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Full Length Article

Biocontrol effect of ginger glycoprotein and essential oil against *Vicia faba* damping-off caused by *Fusarium solani* and *Rhizoctonia solani*

Hossam S. El-Beltagi [a,*,1], Ali Osman [b,**,1], Mahmoud Sitohy [b], Hayfa Habes Almutairi [c], Eman Eldesouky [b], Entsar Abbas [d], Ahmed Mahmoud Ismail [e,f], Hazem S. Elshafie [g], Ippolito Camele [g,***]

[a] *Agricultural Biotechnology Department, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa, 31982, Saudi Arabia*
[b] *Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig, 44511, Egypt*
[c] *Chemistry Department, College of Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia*
[d] *Plant Pathology Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt*
[e] *Department of Arid Land Agriculture, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia*
[f] *Pests and Plant Diseases Unit, College of Agriculture and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia*
[g] *Department of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Via Dell'Ateneo Lucano 10, Potenza, 85100, Italy*

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ABSTRACT

The *Fusarium solani* and *Rhizoctonia solani* are destructive soil-borne pathogens worldwide, significantly impacting crop yields and quality. Synthetic fungicides are usually used for their control despite their adverse environmental and human health impacts. Therefore, there is a growing interest in discovering natural alternatives to synthetic pesticides. This study aims to assess the effectiveness of ginger glycoprotein and essential oil (EO) as antifungal agents against *R. solani* and *F. solani*, correlated with diseases that cause root decay and wilt in *Vicia faba* L. An *in vitro* trial assessed the antifungal efficacy of ginger EO and glycoprotein singularly (at 10 and 25 μg/mL) compared to Rizolex-T 50 WP (at 25, 50, and 75 μg/mL) against *F. solani* and *R. solani* mycelium growth. Whereas, the *in vivo* trial evaluated the efficacy of ginger EO and glycoprotein at 50 and 100 μg/mL, compared to Rizolex-T 50 WP at 50 μg/mL, in reducing pre- and post-emergence damping-off percentages. Sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Fourier transform infrared spectroscopy (FTIR), High-performance liquid chromatography (HPLC), and particle size distribution were carried out to characterize seed glycoprotein. SDS-PAGE of the glycoprotein indicated two bands corresponding to 35 and 29 KDa. An HPLC examination of the glycoprotein carbohydrate component identified glucose, mannose, and fructose. GC-MS analysis of the components of EO identified 28 majors' substances. *R. solani* and *F. solani* mycelial proliferation was dose-dependently inhibited by both EO and glycoprotein. Scanning Electron Microscope (SEM), used for investigating eventually morphological changes in tested fungi after treatment. In the *in vivo* test, it was seen that both EO and glycoprotein at 50 and 100 μg/mL made the symptoms caused by the tested fungi a lot less severe compared to the control. The results showed that ginger glycoprotein and essential oil might be able to be used instead of synthetic fungicides to treat *V. faba* damping off.

1. Introduction

Faba bean (*Vicia faba* L.) is commonly used for animal feed, including poultry, pigs, and horses, as well as human use (Sahile et al., 2011). Faba bean has a rich content of vitamins, carbohydrates, and a high protein content (20–41 %). So, it is recommended as a meat substitute (Abdel-Monaim, 2013). Numerous crops grown in diverse soil types are susceptible to multiple soil-borne fungal pathogens causing wilt and root-rot diseases. Faba beans are susceptible to the attack of many phytopathogens worldwide. Soil-borne infections are among the most

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: helbeltagi@kfu.edu.sa (H.S. El-Beltagi), Aokhalil@zu.edu.eg (A. Osman), ippolito.camele@unibas.it (I. Camele).
[1] These authors share first authorship.

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dangerous diseases infecting crop plants worldwide since they attack different plant species, causing root-rot and damping-off diseases, affecting crop productivity and reducing yield (Abdel-Kader et al., 2011). *R. solani* J.G. Kühn, *F. solani* (Mart.) Sacc., and *Macrophomina phaseolina* (Tassi) Goid. Are known to attack the roots and stems of faba bean and various other economically important crops, resulting in significant reductions in seed germination (Abdel-Kader et al., 2011). *R. solani* can attack more than 2000 plant species (Dawar et al., 2007). Traditionally, controlling these diseases primarily involves soil application, seed treatment, and foliar spraying with systemic fungicides (Kazempour, 2004). These practices cost a lot and adversely affect human health and environment (Vapnek et al., 2007). Despite their rapid efficacy in controlling plant diseases, fungicides are, in fact, one of the main environmental pollutants. It is also impractical to manage synthetic fungicides, as many are only effective at phytotoxic levels (Jarvis, 1988). Thus, there has been a lot of attention regarding the discovery of novel natural substances as potential alternatives for controlling diseases (Khunnamwong et al., 2020; El-Beltagi et al., 2024a). Several researchers reported new biocides-based approaches for the control of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse (Bowers and Locke, 2000; Moretti et al., 2002). Among the most important biocides with promising biological effects are plant essential oils (EOs) and concentrated hydrophobic liquids containing volatile compounds extracted from plants (Camele et al., 2021). Plant EOs are composed of mixtures of terpenoids and other vital secondary metabolites (Elshafie et al., 2023). *Ginger* (*Zingiber officinale* Roscoe) is utilized for a variety of purposes, e.g., cosmetics, pharmaceuticals, and meat products, based on its excellent antioxidant activities (Osae et al., 2019; Hernandez et al., 2021). Consumers are more aware of and inclined towards foods that have elevated levels of antioxidants (Barba et al., 2016). Potential fungicides derived from various sources, such as glycoproteins (Srinivasan, 2017; El-Beltagi et al., 2024b; El-Beltagi et al., 2024c), natural products, and EOs could serve as natural alternatives. The primary goal of this research was to find an economically viable essential oil and glycoprotein isolated from ginger with antifungal properties against *R. solani* and *F. solani* responsible for damping-off.

2. Materials and methods

2.1. Chemicals and plant materials

Ginger rhizomes were obtained from the local market in Zagazig City (Egypt), dried, and ground into fine powder. Electrophoresis reagents (acrylamide, bis-acrylamide, N, N,N′,N' -tetra methyl ethylene diamine, sodium dodecyl sulphate, ammonium per sulphate, Tris, glycine, methanol, acetic acid glacial, and Coomassie Brilliant Blue R-250 dye) were purchased from Bio-Rad laboratories (Richmond, CA, USA). One cultivar of faba bean (Giza843) were kindly obtained from the Legume Crop Research Department, Field Crop Research Institute, Agricultural Research Center, Giza (Egypt).

2.2. Essential oil extraction and identification

2.2.1. Extraction

The extraction of studied EO from ginger rhizomes was carried out as follows. In a conical flask (1 L), 100 g of ground ginger rhizomes were combined with 500 mL of sterile distilled water (SDW). The mixture was then heated to boiling point using the Clevenger apparatus (Glassco Laboratory Equipments Pvt. Ltd., Manglai, P.O. Khudda Kalan, Ambala Cantt, Haryana 133004, India). After 5h of distillation into a graduated cylinder with the steam and EO, the steam and aqueous layer were separated. The EO was refrigerated until it was analyzed (Singh et al., 2008).

2.2.2. GC-MS analysis

The unknown components were identified by comparing their spectrum with the known components registered in NIST and Wiley libraries (Singh et al., 2005). Hewlett Packard GC model HP- 6890 with 30-m 5 % phenyl dimethyl siloxane (HP-SMS) column equipped with Hewlett Packard MS model HP- 5973 detector was utilized to identify single components (Kamaliroosta et al., 2013).

2.3. Glycoprotein extraction and characterization

2.3.1. Extraction

Two g of ground ginger rhizomes were mixed for 3 h with 71 mL extraction buffer (0.1 mol L−1 NaCl solution with 0.2 mol L−1 K2SO4, at 50 °C (35.5:1, v/m) at pH 7.5). The extract was precipitated by adding refrigerated ethanol 95 % (4:1, *v/v*). Supernatant was separated by centrifugation at 5000×*g*/10 min. The residue was mixed with SDW and dialyzed against deionized water at 4 °C for two days. The dialyzed solution was freeze-dried to extract the raw ginger glycoproteins (Zhao et al., 2018).

2.3.2. Characterization

SDS-PAGE gel electrophoresis. The extracted ginger glycoprotein was identified following SDS-PAGE method. A 20 mg of ginger glycoprotein was mixed with 100 μL of β-mercaptoethanol in 1 mL SDS (10 %), vortexed for 15 min, centrifuged at 10,000 g for 5 min. Twenty microliters of recovered precipitate were combined with 20 μL SDS-loading sample buffer and heated for 5 min at 96 °C. Every lane received an electrophoresis aliquot of 10 μL, and SDS-PAGE was run (Laemmli, 1970).

2.3.2.1. FT-IR spectroscopy. Ginger glycoprotein samples were prepared for FT-IR following KBr pellet protocol (Souillac et al., 2002) before tracing by FT-IR Spectroscopy (Nicolet Nexus 470, DTGS, Thermo Scientific, Waltham, USA) at 25 °C. Each resulting spectrum comprised 256 interferograms with resolution of 4 cm^{-1} and 64 scans with a 2 cm^{-1} interval from 4000 to 400 cm^{-1} regions. Second derivation spectra utilized Savitsky–Golay derivative function soft (Surewicz and Mantsch, 1988). The protein secondary structure relative proportion was calculated from infrared second derivative amide spectra by computing areas under bands referring to specific substructures.

2.3.2.2. HPLC. HPLC was used to estimate the carbohydrates in ginger glycoprotein according to Wilson et al. (1981). Mobile phase consisted of 0.002 M sulfuric acid in water. HPLC operating parameters were as follows: column temperature of 60 °C, mobile phase flow rate of 0.8 mL/min, a 10 μL injection volume, and a 40 °C RI detection temperature.

2.3.2.3. Particle size distribution. Ginger glycoprotein's particle size distribution was assessed using a Delsa Nano C Instrument (Malvern Instruments, Westborough, MA) at pH 7, 0.2 % (w/w) concentration, and room temperature, in accordance with Elimelech et al. (1994) methodology.

2.4. Fungal isolation and identification

2.4.1. Isolation

In the winter of 2019, samples of faba bean plants exhibiting symptoms of root rot disease were collected from three different fields in Egypt (Ismailia, El-Huseiniya, and Zagazig). To isolate eventually fungi associated with wilt and root rot symptoms, five symptomatic plants from each location were transported in paper bags to the Plant Pathology Laboratory, Faculty of Agriculture, Zagazig University (Egypt). The roots were washed with tap water, cut into small pieces, and sterilized for 2 min with sodium hypochlorite 3 %. The pieces were then rinsed repeatedly with sterile distilled water (SDW) and dried on sterile filter paper. The root pieces were placed on potato dextrose agar (PDA) enriched with penicillin (20 IU/ml) and incubated at 25 ± 2 °C for 5–7

days, during which they were monitored daily for fungal growth.

2.4.2. Morphological identification

Fungal colonies were purified and preliminary identified based their morphological and microscopical characters using light microscope (Axioskop—ZEISS, Oberkochen, Germany).

2.5. In vitro antifungal activity assay

For this trial, it was selected one isolate of *R. solani* and *F. solani* based on the preliminary morphological and molecular identification. An *in vitro* trial was conducted to assess ginger EO efficacy (at 10 and 25 µg/mL) and glycoprotein (at 10 and 25 µg/mL) in comparison to Rizolex-T 50 WP (at 25, 50, and 75 µg/mL) against *F. solani* and *R. solani* mycelium growth (Euloge et al., 2012). Briefly, 100 mL of PDA was added to conical flasks to which EO, glycoprotein, and Rizolex-T 50 WP were added individually at 45 °C.

One millilitre of Tween 20 (Merck Life Science, Milan, Italy) was mixed with 99 mL of EO to create an emulsion. The nutrient medium was supplemented with 0.19 g/L of chloramphenicol to avoid any bacterial contamination. The sterile Petri dishes were filled with 15 mL of the treated medium and allowed to solidify, then each plate was inoculated with a fresh fungal disc (Ø 5 mm). Control plates were singularly inoculated only with each tested fungi were considered as negative control. All plates were incubated at 24 ± 2 °C until full growth of control. Antifungal activity was calculated as fungal linear growth reduction (%) in each tested treatment compared to controls (Equation (1)).

$$\text{Fungal linear growth reduction (\%)} = \frac{\text{Control linear growth (Cm)} - \text{treatment linear growth (Cm)}}{\text{Control linear growth (Cm)}} \text{ x } 100 \quad \text{(Equation 1)}$$

2.6. Scanning electron microscopy (SEM)

SEM examination was used to evaluate the hyphal deteriorations of *F. solani* and *R. solani* after treatment with ginger EO and glycoprotein at 25 µg/mL for 7 days compared to control (Sitohy et al., 2013). A 500 mL Erlenmeyer flask containing 100 mL of PDB was supplemented with 50 µL of either the tested materials or SDW as a control. Afterward, 1 mL of 5 x 10^7 conidia mL-1 of the fungi being tested was added. After being incubated for 7 days at 25 °C with rotary shaker at 50 rpm, fungal biomass was retrieved through centrifugation at 4500×*g* for 15 min at 4 °C. The biomass was then rinsed with PBS (pH 7.4) and kept in 2.5 % glutaraldehyde in PBS. Fungal pellets were fixed, dehydrated, dried, and mounted onto stubs using double-sided carbon tape. Fungal specimens were gold-coated using a Polaron sputter coater and examined by Quanta FEG-250 SEM instrument (FEI Company, Eindhoven, The Netherlands).

2.7. In vivo experiment (Pot trial)

Seeds were immerged for 8 h either in EO or glycoprotein solutions at 50 and 100 µg/mL, respectively. Based on the initial trial, it was observed that these tested concentrations did not exhibit any detrimental impacts on seed germination. A subset of seeds was subjected to immersion in an emulsion containing Rizolex-T 50 WP at 50 µg/mL as synthetic fungicide, the advisable dose of the producer. Another subset of seeds was immerged only in SDW and considered as negative control. The inoculum for the fungal inoculation was prepared as following. Each studied fungi (*R. solani* and *F. solani*) were singularly cultivated in potato dextrose broth (PDB) and incubated for 5 days at 25 °C under shaking at 120 rpm. The fungal biomass was harvested and rinsed twice with double volume of distilled water. The excess liquid was eliminated and the biomass taken after that for soil inoculation. Five grams of each harvested fungal biomass was suspended in 100 mL of distilled water and mixed with 750 g of sterile soil. The treated seeds were left for drying for 2 h and 6 seeds/pot were planted either in inoculated and uninoculated soils with fungal pathogens (single pathogens or their combination). The experiment was carried out in triplicates with five pots per replication (Somda et al., 2007). Percentages of damping-off either pre- or post-emergence as well as disease incidence (%) were calculated using equations (2)–(4).

$$\text{Pre} - \text{emergence (\%)} = \frac{\text{N. of unmerged seeds}}{\text{Total N. of seeds}} x\ 100 \quad \text{Equation (2)}$$

$$\text{Post} - \text{emergence (\%)} = \frac{\text{Total N. of dead seedlings}}{\text{Total N. of seeds}} x\ 100 \quad \text{Equation (3)}$$

$$\text{Disease incidence (\%)} = (\text{No. of survived plants} / \text{Total No. of sown seeds}) \text{x} 100 \quad \text{Equation (4)}$$

2.8. Statistical analysis

SPSS-25 was utilized to conduct averages of groups, standard error and a One-way analysis of variance (ANOVA) in order to compare means of treatments. Post-hoc test was conducted using Duncan's multiple range test to determine level of significance.

3. Results and discussion

3.1. GC-MS analysis of ginger EO

Fig. 1 presents chemical composition of ginger EO as assayed by GC-MS. Table S1 displays respective retention time and area (%) each single constituent present in ginger EO. In particular, the results showed that ginger EO contains 28 major components, where the principal ones are: cis-sabinene hydroxide (15.62 %), epiglobulol (5.48 %), camphene (4.03 %), linalool (3.84 %), beta-cedren-9alpha-ol (3.53 %), cedrene (2.98 %) whereas the existence of other constituents lower than 2 %. The GC-MS outcomes are in contrast with da Silva et al. (2018) who found that α-zingiberene was principal constituent of ginger EO. These variations attributed to genetic factors, plant age, environmental factors, soil properties, and methods used for extraction.

3.2. Glycoprotein characterization

3.2.1. SDS-PAGE and carbohydrates analysis

The SDS-PAGE identified two distinct bands, measuring approximately 35 and 29 KDa, which correspond to the ginger glycoprotein (Fig. S1A). HPLC examination of ginger glycoprotein carbohydrate component recorded two aldohexoses (glucose and mannose), and one ketohexose (fructose). The content of glucose, mannose, and fructose were 4.45, 4.80, and 10.36 µg/g, respectively (Fig. S1B).

3.2.2. FT-IR and particle size distribution

The IR-spectra between wave numbers 500 and 4000 cm^{-1} for ginger glycoprotein is shown in Fig. 2A.

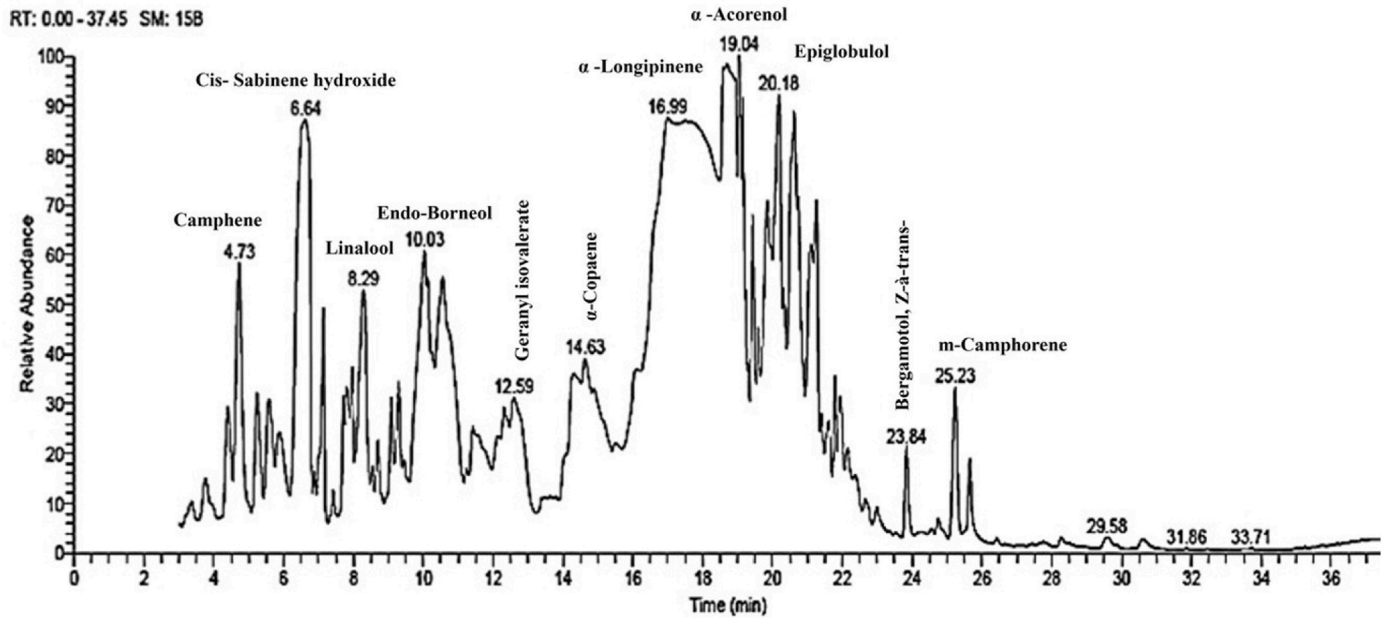

Fig. 1. The GC-MS of profile of the chemical analysis of ginger essential oil.

Primary absorbance peak was included among 1065 and 1630 cm^{-1} (Amide I). Other significant peaks were detected at 1521 cm^{-1} in Amide II region and at 1065 cm^{-1} in carbohydrate region. Protein secondary structure typically relies on spectroscopic technologies such as FT-IR (Lewis et al., 2013). The protein backbone presents several wave number regions of infrared absorption. The Amide I vibration in proteins produces an absorbance peak at 1650 cm^{-1} arising mainly from C═O stretching and at a minor extent from out-of-phase C–N stretching and in-plane N–H bending (Barth, 2007). Several FT-IR studies recorded correlation between wave number positions and protein structural sub-types, contributing to overall Amide I band. α-helices absorption band is centred around 1656 cm^{-1} (Nevskaya and Chirgadze, 1976). β-Sheets exhibited many absorption bands within range of 1624 cm^{-1} to 1642 cm^{-1} (Dong et al., 1990). β-Turn structures exhibit several absorbance bands among 1660 cm^{-1} and 1690 cm^{-1}, 1691 cm^{-1}, 1696 cm^{-1} (Dong et al., 1990). Finally, unordered structures (random coil), produced a peak at 1650 cm^{-1} (Dong et al., 1990).

Fig. 2B shows particle size distribution of ginger glycoprotein. The sample shows bimodal (2 peaks) particle size distribution (% intensity), the 1st one was recorded at a size 214.3 d nm with an intensity 13 %, and the 2nd one was recorded at a size 4617 d nm with an intensity 2 %.

3.3. Morphological identification of isolated fungi

The dominant isolated fungi have been identified based on their morphological characteristics as following. In particular, *R. solani* has a septate mycelium that is typically brown to dark brown, exhibiting a cottony texture. The hyphae are generally thick-walled and irregularly shaped This fungus can produce sclerotia, hard compact masses of hyphae, that are dark brown to black and vary in size and shape (Desvani et al., 2018). Whereas *Fusarium solani* has a septate mycelium that ranges in color from white to light pink or yellow. It produces conidiophores, which are specialized hyphae that bear conidia (asexual spores). The conidia are often cylindrical or ellipsoidal, typically measuring about 10–30 μm in length, and can be produced in chains or clusters at the tips of the conidiophores. *F. solani* can form chlamydospores, which are generally round to oval, thick-walled resting spores that help the fungus survive under adverse conditions (Co'rdova-Albores et al., 2014).

3.4. In vitro antifungal activity

Rizolex-T 50 WP (synthetic fungicide) inhibited *F. solani* and *R. solani* mycelial growth in a concentration-dependent manner on Petri dishes solid agar after incubation (Fig. S2). The highest growth reduction of *F. solani* (95 %) was observed at 75 μg/mL, whereas the highest reduction of *R. solani* (95 %) was observed either 50 or 75 μg/mL concentrations.

On the other hand, ginger EO and glycoprotein inhibited *F. solani* and *R. solani* mycelial growth (Fig. 3). but to a lesser extent than Rizolex-T 50 WP. The EO at 25 μg/ml exhibited highest mycelium growth reduction effect against *F. solani* (76 %) and *R. solani* (36 %). Whereas glycoprotein at 25 μg/ml exhibited most mycelium growth reduction effect *F. solani* (42 %) and *R. solani* (83 %).

Recent studies have provided evidence supporting our findings (Giriraju and Yunus, 2013; Bordoh et al., 2020; Makhuvele et al., 2020). Ginger crude extract at 10 g/L had significant *in vitro* antifungal activity against *Colletotrichum gloeosporioides* (Bordoh et al., 2020). The extract induced effective inhibition extents against mycelial growth and conidial germination amounting to 87.50 % and 88.48 %, respectively. The recorded antifungal activity of glycoproteins and plant essential oil could be achieved by damaging respiratory system, disturbing energy metabolism, enhancing cell membrane permeability, and reducing cell wall-degrading enzyme activities as recorded by Di Pasqua et al. (2007) and Liu et al. (2017).

3.5. Scanning electron microscopy

SEM examination for *F. solani* and *R. solani* after exposure to ginger EO and glycoprotein at 25 μg/mL are shown in Fig. 4.

The mycelium in the control treatment had a normal, straight, and uniform shape. Its cell walls were also regular, uniform, full, and thicker than usual, and the tips of the hyphae were tapered and smooth. However, the application of the studied EO and glycoprotein caused changes in these typical morphological structures. The effectiveness of both ginger essential oil (EO) and glycoprotein can be attributed to their chemical composition, particularly the presence of compounds such as linalool and camphene. Abdullahi et al. (2020) noted that camphene is a dominant constituent in domestic ginger rhizome, accounting for 16.93 % of its composition. Additionally, Shaukat et al. (2023) highlighted

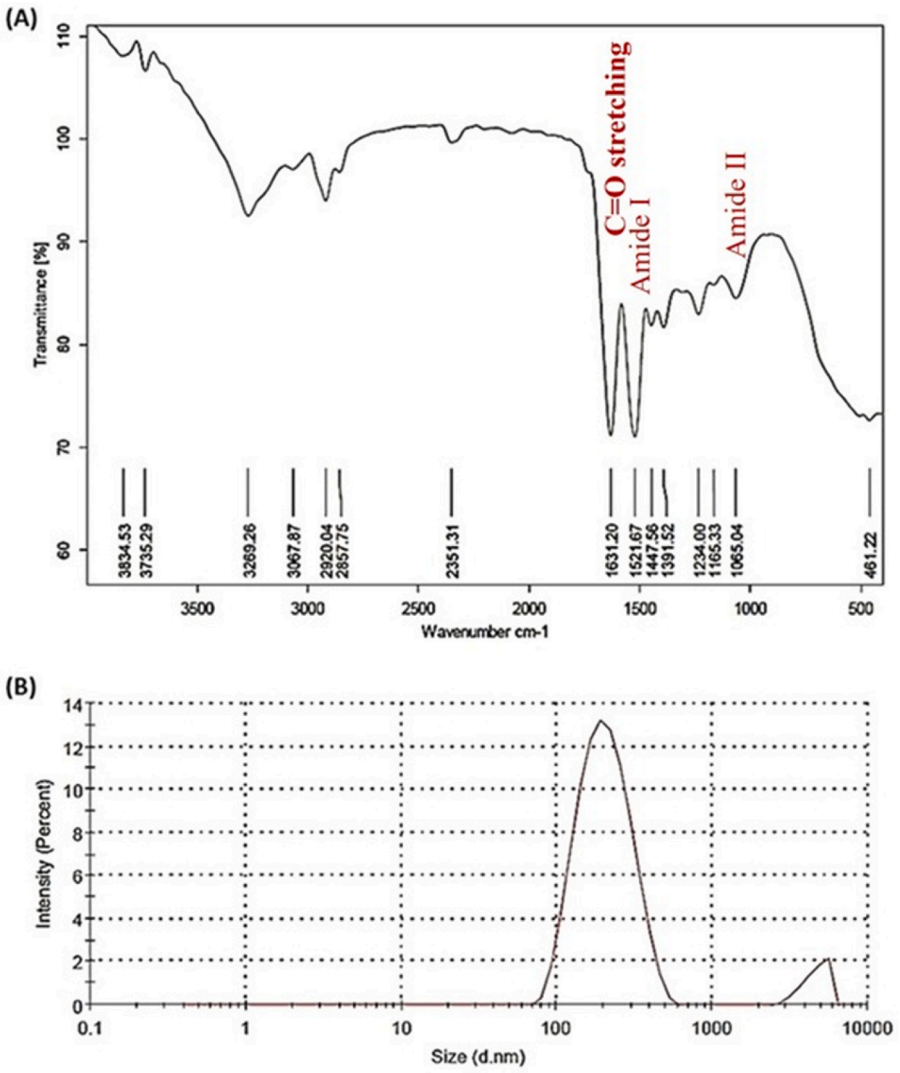

Fig. 2. FT-IR spectra (A), and particle size distribution (B) of ginger glycoprotein.

that linalool is one of the volatile bioactive substances found in ginger. It is concluded that ginger EO and glycoprotein can be effectively used for controlling *F. solani* and *R. solani*. The relative conductivity can serve as an essential indicator of cell membrane permeability (Zhu et al., 2019; Efenberger-Szmechtyk et al., 2021). Research has shown that several antimicrobial compounds can damage the cell membrane integrity in pathogens and as a result, raise the relative conductivity of mycelia (Bouyahya et al., 2019).

3.6. *In vivo antifungal activity*

The findings obtained from the greenhouse trial are presented in Tables 1–3, and Figure S3 (A-C). Overall, all tested treatments (EO, glycoprotein, and Rizolex-T 50 WP) greatly decreased both pre- and post-emergence damping-off caused by *F. solani*, *R. solani*, and their combination, when compared to control treatment.

In particular, regarding *R. solani*, ginger EO at a concentration of 100 µg/mL resulted in a disease incidence of about 11 %, while the glycoprotein at the same concentration showed no disease incidence. While, C + ve and C-ve exhibited a disease incidence of about 33 and 22 %, respectively (Table 1).

In case of *F. solani*, the EO and glycoprotein at a concentration of 100 µg/mL resulted no disease incidence equal to the effect of Rizolex-T 50 WP, compared to C + ve and C-ve which exhibited a disease incidence of about 28 and 22 %, respectively (Table 2).

However, in the case of a combination between the two tested pathogens, the EO at a concentration of 100 µg/mL showed about 11 % of disease incidence and glycoprotein at the same concentration showed no disease incidence, however the Rizolex-T 50 WP showed 22 %, compared to C + ve and C-ve which exhibited a disease incidence of about 17 and 22 %, respectively (Table 3).

During various growth stages, soil-borne fungi cause wilt and damping-off diseases in faba beans (Atwa, 2016). Specific plant extracts can inhibit plant diseases and enhance oxidative enzymes in plants. This increase in enzymes plays a vital role in protecting against disease infections (Staerck et al., 2017). The active ingredients in plant products may either directly attack the pathogen or work on it through other mechanisms that have been studied (Junaid et al., 2013).

Ginger glycoprotein alongside other biochemicals such as essential oil, play an important role in fungal inhibition and disease control. In particular, glycoproteins may contribute in cell wall disruption affecting its structure and integrity, leading to cell death or impaired growth. Glycoproiteins may also increase the permeability of the fungal cell membrane, disrupting vital processes and inhibit the enzyme activity. In addition, the ginger glycoproteins significantly reduce fungal mycelium growth, limiting the spread of some fungal phytopathogens.

In particular, Girish et al. (2006) studied the antifungal effects of a non-toxic glycoprotein isolated from the root tubers of Withania

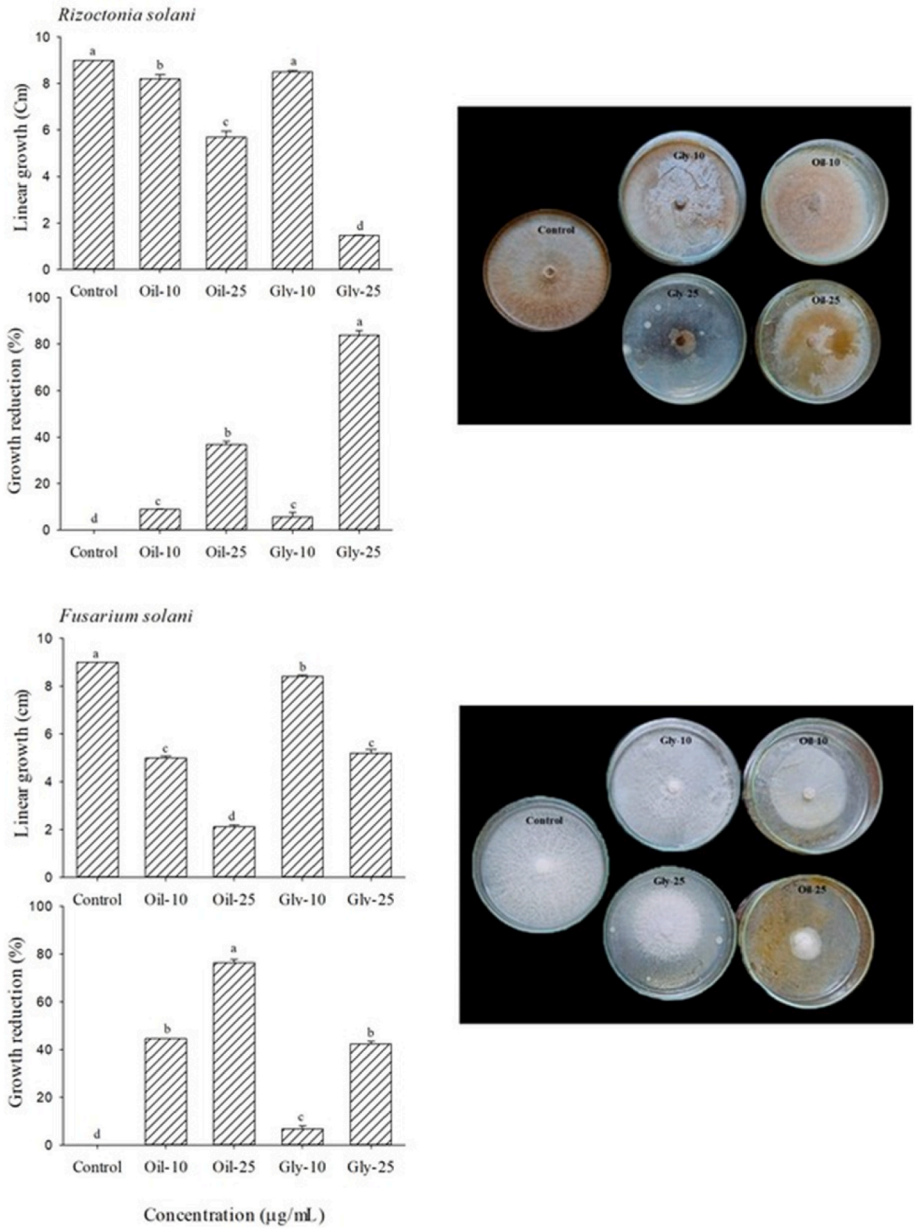

Fig. 3. Antifungal activity of ginger EO and glycoprotein against *F. solani*, and *R. solani* at different concentrations (10 and 25 µg/mL) compared to control. Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

somnifera. They found that this glycoprotein demonstrated potent antifungal activity against Aspergillus flavus, Fusarium oxysporum, and Fusarium verticillioides. The authors concluded that glycoproteins could serve as safe and effective alternatives to synthetic fungicides.

Additionally, Ma et al. (2023) reported that glycoproteins involved in chitin metabolism and cell wall morphogenesis provide an effective strategy for combating fungal pathogens. This approach works by simultaneously altering cell wall plasticity, activating chitin-triggered immunity, and impairing fungal viability.

4. Conclusion

R. solani and *F. solani* are two of most harmful soil-borne diseases in the world, and the quality and productivity of many crops are greatly impacted by these infections. The use of ginger EO and glycoprotein showed a potential inhibition effect on *R. solani* and *F. solani* growth, but to a lesser extent than Rizolex-T 50 WP. Comparing the hyphae structure of the negative control, the microscopical analysis revealed that the treatment with ginger EO and glycoprotein had a significant impact on morphological changes, including distortion, deformation, and fracturing, as well as a loss of smoothness. The antifungal activity observed in the studied glycoproteins and essential oils (EO) may be attributed to their chemical composition, specifically the presence of compounds like linalool and camphene. Additionally, glycoprotein and ginger EO have a high reducing action on the pre- and post-emergence damping induced by *R. solani* and *F. solani.* The obtained promising findings might provide a possible natural alternative to control these pathogens and reduce excessive use of synthetic fungicides, which are harmful to both human health and environment.

CRediT authorship contribution statement

Hossam S. El-Beltagi: Writing – review & editing, Funding acquisition, Data curation. **Ali Osman:** Writing – review & editing, Writing –

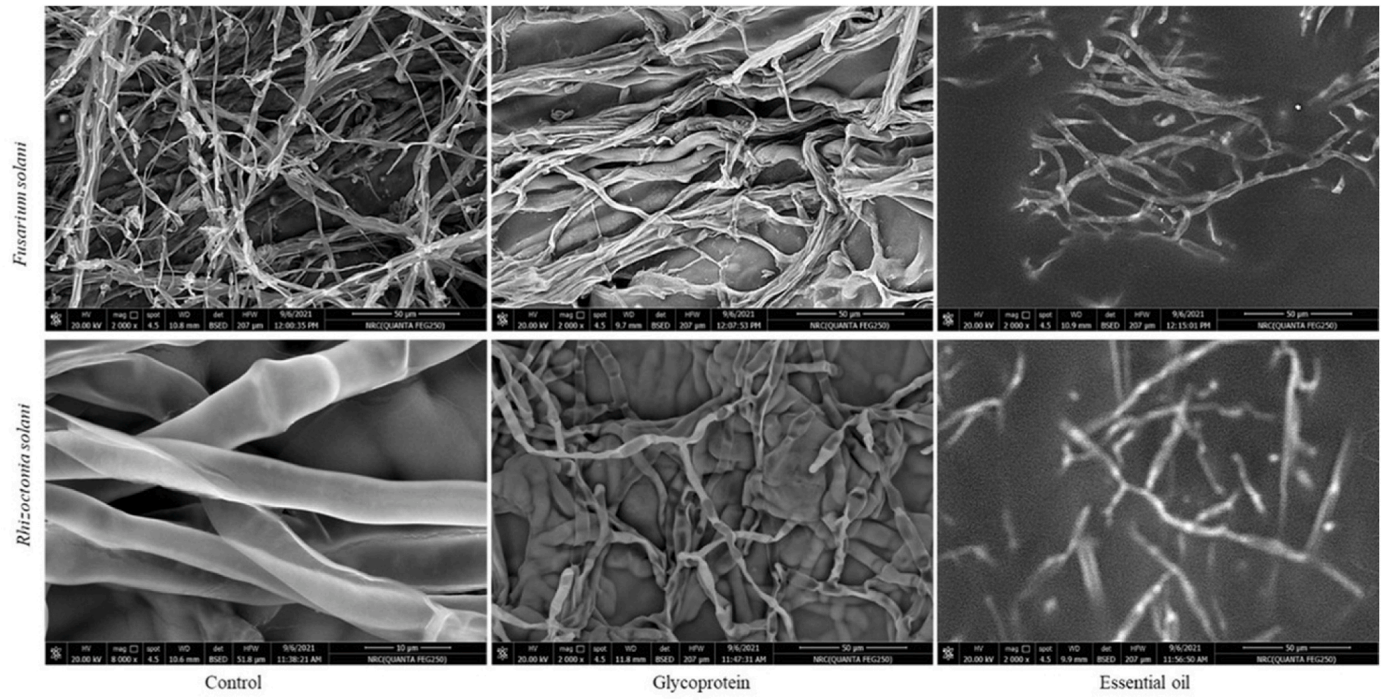

Fig. 4. Scanning electron microscopy of *F. solani* and *R. solani* treated with ginger glycoprotein (25 μg/mL) and EO (25 μg/mL) compared to control.

Table 1
Effect of ginger EO and glycoprotein (50 and 100 μg/mL) on damping-off disease incidence (%) of *V. faba* caused by *R. solani*, under greenhouse condition compared to Rizolex-T 50 WP.

Treatments	Pre-emergence	Post-emergence	Healthy	Disease incidence (%)
C-ve	0.667 ± 0.33 ^{ab}	0.667 ± 0.33 ^{a}	4.667 ± 0.33 ^{bc}	22.23 ^{b}
C + ve	1.333 ± 0.33 ^{a}	0.667 ± 0.33 ^{a}	4.000 ± 0.58 ^{c}	33.34 ^{a}
EO-50	0.667 ± 0.33 ^{ab}	0.000 ± 0 ^{b}	5.333 ± 0.33 ^{ab}	11.12 ^{c}
EO-100	0.000 ± 0 ^{b}	0.000 ± 0 ^{b}	6.000 ± 0 ^{a}	0.00 ^{d}
Gly-50	0.000 ± 0 ^{b}	0.000 ± 0 ^{b}	6.000 ± 0 ^{a}	0.00 ^{d}
Gly-100	0.000 ± 0 ^{b}	0.000 ± 0 ^{b}	6.000 ± 0 ^{a}	0.00 ^{d}
Rizolex-T 50 WP	0.000 ± 0 ^{b}	0.000 ± 0 ^{b}	6.000 ± 0 ^{a}	0.00 ^{d}

Values followed by different letters in each column for each treatment are significantly different at $p < 0.05$ according to Duncan's multiple comparison post hoc test using SPSS software. Data are expressed as the mean value of the damping-off disease incidence (%) for three replicates ± SDs. C-ve: control without fungal infection and treatments; C + ve: fungal infection control and without treatments; EO-50 and EO-100: seeds treated with ginger essential oil at 50 and 100 μg/mL, respectively; Gly-50, and gly-100: seeds treated with ginger glycoprotein at 50 and 100 μg/mL, respectively. Rizolex-T 50 WP was considered as synthetic fungicide and was used at the advisable dose of the producer.

Table 2
Effect of ginger EO and glycoprotein (50 and 100 μg/mL) on damping-off disease incidence of *V. faba* caused by *F. solani*, under greenhouse condition compared to Rizolex-T 50 WP.

Treatments	Pre-emergence	Post-emergence	Healthy	Disease incidence (%)
C-ve	0.667 ± 0.33^{ab}	0.667 ± 0.33^{a}	4.667 ± 0.33^{cd}	22.23 ^{b}
C + ve	1.333 ± 0.33^{a}	0.333 ± 0.33^{a}	4.333 ± 0.33^{d}	27.78 ^{a}
EO-50	1.000 ± 0^{ab}	0.000 ± 0^{a}	5.000 ± 0^{cd}	16.67 ^{c}
EO-100	0.000 ± 0^{b}	0.000 ± 0^{a}	6.000 ± 0^{a}	0.00 ^{e}
Gly-50	0.333 ± 0.33^{ab}	0.333 ± 0.33^{a}	5.333 ± 0.33^{bc}	11.12 ^{d}
Gly-100	0.000 ± 0^{b}	0.000 ± 0^{a}	6.000 ± 0^{ab}	0.00 ^{e}
Rizolex-T 50 WP	0.000 ± 0^{b}	0.000 ± 0^{a}	6.000 ± 0^{ab}	0.00 ^{e}

Values followed by different letters in each column for each treatment are significantly different at $p < 0.05$ according to Duncan's multiple comparison post hoc test using SPSS software. Data are expressed as the mean value of the damping-off disease incidence (%) for three replicates ± SDs. C-ve: control without fungal infection and treatments; C + ve: fungal infection control and without treatments; EO-50 and EO-100: seeds treated with ginger essential oil at 50 and 100 μg/mL, respectively; Gly-50, and gly-100: seeds treated with ginger glycoprotein at 50 and 100 μg/mL, respectively. Rizolex-T 50 WP was considered as synthetic fungicide and was used at the advisable dose of the producer.

original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Mahmoud Sitohy:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Data curation. **Hayfa Habes Almutairi:** Methodology, Formal analysis, Data curation. **Eman Eldesouky:** Methodology, Investigation, Formal analysis. **Entsar Abbas:** Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Ahmed Mahmoud Ismail:** Methodology, Formal analysis, Data curation. **Hazem S. Elshafie:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Ippolito Camele:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis.

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Table 3
Effect of ginger EO and glycoprotein (50 and 100 μg/mL) on damping-off disease incidence of *V. faba* caused by a combination between *F. solani* and *R. solani*, under greenhouse condition compared to Rizolex-T 50 WP.

Treatments	Pre-emergence	Post-emergence	Healthy	Disease incidence (%)
C-ve	0.667 ± 0.33 [a]	0.667 ± 0.33 [a]	4.667 ± 0.33 [b]	22.23 [a]
C + ve	0.667 ± 0.33 [a]	0.000 ± 0 [a]	5.333 ± 0.33 [ab]	11.12 [c]
EO-50	0.667 ± 0.33 [a]	0.333 ± 0.33 [a]	5.000 ± 0.58 [ab]	16.67 [b]
EO-100	0.333 ± 0.33 [a]	0.333 ± 0.33 [a]	5.333 ± 0.33 [ab]	11.12 [c]
Gly-50	0.000 ± 0 [a]	0.000 ± 0 [a]	6.000 ± 0 [a]	0.00 [d]
Gly-100	0.000 ± 0 [a]	0.000 ± 0 [a]	6.000 ± 0 [a]	0.00 [d]
Rizolex-T 50 WP	0.000 ± 0 [a]	0.000 ± 0 [a]	4.667 ± 0.33 [b]	22.23 [a]

Values followed by different letters in each column for each treatment are significantly different at $p < 0.05$ according to Duncan's multiple comparison post hoc test using SPSS software. Data are expressed as the mean value of the damping-off disease incidence (%) for three replicates ± SDs. C-ve: control without fungal infection and treatments; C + ve: fungal infection control and without treatments; EO-50 and EO-100: seeds treated with ginger essential oil at 50 and 100 μg/mL, respectively; Gly-50, and gly-100: seeds treated with ginger glycoprotein at 50 and 100 μg/mL, respectively. Rizolex-T 50 WP was considered as synthetic fungicide and was used at the advisable dose of the producer.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hossam El-Beltagi reports financial support was provided by King Faisal University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.kjs.2025.100437.

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