>	7.1 ± 0.08 <i>g</i>	0.9 ± 0.05 <i>g</i>
CWE + <i>F. oxysporum</i>	64.2 ± 0.87 <i>c</i>	39.2 ± 0.56 <i>c</i>	8.1 ± 0.08 <i>c</i>	3.3 ± 0.02 <i>c</i>	24.6 ± 0.31 <i>c</i>	12.5 ± 0.06 <i>b</i>	2.0 ± 0.04 <i>c</i>
AWE + <i>F. oxysporum</i>	58.3 ± 0.55 <i>d</i>	34.8 ± 0.33 <i>e</i>	7.0 ± 0.12 <i>d</i>	2.9 ± 0.06 <i>d</i>	21.3 ± 0.36 <i>e</i>	11.1 ± 0.11 <i>d</i>	1.5 ± 0.02 <i>e</i>
BPE + <i>F. oxysporum</i>	49.5 ± 0.64 <i>f</i>	30.8 ± 0.15 <i>g</i>	5.6 ± 0.11 <i>f</i>	2.3 ± 0.06 <i>f</i>	16.7 ± 0.26 <i>g</i>	8.2 ± 0.05 <i>f</i>	1.0 ± 0.02 <i>g</i>
CW-AgNPs + <i>F. oxysporum</i>	68.0 ± 0.52 <i>b</i>	41.5 ± 0.44 <i>b</i>	8.9 ± 0.09 <i>c</i>	3.5 ± 0.04 <i>b</i>	26.3 ± 0.34 <i>b</i>	13.7 ± 0.19 <i>ab</i>	2.2 ± 0.03 <i>b</i>
AW-AgNPs + <i>F. oxysporum</i>	61.4 ± 0.57 <i>c</i>	37.4 ± 0.42 <i>d</i>	7.8 ± 0.10 <i>d</i>	3.0 ± 0.03 <i>d</i>	22.8 ± 0.22 <i>d</i>	11.8 ± 0.15 <i>c</i>	1.8 ± 0.02 <i>d</i>
BP-AgNPs + <i>F. oxysporum</i>	54.2 ± 0.73 <i>e</i>	32.6 ± 0.12 <i>f</i>	6.3 ± 0.06 <i>e</i>	2.6 ± 0.02 <i>e</i>	19.1 ± 0.37 <i>f</i>	10.3 ± 0.10 <i>e</i>	1.2 ± 0.03 <i>f</i>

Values are presented as the means ± SE of three independent replicates. Within each column, values with the same letter are not significantly different ($P > 0.05$) according to Tukey's post hoc test

CWE cumin waste extract, AWE anise waste extract, BPE banana peel extract, AgNPs silver nanoparticles

root biomass 2.0 g (80% of healthy control). AWE and BPE extracts showed lower recovery efficacy (Table 3).

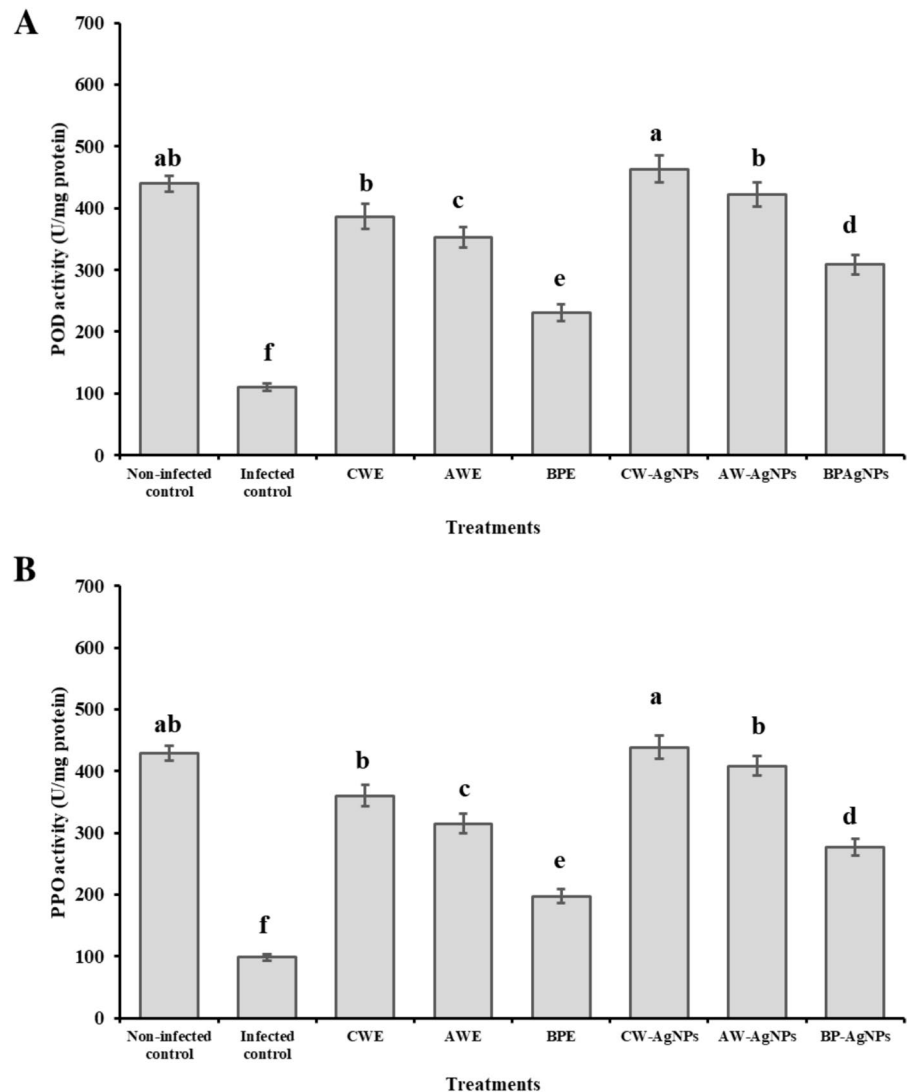
AgNPs treatments substantially enhanced plant recovery. CW-AgNPs achieved near-complete restoration of growth parameters: plant height 68.0 cm (95% of healthy control), leaf area 41.5 cm² (95% of healthy control), 8.9 pods per plant, 3.5 seeds per pod, root length 26.3 cm (94% of healthy control), shoot biomass 13.7 g (96% of healthy control), and root biomass 2.2 g (88% of healthy control) (Table 3). AW-AgNPs and BP-AgNPs similarly demonstrated significant growth enhancement compared to their respective crude extracts (Table 3).

Effect of the tested agro-industrial waste and their AgNPs on antioxidant enzymes

Figure 2 evaluates the effects of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on the activities of antioxidant enzymes.

Infected control plants had a baseline POD activity of 110.53 U/mg protein. Agro-industrial waste crude extracts augmented POD activity, CWE to 386.8 U/mg protein, AWE to 352.8 U/mg protein, and BPE to 231.1 U/mg protein. AgNPs enhanced the activity with CW-AgNPs exhibited the highest POD activity at 463.3 U/mg protein, followed by AW-AgNPs and BP-AgNPs (Fig. 2A).

Fig. 2 Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on the antioxidant enzymes content (A) POD, and (B) PPO of *Fusarium*-infected common bean. Values are means of three independent replicates. Bars represent SE. Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's post hoc test. CWE, cumin waste extract; AWE, anise waste extract; BPE, banana peel extract; AgNPs, silver nanoparticles; POD, peroxidase; and PPO, polyphenol oxidase



Infected control plants demonstrated a baseline PPO activity of 98.4 U/mg protein. Crude extracts elevated PPO activity to 360.46 U/mg protein (CWE), 314.8 U/mg protein (AWE), and 197.7 U/mg protein (BPE). AgNPs significantly improved activity with CW-AgNPs attained 438.6 U/mg protein (Fig. 2B).

Effect of the tested agro-industrial waste and their AgNPs on photosynthetic pigment content

Figure 3 demonstrates the effects of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on photosynthetic pigment restoration in *Fusarium*-infected common bean leaves.

F. oxysporum infection significantly diminished chlorophyll *a* level in comparison to healthy controls (Fig. 3A). Treatment with crude extracts resulted in partial restoration, whereas CW-AgNPs attained nearly 100% recovery of chlorophyll *a* to healthy plant levels, significantly surpassing both the CWE and other AgNPs formulations. AW-AgNPs and BP-AgNPs similarly increased chlorophyll *a* content compared with their respective crude extracts.

Chlorophyll *b* depletion caused by *F. oxysporum* infection was largely restored by agro-industrial waste crude extracts, with additional improvement obtained with AgNPs treatment (Fig. 3B). CW-AgNPs reinstated chlorophyll *b* to levels near those of healthy controls, exhibiting more efficacy than AW-AgNPs and BP-AgNPs.

F. oxysporum infection significantly decreased carotenoid levels (Fig. 3C). Agro-industrial waste crude extracts yielded moderate recovery, whereas AgNPs significantly improved carotenoid restoration. CW-AgNPs attained the highest carotenoid recovery, nearing concentrations found in healthy plants, but AW-AgNPs and BP-AgNPs offered moderate advantages.

Effect of the tested agro-industrial waste and their AgNPs on biochemical constituents of common bean

Table 4 evaluates the effects of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on the restoration of core biochemical components in common bean infected by *F. oxysporum*.

F. oxysporum infection significantly decreased carbohydrate, protein, lipid, and phenolic levels relative to healthy controls (Table 4). Infected control plants

demonstrated significant biochemical depletion in all metabolic indicators. Extracts from agro-industrial wastes facilitated partial biochemical repair. CWE exhibited the highest recovery across crude treatments, elevating all four biochemical constituents toward, but not achieving, healthy control values. AWE and BPE extracts showed lower recovery efficiency than CWE (Table 4).

In contrast, AgNPs formulations markedly enhanced biochemical repair relative to crude extracts. CW-AgNPs achieved the highest recovery, restoring carbohydrate, protein, lipid, and phenolic component levels in plants to nearly ideal levels (Table 4). AW-AgNPs and BP-AgNPs similarly increased all assessed metabolites above the infected controls and their respective crude extracts, although with slightly diminished recovery efficacy relative to CW-AgNPs.

Discussion

The present study demonstrated that green-synthesized AgNPs from agro-industrial wastes from cumin, anise, and banana peel substantially enhanced antifungal and antioxidant activities compared to agro-industrial waste (cumin, anise, and banana peel) crude extracts. There was a strong correlation between the bioavailability of active compounds and their biological efficacy across multiple assay systems.

In the *in vivo* greenhouse trials on infected *P. vulgaris* in our study, all treatments significantly reduced disease severity caused by *F. oxysporum*. The soil drench application of agro-industrial waste extracts reduced disease severity to 59.64% (cumin waste), 66.52% (anise waste), and 74.86% (banana peel), compared with 85.14% in the infected control group. The AgNPs synthesized from agro-industrial byproducts provided improved protection, reducing disease severity to 36.17% (CW-AgNPs), 43.30% (AW-AgNPs), and 51.52% (BP-AgNPs). Our findings are consistent with those of Li et al. (2022) and Qureshi et al. (2023), who reported similar effects.

The use of plant extracts as eco-friendly reducing and stabilizing agents in NPs synthesis is a validated, sustainable approach. In this regard, Qureshi et al. (2023) demonstrated that *Avena fatua* extract can effectively synthesize AgNPs with robust antifungal

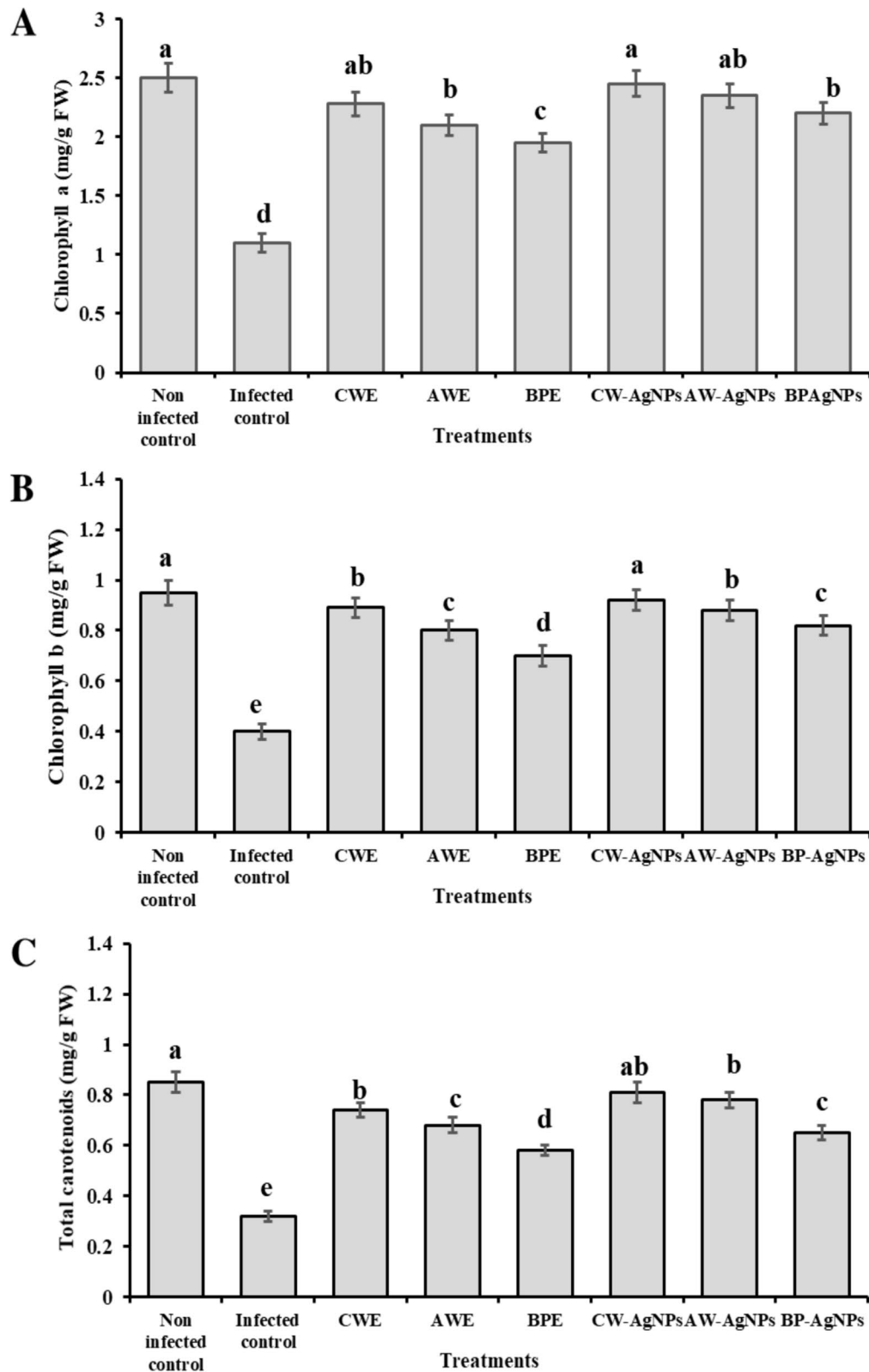


Fig. 3 Effect of agro-industrial waste (cumin, anise, and banana peel) and their AgNPs on pigments content (A) chlorophyll *a* (B) chlorophyll *b*, and (C) total carotenoids of *Fusarium*-infected common bean. Values are means of three independent replicates. Bars represent SE. Mean values with different letters are significantly different ($P < 0.05$) according to Tukey's post hoc test. CWE, cumin waste extract; AWE, anise waste extract; BPE, banana peel extract; AgNPs, silver nanoparticles

efficacy against *F. oxysporum* f.sp. *lycopersici*. Li et al. (2022) also demonstrated that AgNPs inhibit both mycelial growth and spore germination of several plant-pathogenic fungi, including *Fusarium culmarum*, with EC₅₀ values below 15 ppm. Li et al. (2022) also documented that AgNPs inhibit *F. oxysporum* through impaired fungal development, cell structure damage, and disrupted cellular metabolism.

The emerging paradigm of medicinal plant waste utilization addresses environmental sustainability while providing cost-effective biocontrol solutions. Our results agreed with a previous study by Jiang et al. (2025), who established that medicinal plant waste extracts are renewable alternatives to synthetic pesticides and retain 30–50% of essential bioactive compounds, with field-validated disease reduction exceeding 50% across multiple crop systems.

POD and PPO are essential antioxidant defense enzymes, and our results indicated that CW-AgNPs induced POD activity relative to infected controls, representing an approximately fourfold increase. In our investigation, the utilization of crude extracts

from agro-industrial waste enhanced POD activity to 386.8 U/mg protein for CWE, 352.8 U/mg protein for AWE, and 231.1 U/mg protein for BPE. CW-AgNPs demonstrated the highest peroxidase activity at 463.3 U/mg protein, followed by AW-AgNPs and BP-AgNPs (Fig. 2). Simultaneously, the utilization of crude extracts increased PPO activity to 360.46 U/mg protein (CWE), 314.8 U/mg protein (AWE), and 197.7 U/mg protein (BPE). AgNPs markedly enhanced activity, with CW-AgNPs achieving 438.6 U/mg protein (Fig. 2).

Our results agreed with previous studies by Fuerst et al. (2014) and Kumari et al. (2023), which confirmed that POD and PPO serve as systemic resistance markers, with their expression elevated in pathogen-infected plants that are effectively mobilizing defense mechanisms. Fuerst et al. (2014) established that POD, PPO, and chitinase are defense enzymes induced during fungal pathogen challenge, with four antimicrobial mechanisms: (1) quinone toxicity, (2) nutrient reduction, (3) physical barrier creation, and (4) ROS production. Kumari et al. (2023) have also documented that defense-induced compounds and NPs can significantly increase POD and PPO activity, with the highest peroxidase increases at 72 h from chitosan and 96 h from AgNPs.

In the current study, the severe pigment depletion in infected controls, chlorophyll *a* reduced to 1.1 mg/g (63% reduction), chlorophyll *b* to 0.4 mg/g (69% reduction), and carotenoids to 0.32 mg/g (70% reduction), reflects the metabolic damage caused

Table 4 Effects of agro-industrial waste (cumin, anise, and banana peel) and their silver nanoparticles on biochemical constituents in *Fusarium oxysporum*-infected common bean (*Phaseolus vulgaris*)

Treatments	Carbohydrates (mg/g FW)	Proteins (mg/g FW)	Lipids (mg/g FW)	Phenolic compounds (mg GAE/g FW)
Healthy control (no <i>F. oxysporum</i>)	44.2 ± 0.7 a	57.5 ± 0.27 a	12.3 ± 0.10 a	3.24 ± 0.03 a
Infected with <i>F. oxysporum</i>	28.6 ± 0.1 h	33.2 ± 0.37 g	6.8 ± 0.07 f	1.56 ± 0.02 g
CWE + <i>F. oxysporum</i>	40.8 ± 0.06 c	50.7 ± 0.23 c	10.7 ± 0.09 b	2.68 ± 0.06 c
AWE + <i>F. oxysporum</i>	37.9 ± 0.11 e	45.8 ± 0.29 d	9.5 ± 0.11 cd	2.21 ± 0.04 e
BPE + <i>F. oxysporum</i>	32.4 ± 0.07 g	38.5 ± 0.62 f	8.2 ± 0.08 e	1.78 ± 0.03 f
CW-AgNPs + <i>F. oxysporum</i>	42.3 ± 0.05 b	53.6 ± 0.67 b	11.2 ± 0.12 b	2.98 ± 0.04 b
AW-AgNPs + <i>F. oxysporum</i>	39.4 ± 0.07 d	48.7 ± 0.28 c	10.0 ± 0.13 c	2.46 ± 0.06 d
BP-AgNPs + <i>F. oxysporum</i>	35.2 ± 0.04 f	42.3 ± 0.25 e	9.0 ± 0.08 d	1.98 ± 0.05 f

Values are presented as the means ± SE of three independent replicates. Within each column, values with the same letter are not significantly different ($P > 0.05$) according to Tukey's post hoc test

CWE cumin waste extract, AWE anise waste extract, BPE banana peel extract, AgNPs silver nanoparticles

by *Fusarium* vascular invasion (Fig. 3). Our results are consistent with previous studies confirming that pathogenic infections suppress photosynthesis through multiple mechanisms: stomatal closure, chloroplast degradation, and resource reallocation to defense responses (Cheaib & Killiny, 2025).

Pathogenic infection reduces photosynthetic capacity through chlorosis and chlorophyll depletion (often > 50% reduction), with chloroplasts functioning as key sites for recognizing pathogen invasion through ROS bursts, calcium fluxes, and salicylic acid induction. However, treatment with crude extracts resulted in partial restoration, whereas CW-AgNPs achieved nearly 100% recovery of chlorophyll *a* to healthy plant levels, greatly exceeding both CWE and other AgNP formulations. AW-AgNPs and BP-AgNPs increased chlorophyll *a* content relative to their respective crude extracts (Fig. 3). Chlorophyll *b* depletion resulting from *F. oxysporum* infection was predominantly reinstated by crude extracts from agro-industrial waste, with further enhancement achieved through AgNPs treatment. CW-AgNPs restored chlorophyll *b* to levels comparable to healthy controls, demonstrating more efficacy than AW-AgNPs and BP-AgNPs (Fig. 3).

The use of crude extracts from agro-industrial waste yielded limited recovery, whereas AgNPs markedly enhanced carotenoid restoration. CW-AgNPs achieved the maximum carotenoid recovery, approaching levels observed in healthy plants, whereas AW-AgNPs and BP-AgNPs provided moderate benefits. Our findings align with prior research, affirming that the utilization of nanoparticles enhanced photosynthetic pigments in the presence of *F. oxysporum* (Parveen & Siddiqui, 2021). The restoration of carbohydrates, proteins, lipids, and phenolic compounds to near-optimal levels in the present study signifies a recovery of essential metabolic activities (Table 4). Our results indicated that CW-AgNPs attained carbohydrate recovery of 42.3 mg/g (96% of healthy control: 44.2 mg/g), protein at 53.6 mg/g (93% of healthy: 57.5 mg/g), lipid at 11.2 mg/g (91% of healthy: 12.3 mg/g), and phenolics at 2.98 mg GAE/g (92% of healthy: 3.24 mg/g), suggesting significant metabolic normalization. Our results corroborate those of Attia et al. (2023), who reported that treatment with CuO-ZnO NPs significantly reduced the disease index of *Fusarium* wilt in *Vicia faba*, accompanied by notable improvements in

growth parameters and photosynthetic pigments, as well as elevated levels of carbohydrates and proteins.

Several studies consistently reported partial restoration of metabolic elements following treatment of phytopathogen infection, with a general recovery of 70–80%. The present findings showed a 90–96% recovery across all four biochemical measures, implying either higher disease-control efficiency or improved recovery mechanisms beyond mere pathogen inhibition. The findings aligned with those of Onaran and Başaran (2018), who indicated that phenolic compounds, such as rutin (693.2 µg/g), attained complete antifungal suppression at concentrations of 10–20 mg/mL.

In conclusion, the present study explicitly indicates that AgNPs synthesized from agro-industrial waste extracts of cumin, anise, and banana peels may serve as efficient, sustainable nano-biopesticides, surpassing the efficacy of individual extracts in the integrated management of *Fusarium* root rot and vascular wilt in common beans.

The combination of green chemistry, nanotechnology, disease control, plant physiology, and sustainable agriculture confirms that plant waste-derived AgNPs are effective biocontrol agents that act through direct antimicrobial action, improved bioavailability via controlled release, activation of plant defenses, metabolic normalization, and synergistic interactions between plant extracts and NPs. This integrated mechanistic approach progresses beyond empirical findings to provide predictive models for formulation optimization and agricultural application.

Acknowledgements The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R443), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number (R. G. P. 2/398/46).

Authors contributions Rehab Oraby: Investigation, methodology, validation, writing – original draft. Amara N. Alqahtani, Mari Sumayli, and Ramadan Taha: Investigation, methodology, validation. Laila A. AL-Essa, and Hanan AlOmari: methodology. Merfat O. Aljhdli: validation. Ahmed M. Saad: methodology, validation. Taha F. Taha: Formal analysis. Mohamed A. Taha: validation, writing – original draft. Walid F.A. Mosa, and Mohamed T. El-Saadony: Investigation, methodology, validation, writing – original draft. Lamia Elmaghraby: Investigation, methodology. Betty T. Mathew: Formal analysis, writing

original draft. Synan AbuQamar: Conceptualization, funding acquisition, supervision, writing original draft, writing, review, and editing. Khaled El-Tarabily: Conceptualization, funding acquisition, supervision, writing, review, and editing.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R443), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. This research was funded by Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number (R. G. P. 2/398/46). This work was supported by Khalifa Center for Genetics Engineering and Biotechnology-UAEU (Grant number: 12R255) and UAEU Program for Advanced Research (UPAR; 12S273) to SAQ; and UPAR (Grant numbers: 12S169 and 12S291) to KE-T.

Data availability The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors declare no conflict of interest. Professor Khaled A. El-Tarabily declares that he is an associate editor for the European Journal of Plant Pathology.

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