



Biosynthesis of Algae based nanoparticles and their potential effect against Charcoal rot disease in common bean (*Phaseolus vulgaris* L.)

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Abstract *Macrophomina phaseolina* is a soil- and seed borne pathogen causing root rot disease. Disease management using marine macroalgae (*Ulva fasciata*) as well as nanotechnology practices can be a method for disease control. *In vitro* tests of *M. phaseolina* control using algal extracts, MgNPs and CH-Mg-alg NC (chitosan- magnesium- algae nanocomposite) as well as CH –alg NC (chitosan- algae nanocomposite) decreases fungal radial growth. We found that these extracts cause distortion in mycelium structure. HPLC analysis of the algal extract showed the presence of eight major phenolic compounds that may have an antifungal activity. The CH-Mg-alg NC treatment had the largest inhibitory effect decreasing radial growth by 88.9% compared to the control. The hydrodynamic diameter of the CH-Mg-alg NC particles includes two peaks at 7.36 nm and 898.9 nm with

a polydispersity index (PDI) of 0.34 and a zeta potential of -11.0 mV. In pot experiments, disease severity decreased after the CH-Mg-alg NC seed soaking treatment with a severity reduction of 56.43 to 23.81 and 53.53 to 25.02% in the first and second season, respectively. The treatments also improved vegetative growth parameters compared to the positive untreated control. To track the distribution of the nanocomposite within the pods and soil, data obtained from Inductively Coupled Plasma (ICP) spectrometry indicated that the magnesium (Mg) ratio not increases beyond the natural/safe levels in both the pods and soil. These findings support the safety of the application of CH-Mg-alg NC in disease management strategies.

Keywords *Macrophomina phaseolina* · *Ulva fasciata* · MgNPs · Chitosan · Nanocomposite · Seed soaking · Soil drenching

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Introduction

Phaseolus vulgaris L. (common bean) is the predominant legume crop, accounting for approximately 85% of global bean production (Machiani et al., 2019). The consumption of common beans is particularly significant in many tropical and subtropical countries, where they serve as a vital source of food for both humans and animals, second only to cereals. Common beans are rich in protein, vitamins, minerals, and dietary fiber, and they play a

crucial role in nitrogen fixation, thereby enhancing soil quality and supporting effective crop rotation systems (Abdou et al., 2019). The annual global yield of common beans is around 34.8 million hectares, producing nearly 27.5 million tons per year. In Egypt, common beans are cultivated on approximately 36.7 thousand hectares, yielding about 144.8 thousand tons (FAOSTAT, 2020).

Production and quality losses in common bean to seed-borne pathogenic fungi is common, including *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, and *Rhizoctonia solani*. Charcoal rot or dry root rot (DRR), caused by *Macrophomina phaseolina*, can result in yield losses ranging from 25 to 48% (Iqbal, & Mukhtar, 2014). The disease caused by this soil- and seed-borne pathogen can lead to plant wilting and eventual plant death, with symptoms becoming apparent from the time of emergence. Disease progression causes mechanical blockage of xylem vessels by microsclerotia, toxin production, enzymatic activity, and mechanical pressure during fungal penetration. The fungus produces several cell wall-degrading enzymes, hydrolytic enzymes, and phytotoxins, such as phaseolin and botryodiplodin (Singh et al., 2021).

To decrease disease incidence in soil, various management strategies have been used, including biological control and nanotechnology applications. Some marine macroalgae, or seaweeds, serve as biocontrol agents, producing secondary metabolites that inhibit the growth of numerous soil-borne pathogens. These metabolites, which include polyphenols, alkaloids, glycerol, sterols, and quinones, have antimicrobial properties. Specifically, *Ulva fasciata* has demonstrated antifungal activity against fungal pathogens such as *M. phaseolina* and *Fusarium oxysporum* (Lotfi et al., 2021). In addition to their antimicrobial effects, macroalgae provide antioxidant benefits that contribute positively to human health (Garbary, 2010).

The application of nanotechnology in agriculture offers innovative approaches for developing safe alternatives to conventional fungicides. Metal nanoparticles, such as magnesium nanoparticles (MgNPs), exhibit remarkable antimicrobial potential against soil-borne fungal pathogens while showing minimal cytotoxicity to mammalian cells. Furthermore, magnesium is an essential

macronutrient necessary for plant growth and photosynthesis (Ismail & Abd El-Gawad, 2021).

The main objective of this research is to investigate the efficiency of the green-synthesized nanocomposite and compare it to the efficacy of synthetic fungicides.

Material and methods

Common bean seeds

Seeds of *Phaseolus vulgaris* L. (Dokki 017 cultivar, winter crop) were obtained from the horticulture research institute, agricultural research center, Giza, Egypt.

Pathogenic fungi

The *Macrophomina phaseolina* (Tassi) Goid. strain (M 14, with the genebank accession number of OR243969) was kindly provided by the culture collection of the Mycology and Disease Survey Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Algal material collection

From the Port Said and Alexandria coasts, *U. fasciata* was collected. The collected algae were washed carefully under running tap water to eliminate deposit sand and soil particles or any other irrelevant matter. The algae were drained and wiped by blotting sheet and left to air-dry at 45 °C for five days. Algae were grounded mechanically (Soliman et al., 2018).

Synthetic fungicide

Rhizolex-T 50% (WP) (Sumi Agro Company, Japan) was used as seed treatment fungicide and applied following the manufacturer's recommended dose (3 g/kg seed).

Seed germination and vigor index

Common bean seeds were surface sterilized using sodium hypochlorite (2%) for ten min, then washed

several times with sterile H₂O and desiccated using filter paper. Seeds were sown in disinfected soil (sand: clay at 1:2 v/v) and after fifteen days, the germination percentage and growth parameters including shoot and root lengths were determined. The germination

(%) and vigor index were calculated according to Masum et al. (2012)

$$\text{Germination (\%)} = \frac{\text{number of germinated seeds}}{\text{total number of cultivated seeds}} \times 100$$

$$\text{Vigor index (I)} = \text{Germination (\%)} \times \text{seedling length (root + shoot)}$$

Macroalgae identification

Ulva fasciata Delile, green macroalgae, was collected and identified (El-sheekh et al., 2021).

Preparation of the algal extracts

20 g of algae powder was dissolved in a flask with 170 mL of pure ethanol and 30 mL water (1:10 w/v) for 6 h at 60 °C while stirring. Next the extract was filtered using cheesecloth followed by Whatman paper No. 2, then filtered through a membranous syringe filter (pore size 0.22 µm) and sonication at 750 W for one hour (Hamouda et al., 2023; Kumar et al., 2008).

Determination of the phenolic compounds in *Ulva fasciata* extract

Phenolic compounds were determined in the algal extract using HPLC analysis that was conducted at the Faculty of Agriculture, Cairo University, Giza, Egypt. This was done according to the Agilent Application note (Publication number 5991–3801 EN, 2014) at 20 °C and 38% RH. The used column: aKinetex® 1.7 µm EVO C150 mm × 4.6 mm, (Phenomenex, USA) was operated at 30 °C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.1% trifluoro acetic acid (TFA), (B) Acetonitrile, (C) methanol. Flow rate 1 ml/min. The injected volume was 20 µl. Detection: variable wavelength detector (VWD) set at 280 nm.

Chemical Synthesis of nanoparticles

A. Chemical Reagents Magnesium chloride (MgCl₂), Chitosan (C₅₆H₁₀₃N₉₀₃₉), Sodium tri poly phosphate (STPP), Ammonia solution 30% conc. (NH₄OH), Glacial acetic acid (CH₃COOH), sulphoric acid (H₂SO₄) and perchloric acid (HClO₄).

B. Synthesis of nanoparticles

Magnesium nanoparticles (MgNPs)

In order to produce magnesium nanoparticles, 0.1 M of MgCl₂ was used as precursor of Mg, and an ammonia solution (30% conc.) (NH₄OH) was used as reducing agent following the co-precipitation method as described by Pachiyappan et al. (2022). Briefly, about 0.1 M of MgCl₂ was gently mixing with a NH₄OH solution while stirring (700 rpm) till the development of a turbid cloud. The mixture was left to precipitate. The solution was kept at 25 ± 2 °C to remove the precipitated materials. The precipitated nanoparticles were then washed five times with deionized water and air dried. Finally, the resultant MgNPs were calcinated at 400 °C for 4 h for further use (Pachiyappan et al., 2022).

Chitosan nanoparticles (CH NPs)

Chitosan nanoparticles were prepared based on the ionic gelation of chitosan with sodium tri-polyphosphate (STPP) anions following a modified protocol (Almukhtar & Karam, 2020). Chitosan was stirred in 1% (v/v) of acetic acid at 400 rpm overnight in a magnetic stirrer. Then it was filtered through a Polyvinyl Difluoride (PVDF) syringe filter (0.22 µm pore size). Meanwhile, STPP was prepared at a concentration of 0.25% (w/v) in sterile distilled H₂O, and then filtered using a PVDF syringe filter. With a magnetic stirrer rotating at 800 rpm, chitosan and STPP of equal volume were cross-linked, followed by a 10-min centrifugation at 12,000 rpm. The obtained formulation was re-suspended in sterile distilled water and ultra-sonified at a 28% pulse ratio for 100 s at 4 °C for producing pellets. Finally, Chitosan nanoparticles were purified and evenly dispersed through centrifugation and ultra-sonication, which was performed three times.

Green synthesis of nanoparticles

- A. Preparation of algae based MgNPs (MgNPs-alg)
Magnesium nanoparticles were biosynthesized following a modified protocol of Pachiyappan et al. (2022). The aqueous extract of green marine *Ulva fasciata* was used as a reducing agent.
- B. Preparation of chitosan loaded algae extract (CH–alg NC)
As indicated above, chitosan nanoparticles were prepared based on the ionic gelation of chitosan with STPP anions following a modified protocol of Almukhtar and Karam (2020). Additionally, 10 mL of prepared *Ulva fasciata* aqueous extract was filtered (pore size 0.22 µm) and mixed with to 10 mL of the STPP solution (1:1 v/v). The cross-linking of chitosan with STPP and algae at equal volume was made drop by drop using a magnetic stirrer at 1200 rpm. The resultant mixture was centrifuged at 10,000 rpm for 10 min, and the produced pellets were resuspended in deionized water for washing, followed by ultra-sonication at 28% pulse ratio for 100 s. Centrifugation followed by the ultra-sonication step was repeated five times, and the precipitated nano-formula was freeze-dried and stored in a desiccator for further analysis (El-Naggar et al., 2022; Oh et al., 2019).
- C. Preparation of chitosan loaded (MgNPs-alg) nanocomposite (CH-Mg-alg NC)
About 0.1 g of the algal based MgNPs was dispersed in 45 mL of distilled water, and loaded in drops on 5 ml nano-chitosan under continuous stirring at 1000 rpm for 20 min. Next *Ulva fasciata* extract (5 ml) was added in drops with stirring at 1500 rpm over the course of time (60 min). The mixture solution was kept at 25 ± 2 °C and centrifuged to remove any wastes. The precipitated nanoparticles were washed in distilled water and then dried. The resultant NPs composite was calcinated at 400 °C for 4 h and stored for further use (Hamouda et al., 2023; Pachiyappan et al., 2022).

Characterization of nanoparticles

All the produced nano-samples were characterized after dilution with deionized water using spectroscopic analyses. The size distribution and zeta

potential analysis use dynamic light scattering (DLS) with a Zetasizer Ultra (Malvern Panalytical Ltd., Malvern, UK) as described by Mehanathan et al. (2023). Fourier transform infrared (FTIR) spectrophotometry (Avatar-300, Nicolet, USA) analysis was conducted to determine the functional groups. The chemical functional groups were responsible for the interaction of MgNPs and algae in the formation of CH-alg NC. FTIR spectrophotometry was done at wavelengths ranging from 4000 to 400 cm^{-1} with 16 average scans. Microstructures were recorded on a MSAL-XD2 X-ray diffractometer (XRD, Bruker, Karlsruhe, Germany) using Cu target in the 2 θ range from 5 to 80 ° (40 kV, 30 mA, λ 1.54051 Å) as described in Hamouda et al. (2023).

In vitro experiments

PDA growth medium was supplemented individually with concentrations of 100, 500 and 1000 µg/ml of each treatment (algae extract, MgNPs, CH-Mg-alg NC, CH–alg NC and MgCl_2). Media were poured in petri dishes of 9 cm diameter. A disc (0.5 cm diameter) of a 6 days actively grown fungal culture (25 ± 2 °C) was placed on the center of the PDA plate, and incubated at 25 ± 2 °C for 6 days. Fungal radial growth was measured and examined under a light microscope for all treatments. Recommended dose of the synthetic fungicide (Rhizolex-T 50%, 3 g/l) was applied to PDA medium in the same manner.

In vivo experiments

Seed soaking

Seeds were surface sterilized as mentioned in germination tests prior to soaking in the different treatments. Seeds were immersed individually in (algal extract, MgNPs, CH-Mg-alg NC, CH–alg NC and MgCl_2) with dilutions of 500, 500, 100, 100 and 1000 µg/ml respectively, for 30 min before sowing. The fungicide, Rhizolex-T 50% WP was applied as seed treatment (3 g/kg).

Pot experiments

Pot experiments were conducted at the Agricultural Research Centre in Giza, Egypt (latitude: 30° 01' 16.99''N, longitude: 31° 12' 30.01''E). Pots were sterilized using 5% formalin solution for 15 min and allowed to rest for 7 days prior to use. Following sterilization, the pots were filled with disinfected soil (a 1:2 v/v mixture of sand and clay) and arranged in five replicates for each treatment with twenty seeds sown for each replicate. The negative control pots were irrigated with water only, while the experimental pots were infested with *Macrophomina* inoculum. This inoculum was prepared from inoculating discs from 7-day-old cultures grown on potato dextrose agar (PDA) plates (incubated at 25 ± 2 °C) into a sterilized

mixture of sorghum, coarse sand, and water (2:1:2 v/v) in 500 mL Erlenmeyer flasks, which were then incubated at 25 °C for 15 days. The *M. phaseolina* sand corn inoculum was added to the soil at a rate of 3% of the total soil weight in each pot and thoroughly mixed. Regular irrigation was maintained for one week to facilitate the spread of the tested fungus prior to sowing the common beans. Results were recorded over two consecutive growing seasons.

Disease assessment

Percentages of pre-and post-emergence damping-off were recorded at 15 and 30 days after sowing respectively, in all treatments according to the following formulas:

$$\text{Pre – emergence damping – off (\%)} = \frac{\text{Number of non – germinated seeds after 15 days}}{\text{Total number of planted seeds}} \times 100$$

$$\text{Post – emergence damping – off (\%)} = \frac{\text{Number of dead seedlings after 30 days}}{\text{Total number of planted seeds}} \times 100$$

The surviving plants percentage was calculated 60 days after sowing in all treatments according to El-Rayes et al. (2022).

$$\text{survived plants (\%)} = \frac{\text{Number of survived seedlings after 60 days}}{\text{Total number of planted seeds}} \times 100$$

At the end of the growing season, disease severity was determined with a diseases scale proposed by Mengistu et al. (2007). Diseases severity (%) was calculated using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times v)}{5 N} \times 100$$

where: n= number of plants in each category, v= numerical values of symptoms category N= total number of plants, (5) =the maximum numerical value of the symptom category (Zanella et al., 2020).

Vegetative growth parameters

Seven growth parameters were measured after sixty days after sowing including shoot and root lengths (cm), plant fresh and dry weights (g), number of pods, fresh and dry weights of pods.

Residual quantity of Mg element in soil and common bean pods

Soil analysis

Soil samples were digested by aqua regia (hydrochloric acid and nitric acid) according to Cottenie et al. (1982)

Table 1 Detected phenolic compounds in the algal extract based on HPLC qualitative and quantitative analysis

Compound name	Retention time (RT) (min)	Area (%)	Concentration (mg/kg)
Gallic acid	3.27	68.2447	16.923
P.hydroxybenzoic	5.81	13.7204	8.719
Caffeic acid	8.08	85.6901	24.878
Syringic acid	9.97	22.2057	5.277
P. Coumaric	10.27	3.7537	0.694
Ferulic	12.23	5.4114	5.113
O. cumaric	12.96	1.5287	0.224
Apigenin	19.76	103.8071	3.883

and analyzed by Inductively Coupled Plasma (ICP) spectrometry (Ultima 2 JY Plasma).

Pod analysis

0.5 g of ground pods was wet digested using a sulphuric- perchloric- acids mixture ($\text{HClO}_4 + \text{H}_2\text{SO}_4$) according to procedure of Chapman and Pratt (1961) and pod analysis was performed using Inductively Coupled Plasma (ICP) spectrometry (Ultima 2 JY Plasma).

Statistical analysis

The data were analyzed using one-way ANOVA. Tukey–Kramer test for multiple comparisons at 0.05 level of probability. The statistical software MINITAB (Minitab® 19.2020.1 version, Minitab Inc., State College, PA, USA) was used.

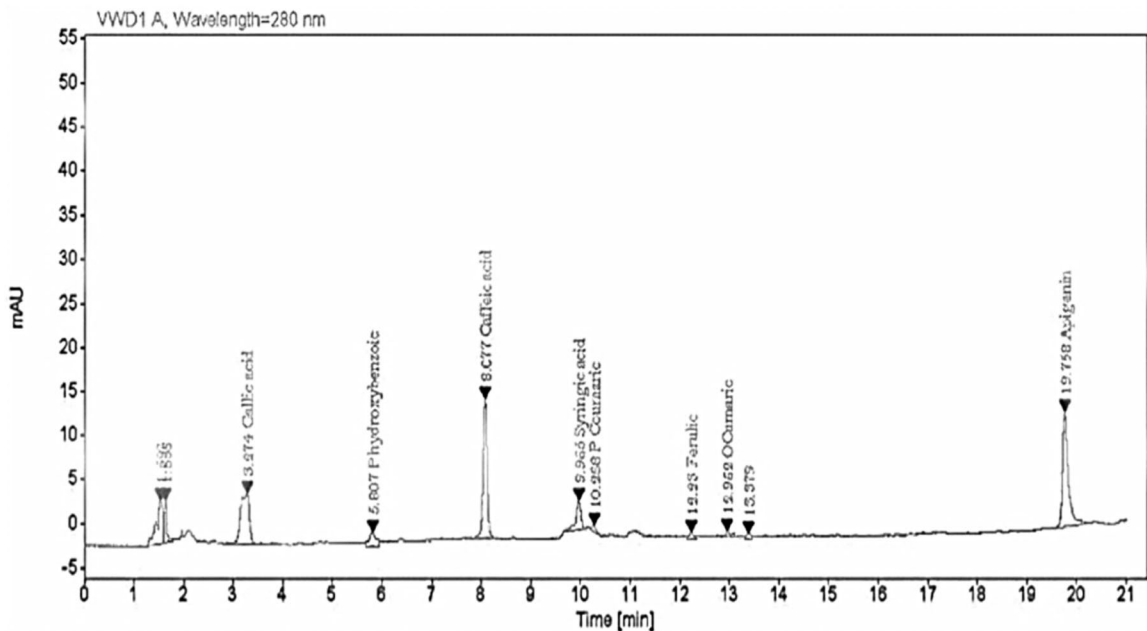
Results

Seed germination and vigor index

After fifteen days from sowing, seed germination and seedling growth properties were assessed showing shoot and root lengths of 14.52 and 4.22 cm respectively with germination percentage of 90% and vigor index of 1686.6.

Determination of phenolic compounds in algal extract

HPLC analysis was performed to determine the antifungal metabolites in the *Ulva fasciata* extract as phenolic compounds. Table 1 and Fig. 1 showed eight major phenolic compounds. Caffeic acid had the highest concentration while o. cumaric had the lowest value.

**Fig. 1** HPLC chromatogram of phenolic compounds recorded in algal extract

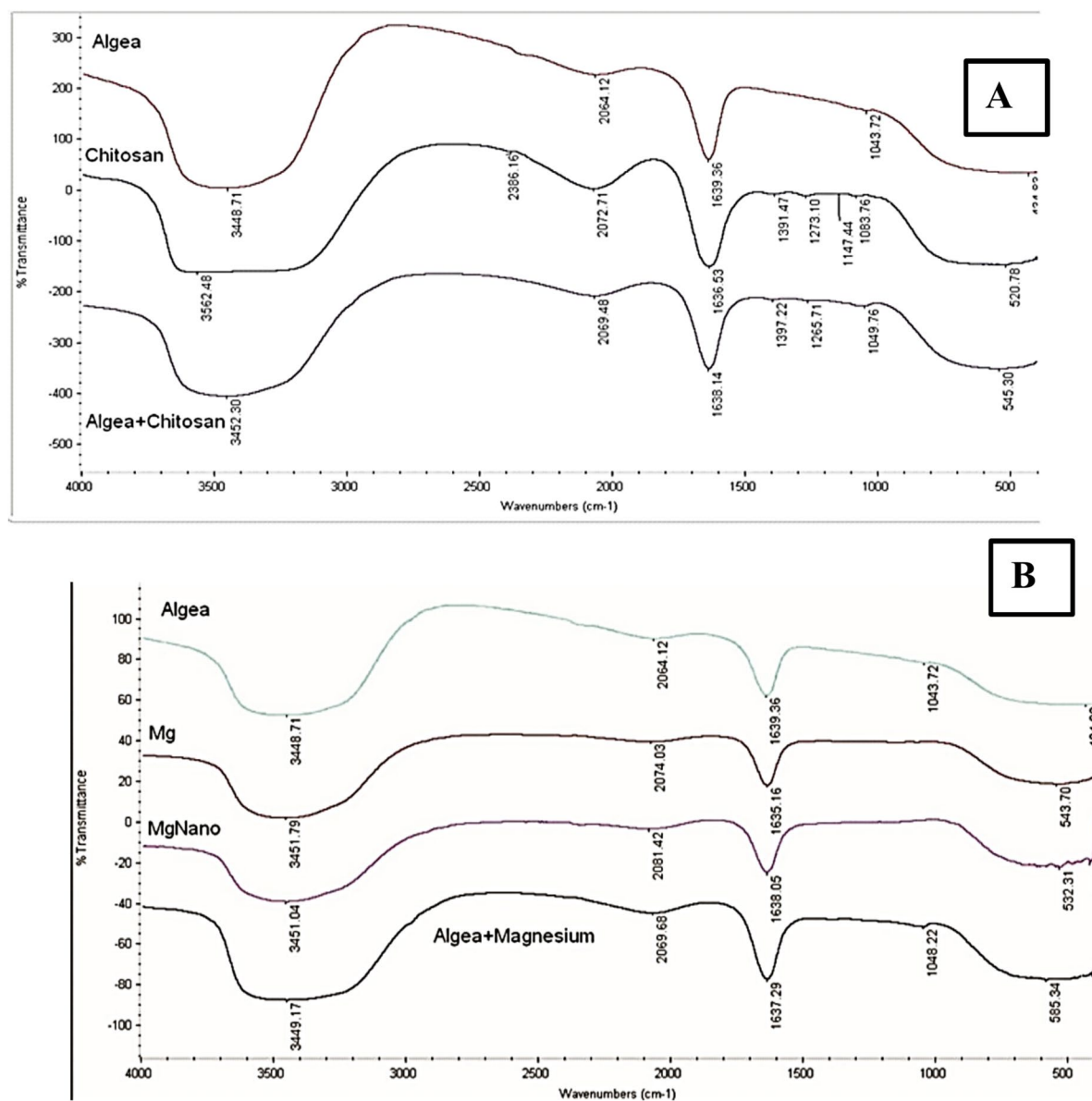


Fig. 2 FTIR spectra of **A:** algae, CH NPs and CH-alg NC. **B:** algae, pure MgCl₂, MgNPs and CH-Mg-alg NC

Characterization of the nanoparticles

A- fourier transform infrared (FTIR) analysis

FTIR analysis was employed to identify the functional groups responsible for the interaction between algal extracts and the chemically synthesized chitosan nanoparticles (CH NPs) and magnesium nanoparticles

(MgNPs). The FTIR spectrum of the algal extracts exhibited characteristic bands at 3448 cm⁻¹ (O–H stretching and bending vibrations), 2064 cm⁻¹ (C=O stretching), 1639 cm⁻¹ (C=C stretching), and 1043 cm⁻¹ (C–CH₃), indicating the presence of key functional groups. Similarly, chitosan molecules showed prominent peaks at 3562 and 2386 cm⁻¹ related to O–H stretching and hydrogen bonding, while peaks

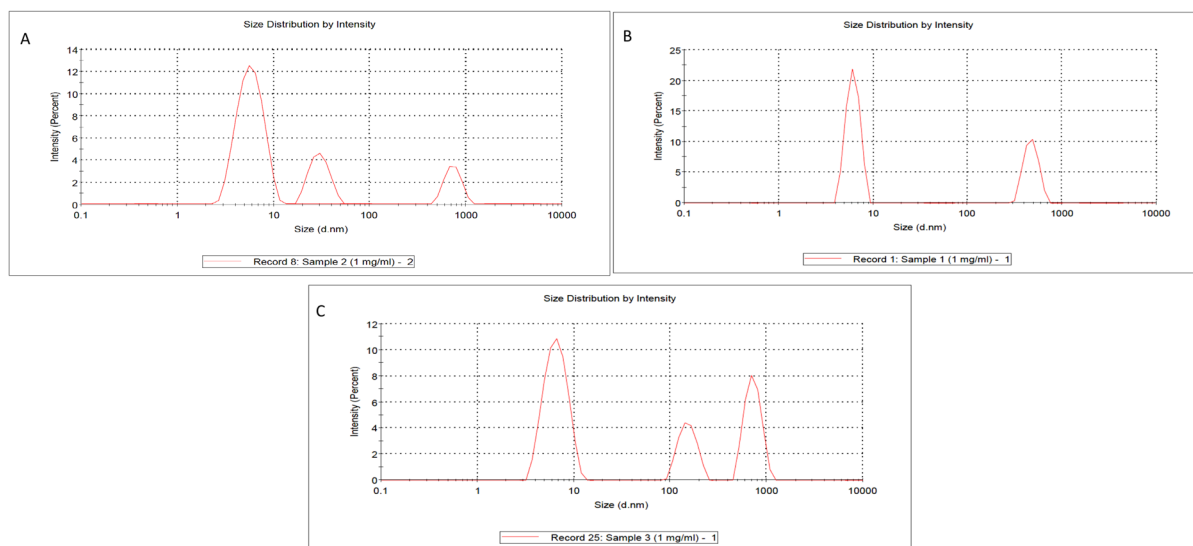


Fig. 3 Size distribution by the intensity graph of **A-** MgNPs, **B-** CH-Mg-alg NC and **C-** CH-alg NC

at 2072, 1636, and 1391 cm^{-1} corresponded to N=C=S, C=C, and C–N stretching of amide, respectively. Additional bands at 1273, 1147, and 1083 cm^{-1} were linked to $\text{CH}_3 + \text{COC}$, CH_3 , and C–O–C stretching, respectively. The FTIR spectra of pure MgCl_2 , MgNPs, algae, and the CH-Mg-alg nanocomposite (NC) revealed consistent O–H stretching vibrations around 3448–3451 cm^{-1} , N=C=S and C=O stretching around 2064–2081 cm^{-1} , and C=C stretching near 1635–1639 cm^{-1} . Notably, the Mg-containing samples (MgCl_2 , MgNPs, and CH-Mg-alg NC) displayed strong absorption bands at 543–585 cm^{-1} , confirming the presence of magnesium. The CH-Mg-alg NC specifically exhibited bands at 3449, 2069, 1637, and 1048 cm^{-1} , corresponding to O–H, N=C=S, C=C, and C– CH_3 vibrations, respectively, with a distinct band at 585 cm^{-1} indicating successful incorporation of MgNPs into the nanocomposite (Fig. 2).

B- Dynamic light scattering (DLS) and zeta potential measurements

To elucidate the particle size distribution and zeta potential of the nanomaterials, Dynamic light scattering (DLS) was considered to be an efficient and statistically reliable method to determine the surface

charge of the particle Fig. 3 and 4. The hydrodynamic diameter of the particle size distribution of MgNPs gave three picks at 8.19 nm, 67.4 nm and 961 nm with a polydispersity index (PDI) of 0.48 and had a zeta potential of –3.59 mV. CH-Mg-alg NC showed a hydrodynamic diameter of particle size at 7.36 nm and 898.9 nm with a polydispersity index (PDI) of 0.34 and had a zeta potential of –11.0 mV. The CH-alg NC gave three picks at 7.88 nm, 153.3 nm and 916.5 nm with a polydispersity index (PDI) of 0.51 and had a zeta potential of –22.0 mV due to the effect of Brownian motion, in addition to the hydrodynamic diameter in the calculation of particle size.

C- XRD spectrum analysis

The X-ray diffraction (XRD) patterns of the chitosan, MgNPs, CH-alg NC and CH-Mg-alg NC are presented in Fig. 5. The diffraction pattern of chitosan showed a prominent peak centered at 19.2°, indicating a specific arrangement of atoms or molecules within the CH NPs structure at that particular angle. This peak suggests the presence of a crystalline phase in the CH NPs material, as it corresponds to a characteristic crystal plane.

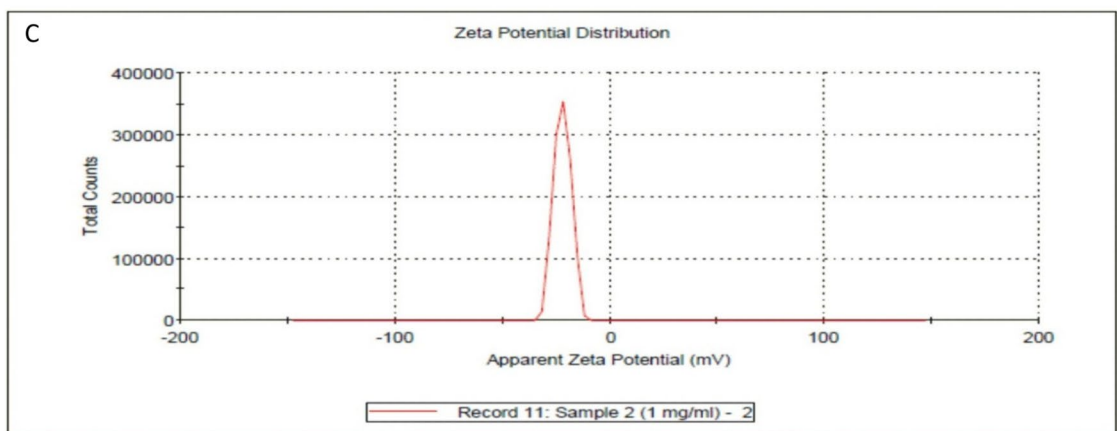
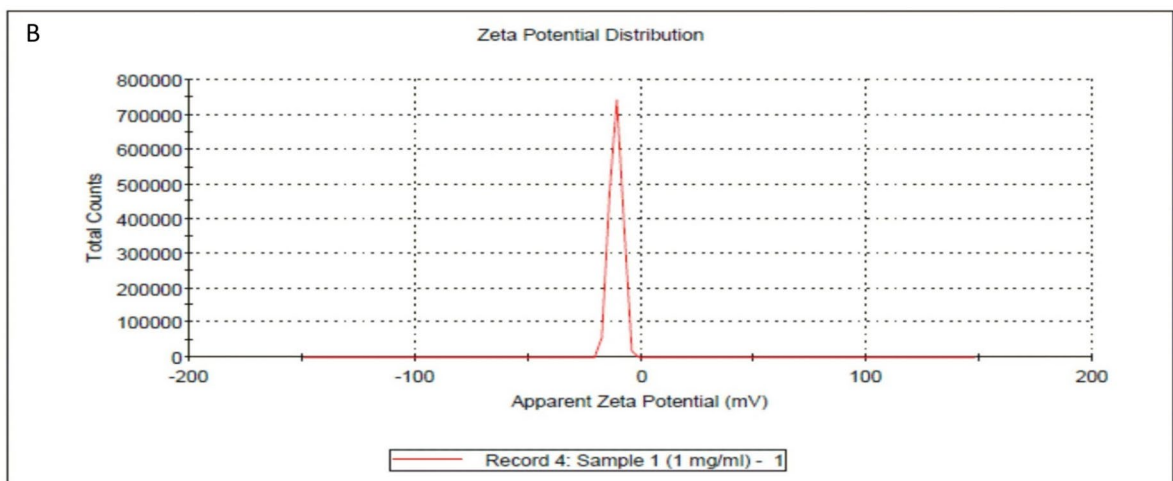
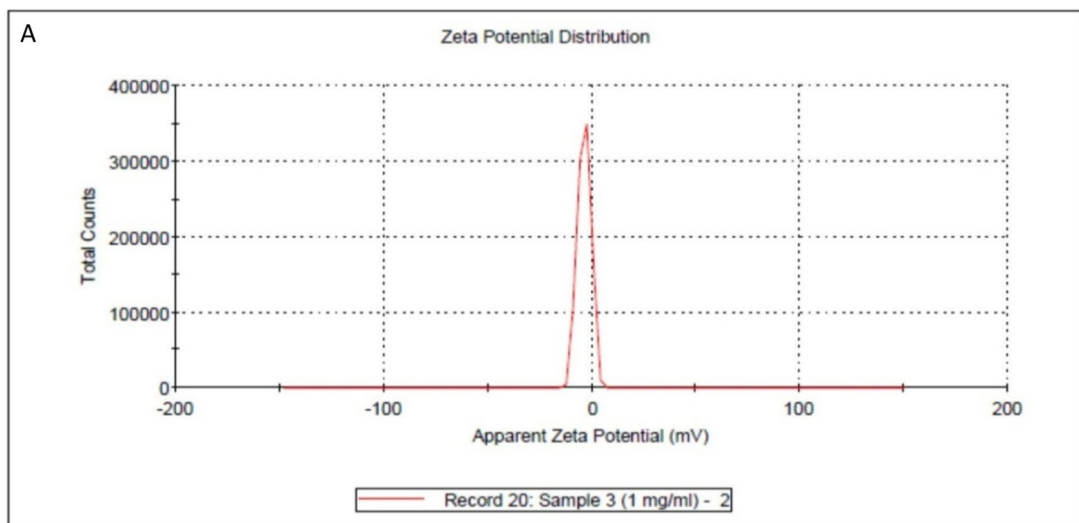


Fig. 4 Zeta potential analysis of **A**-MgNPs, **B**- CH-Mg-alg NC and **C**- CH-alg NC

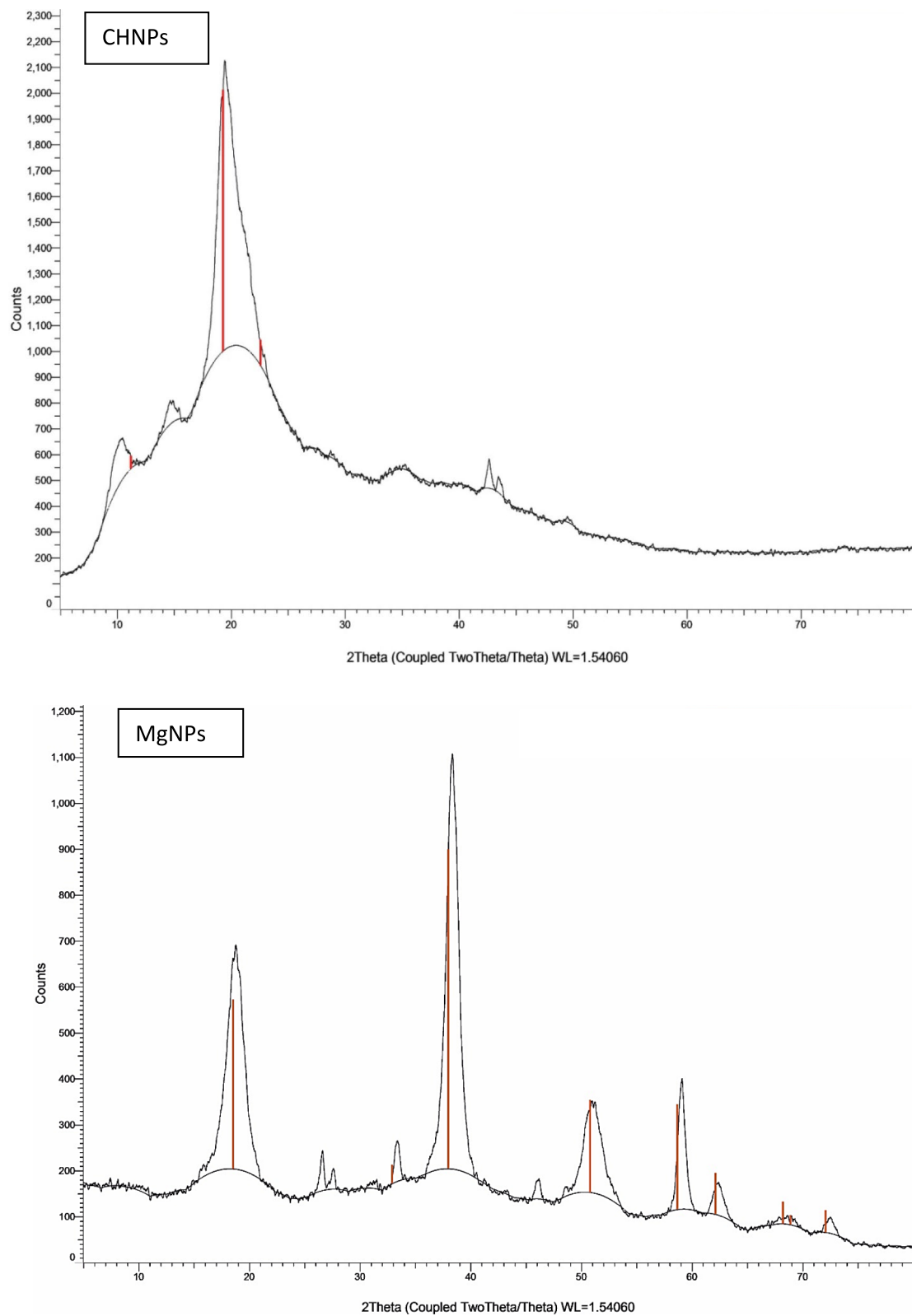


Fig. 5 XRD spectra: CH NPs, MgNPs, CH-alg NC and CH-Mg-alg NC

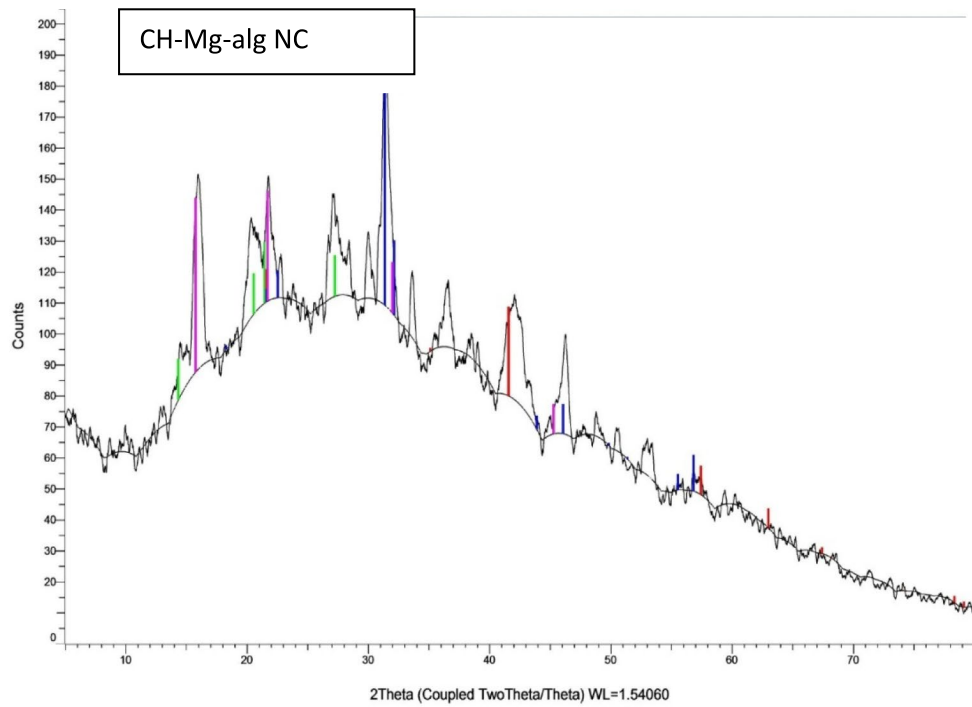
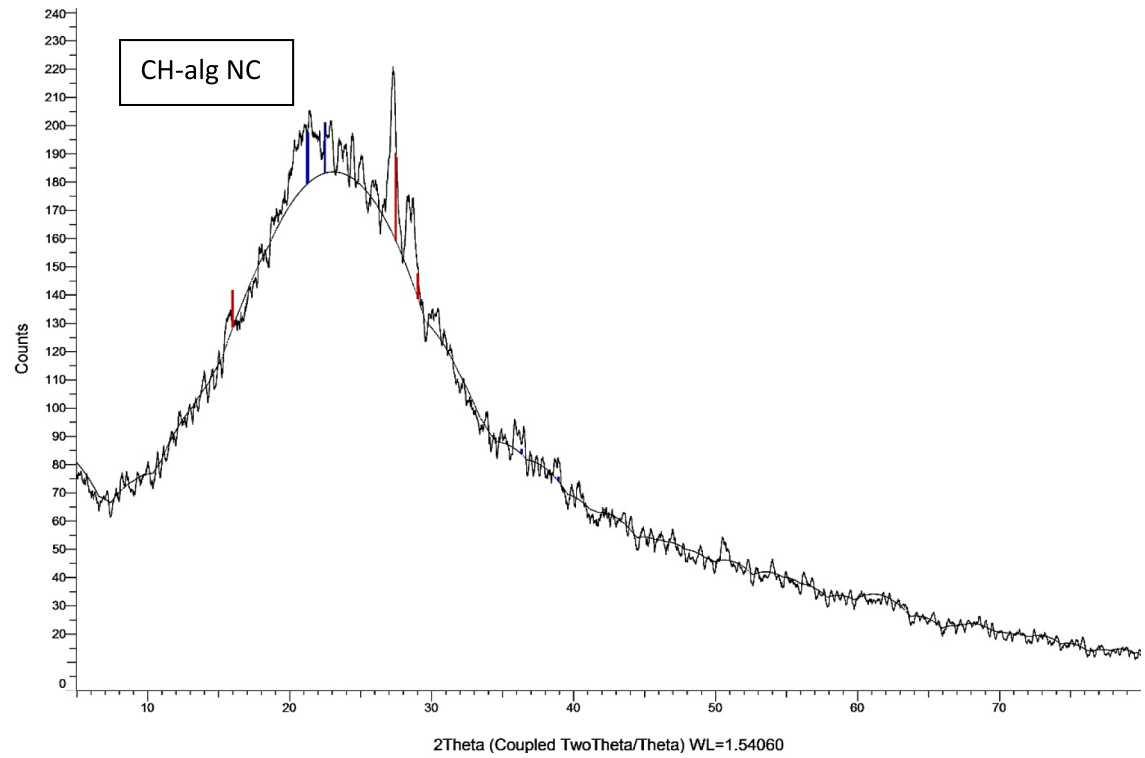


Fig. 5 (continued)

Table 2 Variations in *M. phaseolina* radial growth upon treatments application

treatment	Radial growth (cm) and treatment concentrations ($\mu\text{g/ml}$)		
	100	500	1000
CH-Mg-alg NC	1.00 ^a	4.00 ^d	8.50 ^d
CH-alg NC	1.50 ^b	8.10 ^e	9.00 ^e
Algae	5.20 ^d	1.97 ^a	6.40 ^b
MgNPs	8.20 ^e	2.53 ^b	9.00 ^e
MgCl ₂	9.00 ^f	8.50 ^{ef}	8.10 ^c
Chemical Fungicide (3 g/l)	3.50 ^c	3.50 ^c	3.50 ^a
Control	9.00 ^f	9.00 ^f	9.00 ^e

Results were represented as mean for five replicates (n = 5)

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

In vitro inhibitory effect of tested treatments against *M. phaseolina*

In vitro suppressive potential of different tested treatments against *M. phaseolina* was assessed as a decrease in the fungal radial growth as shown in Table 2 and Fig. 6. Radial growth was measured only after 6 days due to the rapid growth rate of *M. phaseolina*. The lowest radial growth values was obtained upon treatment with CH-Mg-alg NC (100 $\mu\text{g/ml}$) followed by CH-alg NC (100 $\mu\text{g/ml}$), algal extracts (500 $\mu\text{g/ml}$), MgNPs (500 $\mu\text{g/ml}$), MgCl₂ (1000 $\mu\text{g/ml}$) respectively, compared to the untreated control. Figure 7 illustrates the microscopic variation among the different treatments in suppressing microsclerotia formation of *M. phaseolina*. The CH-Mg-alg NC (100 $\mu\text{g/ml}$) treatment had the highest inhibitory effect where it decreased radial growth by 88.9% compared to the control.

Disease assessment under *in-vivo* conditions

Macrophomina root rot development was evaluated as pre-emergence, post-emergence damping off (%) and plant survival (%) which were determined compared to the uninfected seedlings (negative control). The obtained data revealed that all the tested treatments reduce disease severity significantly, compared to the positive untreated control but to

different degrees. The application of CH-Mg-alg NC via seed soaking gave the highest percentage decrease of disease severity while MgCl₂ treatment (soil drenching) had the lowest percentage reduction compared to the positive control. These findings were consistently confirmed throughout the two growing seasons, demonstrating a strong correlation, as presented in Tables 3, 4 & 5.

Measurement of the vegetative growth parameters under the infection stress

Our results revealed that growth parameters were affected by the application of different treatments under the infection stress during the first season and the results were confirmed in the second one with significantly strong correlation as shown in Tables 6, 7, 8 and Fig. 8.

Moreover, it was found that growth parameter decreased significantly with the increase in disease severity through the two seasons with a strong correlation as shown in Table 9.

Residual quantity of Mg element in soil and pods

Data in Table 10 demonstrated that the application of CH-Mg-alg NC; MgNPs and MgCl₂ decreases the Mg levels in soil and pods beyond the naturally occurred ratio.

Discussion

Macrophomina phaseolina, is the causal agent of charcoal rot or root rot, which leads to plant death going through a variety of disease symptoms at the different stages of plant development. Metal nanoparticles such as MgNPs, presents a novel strategy to control soil-borne fungi with low cytotoxicity towards humans and animals. Marine macro algae, such as *Ulva fasciata*, were used as biocontrol agents that produce secondary metabolic bioactive compounds which can inactivate soil borne fungi (Lotfi et al., 2021).

Our *In vitro* results show a decrease in growth of mycelium upon the application of algal extracts and nanoparticles. It also leads to reduced microsclerotia formation that is in accordance with the data obtained

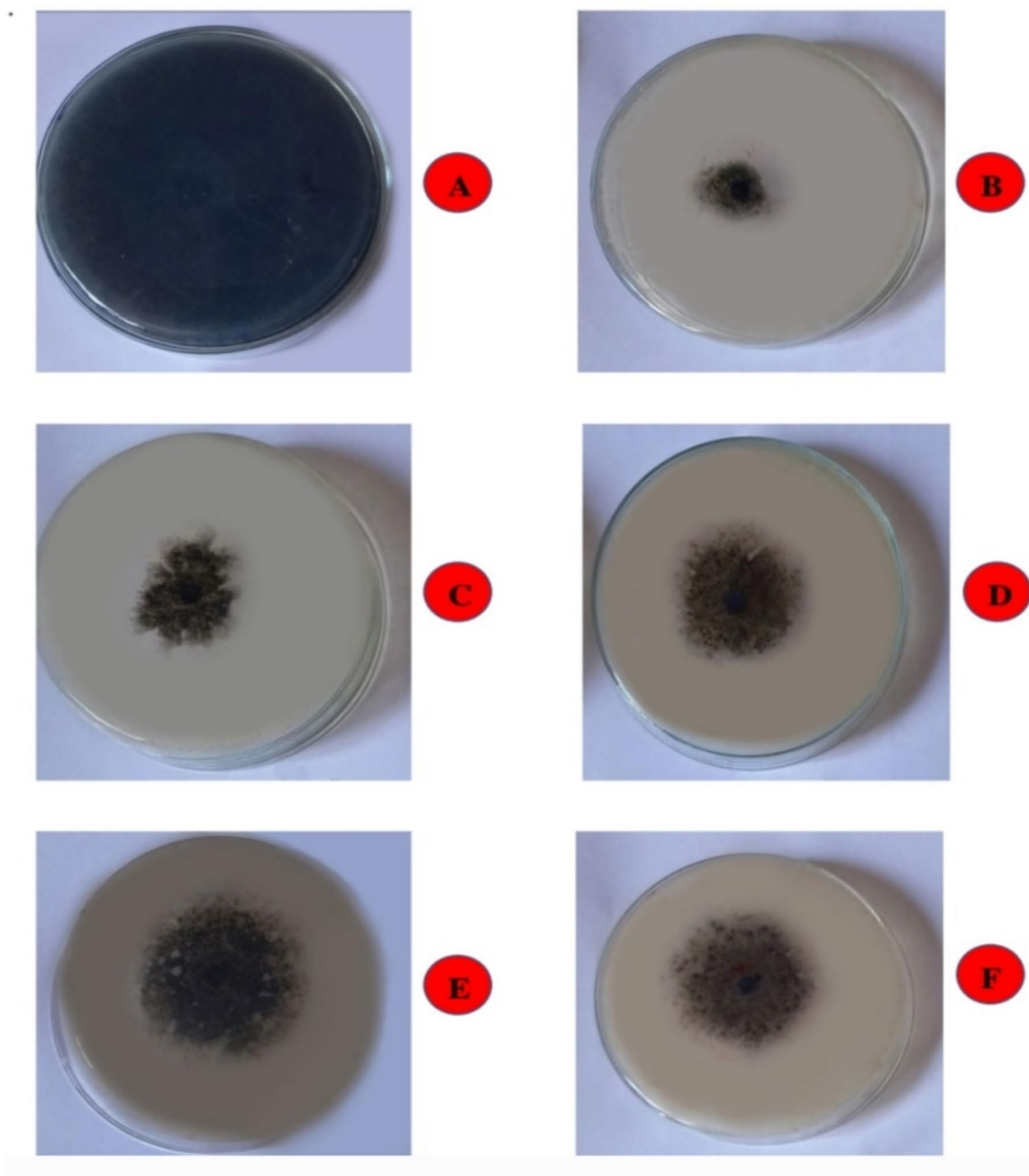


Fig. 6 *In-vitro* *M. phaseolina* growth inhibition with the application of different treatments dilutions. Untreated control **A**; CH-Mg-alg NC **B**; CH-alg NC **C**; Algae extract **D**; fungicide **E** and MgNPs **F**

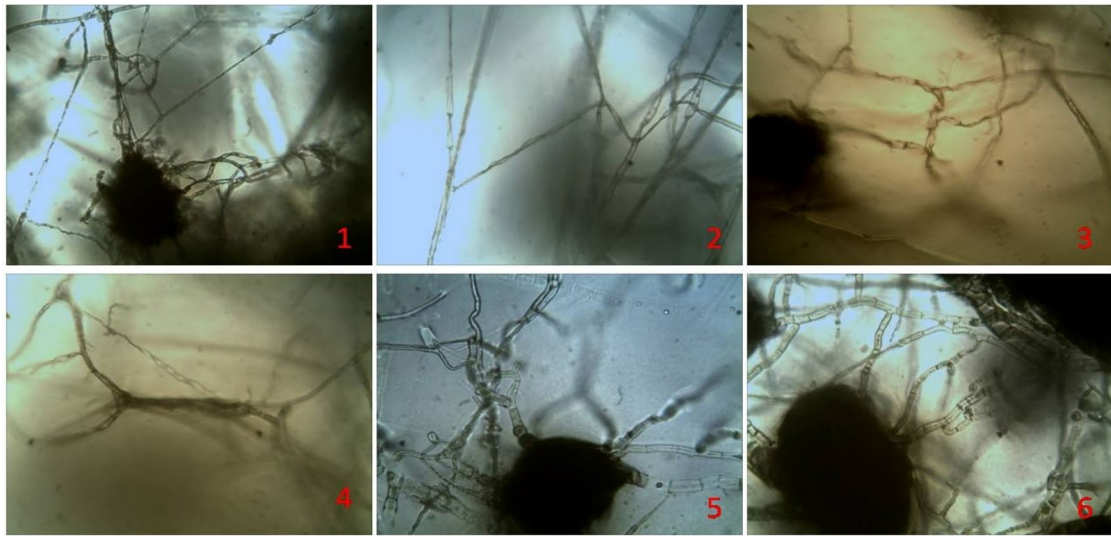


Fig. 7 Microscopic image, high power 40X (magnification power 400 X), showing the potential antifungal activity of concentrations of the following treatments: 1- Algae extract (500 µg/ml), 2-CH-Mg-alg NC (100 µg/ml), 3- MgNPs (500 µg/ml),

4- CH-alg NC (100 µg/ml), 5- fungicide and 6- Control against *M. phaseolina* hyphae after cultivation on PDAgrowth plates and incubation at $25 \pm 2^\circ\text{C}$ for 3–6 days

Table 3 Effect of different treatments on disease development on common bean seedlings during the 1st season

Treatment	Pre-Emergence Damping Off (%)	Post-Emergence Damping Off (%)	Plant Survival (%)	Disease severity (%)
- ve control	6.00 ^a	3.00 ^a	91.00 ^g	0.00 ^a
+ ve control	36.00 ^g	25.00 ^f	39.00 ^a	56.43 ^g
Fungicide	13.00 ^{abcd}	6.00 ^{ab}	81.00 ^{ef}	29.85 ^c
Seed soaking				
Algae	16.00 ^{bcde}	10.00 ^{abc}	74.00 ^{de}	34.22 ^d
MgNPs	19.00 ^{cdef}	12.00 ^{bcd}	69.00 ^{cd}	36.48 ^{de}
MgCl ₂	22.00 ^{ef}	17.00 ^{de}	61.00 ^{bc}	41.83 ^f
CH -alg NC	11.00 ^{ab}	8.00 ^{ab}	81.00 ^{ef}	25.32 ^b
CH-Mg-alg NC	10.00 ^{ab}	4.00 ^a	86.00 ^{fg}	23.81 ^b
Soil drenching				
Algae	20.00 ^{def}	16.00 ^{cde}	64.00 ^{bc}	38.21 ^e
MgNPs	19.00 ^{cdef}	16.00 ^{cde}	65.00 ^c	37.52 ^{de}
MgCl ₂	24.00 ^f	20.00 ^{ef}	56.00 ^b	44.32 ^f
CH -alg NC	12.00 ^{abc}	7.00 ^{ab}	81.00 ^{ef}	25.65 ^b
CH-Mg-alg NC	12.00 ^{abc}	8.00 ^{ab}	80.00 ^{ef}	26.48 ^{bc}

Values were represented as mean with n = 5

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

by Senthilkumar et al. (2014) and Mostafa et al., (2022).

Profile analysis of *U. fasciata* extract showed the presence of phenolic compounds that may disrupt

the physiological processes in *Macrophomina phaseolina*, such as cell wall formation, membrane stability, or enzymatic functions Abbassy et al., (2014). Antioxidant properties involved the neutralization

Table 4 Effect of different treatments on disease development on common bean seedlings during the 2nd season

Treatment	Pre-Emergence Damping Off (%)	Post-Emergence Damping Off (%)	Plant Survival (%)	Disease severity (%)
-ve Control	9.00 ^a	3.00 ^a	88.00 ^g	0.00 ^a
+ ve control	37.00 ^e	29.00 ^f	34.00 ^a	53.53 ^f
Fungicide	14.00 ^{ab}	10.00 ^b	76.00 ^{ef}	28.64
Seed soaking				
Algae	17.00 ^b	12.00 ^{bcd}	71.00 ^{de}	35.86 ^c
MgNPs	18.00 ^{bc}	16.00 ^{cde}	66.00 ^{cd}	39.23 ^{cd}
MgCl ₂	24.00 ^d	20.00 ^e	56.00 ^b	41.32 ^{de}
CH -alg NC	13.00 ^{ab}	11.00 ^{bc}	76.00 ^{ef}	26.42 ^b
CH-Mg-alg NC	11.00 ^a	8.00 ^{ab}	81.00 ^{fg}	25.02 ^b
Soil drenching				
Algae	23.00 ^{cd}	17.00 ^{de}	60.00 ^{bc}	39.43 ^{cd}
MgNPs	23.00 ^{cd}	18.00 ^e	59.00 ^{bc}	38.22 ^{cd}
MgCl ₂	24.00 ^d	21.00 ^e	55.00 ^b	43.68 ^e
CH -alg NC	13.00 ^{ab}	11.00 ^{bc}	76.00 ^{ef}	25.91 ^b
CH-Mg-alg NC	13.00 ^{ab}	9.00 ^b	78.00 ^{ef}	27.02 ^b

Values were represented as mean with n = 5

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

Table 5 Correlations between disease measurements taken in first and second seasons

Disease assessment parameters	Correlation ⁺
Disease severity (%)	0.972**
Pre-Emergence Damping Off (%)	0.887**
Post-Emergence Damping Off (%)	0.830**
Plant Survival (%)	0.942**

**Significantly Strong Correlation (Pearson correlation at probability ≤ 0.01)

of the reactive oxygen species (ROS) protect the plant against oxidative stress (Topolovec-Pintarić, 2020). Moreover, phenolic compounds can stimulate immune responses in the plant via activation of defense-related genes and the production of phytoalexins as antimicrobial substances synthesized by the plants. Furthermore, some phenolic compounds can directly affect the virulence factors of plant pathogens minimizing their potential to infect the plant (Ramírez-Gómez et al., 2020).

Phenolic compounds include caffeic acid produced with a concentration of 24.878 mg/kg is known for its antifungal activity. Caffeic acid and its derivatives were able to form insoluble complexes with iron ions, which leads to the prevention of iron absorption

by living cells which is known as intracellular iron starvation (Sun et al., 2018) as well as their action on nucleic acid of fungal cells and cellular proteins. Additionally, caffeic acid derivatives cause a distortion of the cell wall and membranes which in turn change the surface micromorphology and could change the permeability of the cell membrane leading to an osmotic imbalance (Alfarrayeh et al., 2021).

Gallic acid (16.923 mg/kg) has antioxidant properties through the elimination of the reactive oxygen species or inactivation of enzymes that produce ROS and improve the activity of antioxidant enzymes, such as glutathione (Baite et al., 2024).

The peak assignments provide insights into the molecular composition and bonding characteristics in the samples and contribute to our understanding of their chemical properties and potential applications (Tomaszewska et al., 2013). The presence of sharp intense peaks in an XRD pattern is commonly associated with a high level of crystallinity in a given material (Günister et al., 2007; Julkapli et al., 2008; Dey et al., 2016). Similarly, the XRD pattern of MgNPs displayed broad peaks with high intensities, particularly at a 2θ angle of 38.017 degree, which aligns with the findings reported by Kroftová et al. (2019).

Our findings are consistent with previous research conducted by Dey et al. (2016); Radha and Murugesan

Table 6 Common bean growth parameters as influenced by different treatments during the first season

Treatment	Shoot length (cm)	Root length (cm)	Plant fresh wt.(g)	Plant dry wt. (g)	Number of pods	Pods fresh wt. (g)	Pods dry wt. (g)
-ve Control	28.89 ^{cde}	7.63 ^d	41.97 ^{cd}	8.99 ^{de}	12.2 ^c	24.54 ^d	5.13 ^c
+ ve control	17.36 ^a	3.24 ^a	13.05 ^a	4.21 ^a	2.2 ^a	3.49 ^a	0.88 ^a
Fungicide	29.40 ^{cde}	9.22 ^e	45.07 ^d	9.67 ^e	18.2 ^e	25.65 ^d	5.23 ^c
Seed soaking							
Algae	29.34 ^{cde}	7.49 ^d	40.93 ^c	8.90 ^{de}	15.2 ^d	24.05 ^d	5.61 ^c
MgNPs	26.20 ^{bcd}	6.44 ^{cd}	31.36 ^b	8.32 ^{cde}	12.2 ^c	15.50 ^c	3.53 ^b
MgCl ₂	25.88 ^{bc}	5.44 ^{bc}	29.09 ^b	6.03 ^b	11.0 ^{bc}	13.08 ^{bc}	2.99 ^b
CH -alg NC	30.22 ^{de}	10.41 ^{ef}	58.40 ^e	14.56 ^f	28.0 ^{gh}	40.21 ^g	10.21 ^e
CH-Mg-alg NC	32.77 ^e	11.36 ^f	58.54 ^e	15.29 ^f	29.6 ^h	45.60 ^h	12.12 ^f
Soil drenching							
Algae	27.31 ^{bcd}	6.52 ^{cd}	29.82 ^b	7.71 ^{bc}	8.2 ^b	12.20 ^b	3.09 ^b
MgNPs	26.07 ^{bcd}	6.23 ^c	29.73 ^b	7.64 ^{bc}	9.0 ^{bc}	12.01 ^b	3.09 ^b
MgCl ₂	24.26 ^b	4.95 ^b	28.70 ^b	6.86 ^{bc}	10.2 ^{bc}	16.20 ^c	2.94 ^b
CH -alg NC	29.57 ^{cde}	9.80 ^e	56.11 ^e	14.17 ^f	26.2 ^g	36.74 ^f	9.69 ^e
CH-Mg-alg NC	29.52 ^{cde}	9.37 ^e	57.50 ^e	13.75 ^f	23.4 ^f	32.65 ^e	7.95 ^d

Values were represented as mean with n = 5

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

Table 7 Common bean growth parameters as influenced by different treatments during the second season

Treatment	Shoot length (cm)	Root length (cm)	Plant fresh wt.(g)	Plant dry wt. (g)	Number of fruits	Pods fresh wt. (g)	Pods dry wt. (g)
-ve Control	28.34 ^{def}	7.47 ^e	41.68 ^e	8.89 ^{ef}	12.0 ^e	24.47 ^d	4.98 ^d
+ ve control	16.63 ^a	3.22 ^a	12.56 ^a	5.19 ^a	1.8 ^a	3.57 ^a	0.83 ^a
Fungicide	28.06 ^{def}	8.76 ^f	44.40 ^f	9.39 ^f	17.6 ^g	25.08 ^d	5.11 ^d
Seed soaking							
Algae	27.89 ^{de}	7.38 ^e	39.46 ^d	8.42 ^e	15.6 ^f	23.65 ^d	5.36 ^d
MgNPs	25.77 ^{bc}	6.28 ^d	30.98 ^c	8.29 ^{de}	11.6 ^{de}	15.23 ^c	3.43 ^c
MgCl ₂	25.15 ^{bc}	5.03 ^{bc}	28.11 ^b	6.16 ^b	10.8 ^{cde}	12.51 ^b	2.75 ^b
CH -alg NC	29.93 ^f	10.18 ^{gh}	57.68 ^g	14.00 ^{gh}	28.2 ⁱ	40.45 ^g	10.28 ^g
CH-Mg-alg NC	32.37 ^g	10.80 ^h	57.50 ^g	14.83 ^h	29.8 ⁱ	45.02 ^h	11.62 ^h
Soil drenching							
Algae	26.71 ^{cd}	6.44 ^d	28.59 ^b	7.21 ^c	7.8 ^b	11.79 ^b	3.05 ^{bc}
MgNPs	25.44 ^{bc}	5.79 ^{cd}	29.50 ^{bc}	7.42 ^{cd}	8.8 ^{bc}	11.51 ^b	3.00 ^{bc}
MgCl ₂	24.33 ^b	4.84 ^b	28.04 ^b	6.72 ^{bc}	9.8 ^{bcd}	15.63 ^c	2.86 ^{bc}
CH -alg NC	29.53 ^{ef}	9.88 ^g	56.18 ^g	14.24 ^{gh}	25.8 ^h	36.41 ^f	9.44 ^f
CH-Mg-alg NC	29.35 ^{ef}	9.58 ^g	56.72 ^g	13.37 ^g	24.2 ^h	32.15 ^e	7.74 ^e

Values were represented as mean with n = 5

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

Table 8 Correlations between growth parameters through the two growing seasons

Growth parameter	Correlation ⁺
Shoot length	0.85**
root length	0.95**
Plant fresh weight	0.99**
Plant dry weight	0.95**
Number of pods	0.98**
Pods fresh weight	0.99**
Pods dry weight	0.98**

** Significantly Strong Correlation (Pearson correlation at probability ≤ 0.01)

(2017); Hassaana et al. (2021); Makhlof et al. (2022) supporting the agreement between the obtained XRD results and the data reported in the literature.

The samples (MgNPs, algae extract, CHNPs, CH-Mg-alg NC, and CH-alg NC) exhibited certain characteristics for concentrated biomolecules, molecular and strong adhesion forces; aggregation and electrostatic attraction and coagulation. Concentrated biomolecules in algae extract and chitosan could comprise proteins, polysaccharides, nucleic acids, etc., depending on the samples composition (Bedi et al., 2022).

Moreover, the observed aggregation in the solution can be attributed to strong adhesion forces acting between the particles. This suggests a propensity for particle–particle sticking, potentially resulting from intermolecular forces such as van der Waals forces, hydrogen bonding, or other similar interactions (González-Aviña et al., 2024). Furthermore, an investigation revealed that electrostatic attraction between the particles led to coagulation. This phenomenon signifies that particles possess opposite charges resulting in their aggregation and the formation of larger clusters (Bouguerra et al., 2024). Those observations reveal the complex interactions among the various components in the solution including biomolecules, water, and nanoparticles. Understanding and effectively controlling these interactions is essential for the application of nanoparticle synthesis and bio-composite formation (Chen et al., 2020).

Based on these observations, Mg nanoparticles possess antifungal properties by modifying several cellular processes in fungi, which leads to growth inhibition. Additionally, chitosan (derived from chitin found in crustacean shells) exhibits both direct

and indirect antifungal activities by disrupting fungal cell membranes, enhancing plant resilience against infections, inducing stress responses, and promoting the production of defense-related compounds (Chen et al., 2020). The observed infection suppression following the application of the CH-Mg-algae NC or CH NPs highlights their significant potential against *Macrophomina phaseolina*, corroborating findings from Abd-El-Kareem et al. (2018) and Roohallah et al. (2022). Thus, the combination of algae and nanoparticles may produce a synergistic effect, resulting in enhanced antifungal activity compared to each component alone (Praveen & Kannan, 2023; Roohallah et al., 2022). Furthermore, the CH-Mg-alg NC may strengthen physical barriers in plants, such as the cell wall, thereby hindering pathogen invasion and limiting the spread of disease (Wasule et al. 2024).

Likewise, nanocomposites can modulate the production and signaling of phytohormones such as salicylic acid, jasmonic acid, and ethylene, which play crucial roles in regulating plant defense responses. Additionally, nanocomposites containing minerals like magnesium nanoparticles (MgNPs) can enhance plant nutrient uptake and metabolism, which are essential for maintaining plant health and resilience against diseases. The presence of magnesium in MgNPs is essential for plant growth and the photosynthesis process, thereby improving vegetative growth characteristics such as shoot length, root length, plant fresh weight, plant dry weight, number of fruits, fruit fresh weight, and fruit dry weight (Ismail & Abd El-Gawad, 2021). Furthermore, nanocomposites may assist plants in mitigating environmental stresses, such as drought or high temperatures, which can compromise their defense mechanisms (Noman et al., 2023).

In the present work, the experimental treatments decrease magnesium levels beyond those naturally found in the environment. Magnesium absorption by plants was influenced not only by the available magnesium level in soil but also by the occurrence of various soil ions and by ion antagonism within the plant particularly the antagonism between potassium and magnesium (Yan & Hou, 2018). Correspondingly, treatment with CH-Mg-alg NC including *U. fasciata* extract (which was rich in potassium and used as organic fertilizer) (El-Gendy et al., 2023) increases potassium level, thus inhibiting absorption or breakdown of magnesium salts. Moreover, application of

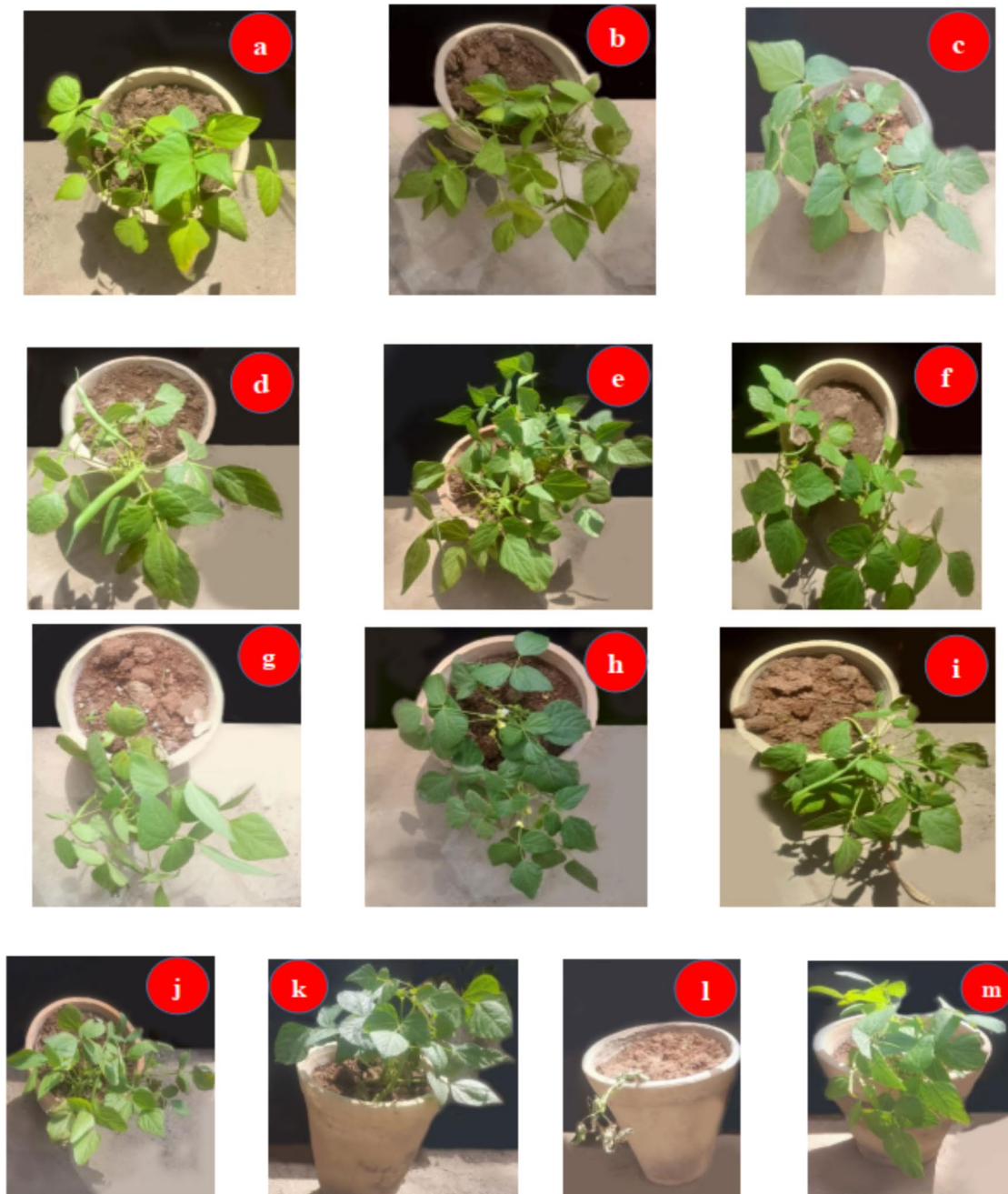


Fig. 8 Common bean growth features as influenced by application of different treatments under *in vivo* conditions. **(a)** Algae (seed soaking); **(b)** CH-alg NC (soil drenching); **(c)** Algae (soil drenching); **(d)** CH-Mg-alg NC (seed soaking); **(e)** nega-

tive control; **(f)** CH-Mg-alg NC (soil drenching); **(g)** $MgCl_2$ (seed soaking); **(h)** CH-alg NC (seed soaking); **(i)** $MgCl_2$ (soil drenching); **(j)** MgNPs (seed soaking); **(k)** Fungicide; **(l)** positive control and **(m)** MgNPs (soil drenching)

Table 9 Correlations between growth parameters and disease severity through the two growing seasons

Growth parameter	DS (%) (First season)	DS (%) (Second season)
Shoot length	0.80**	0.89**
root length	0.85**	0.86**
Plant fresh weight	0.83**	0.83**
Plant dry weight	0.79**	0.76**
Number of fruits	0.76**	0.75**
Pods fresh weight	0.77**	0.79**
Pods dry weight	0.78**	0.78**

**Significantly Strong Correlation (Pearson correlation at probability ≤ 0.01)

Table 10 Residues of Magnesium element in soil and pods

treatment	In soil (mg/kg soil)	In pods (%)
-ve control	100.40	0.28
+ ve control	101.20	0.26
fungicide	78.80	0.22
Seed soaking		
CH-Mg-alg NC	88.6	0.27
MgNPs	86.0	0.15
MgCl ₂	25.80	0.21
Soil drenching		
CH-Mg-alg NC	100.6	0.21
MgNPs	49.0	0.22
MgCl ₂	29.0	0.22

Mg compounds like, MgCl₂ or MgNPs lowers the pH level which further reduces magnesium uptake (Ishfaq et al., 2022). Those findings support the safe application for disease management without the risk of excessive magnesium accumulation in the soil or plant tissues and offer sustainable approach to alleviate concerns related to magnesium buildup (Rashid et al., 2023; Yousaf et al., 2021).

Our study introduced two application methods: seed soaking and soil drenching. The results indicated that seed soaking was more effective than soil drenching in managing disease and improving vegetative growth parameters under infection stress (Mohamadineia et al., 2015). Seed soaking has been proposed by Rocha et al. (2019) as a method for delivering beneficial microbes to agricultural crops,

offering direct protection to seeds and emerging seedlings while minimizing environmental impacts.

In contrast, soil drenching involves the application of liquid substances directly to the soil at the base of the plant at various developmental stages. This method tends to have a slower impact, as the applied chemicals require time to be absorbed by the roots and transported to other parts of the plant (Youssef et al., 2022). Soil drenching may also pose environmental risks related to leaching or runoff and requires careful application to prevent phytotoxicity or root damage (Asmawi et al., 2024).

In conclusion, further research is needed to fully elucidate the mechanisms by which nanocomposites influence plant defense responses. Nevertheless, the potential of nanocomposites to enhance plant immunity holds promise for developing sustainable and effective disease management strategies in agriculture.

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Author contributions M. F. developed the research conception, and performed statistical analysis. All authors contribute in methodology and wrote the first draft of the manuscript. M.F. and A. Y. substantially revised the intellectual content of the manuscript and prepared the final written draft. All authors approved the final manuscript.

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Data Availability Data of this study is completely available in manuscript.

Declarations

Ethical approval Not applicable.

Competing interests Authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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