Zagazig J. Agric. Res., Vol. 51 No. (3) 2024 519-528

Biotechnology Research

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GINGER RHIZOME ETHANOLIC EXTRACT'S ANTIOXIDANT ACTIVITY AND ANTIFUNGAL ACTIVITY AGAINST *Fusarium solani* AND *Rhizoctonia solani*

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Received: 09/05/2024; Accepted: 19/05/2024

ABSTRACT: *Fusarium solani* and *Rhizoctonia solani* are highly destructive soil-borne diseases that have a significant impact on agricultural productivity and quality on a global scale. Ginger, a significant medicinal plant, is utilized as a substitute for chemical fungicides and bactericides due to the properties of its rhizome extract. This work aims to demonstrate the antifungal and antioxidant effects of the phytochemical components found in ethanolic crude extracts derived from ginger rhizomes. The ethanolic extract of ginger rhizome underwent phytochemical examination, which detected the presence of flavonoids, alkaloids, terpenoids, tannins, polysaccharides, saponins, and glycosides. The ginger rhizome's ethanolic extract has a concentration of 573.87 mg GAE/100 g extract for total phenols (TPCs) and 232.43 mg QE/100 g extract for total flavonoids (TFCs). The DPPH radical scavenging activity exhibited a significant increase, rising from 13% to 89%, as the concentration of the extract was raised from 100 to 600 µg/mL. Our observation revealed that the ethanolic extract derived from ginger rhizome effectively suppressed the growth of *F. solani* and *R. solani* mycelium on solid agar medium Petri dishes. The degree of inhibition was found to be directly proportional to the concentration of the extract. Our research suggests that ginger has the potential to be a valuable source of natural antioxidants and antifungal substances.

Key words: Ginger, ethanolic extract, antifungal, antioxidant, phytochemical.

INTRODUCTION

Plant diseases can have devastating effects on crops during both the production and post-harvest stages. They can reduce global yields by as much as 30%, leading to the production of inferior-quality grains, fruits, and vegetables. Pathogens, which attack and damage the plants, cause abiotic and biotic stresses, which pose serious threats to crop growth and health (**Li *et al.*, 2022**). *Fusarium oxysporum* and *Fusarium solani*, the causes of Fusarium wilt, pose significant threats to the production and post-harvest storage of many fruits and vegetables (**Zakaria, 2023**). Most Fusarium species reside in the soil, where they can function as either saprophytes or pathogens. Fusarium can infect both above-ground and below-ground plant parts, either as the primary or secondary pathogen **(Summerell *et al.*, 2003**). Fusarium species produce conidia that can spread through air, rain, and irrigation water. Some species also produce chlamydospores that can survive in soil and plant debris for extended periods (**Lin *et al.*, 2013**). Rhizoctonia solani is well known as a soil borne pathogen that can attack a wide range of plant species causing seed decay, damping off, hypocotyl rot and web blight. Furthermore, *R. solani* (AG-4) is the causal agent of root rot disease of bean under greenhouse and field conditions that causes great damage to beans and severe economic losses **Ahed and Kamil (2013)**. Chemical fungicides are currently mainly used to control Fusarium wilt; however,

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because of anthropogenic activities, such as incorrect fertilizer application, the extensive use of chemical fungicides has led to numerous serious issues, including ecological imbalances, pollution of the environment, and health risks for both humans and animals. Additionally, infections will become less sensitive to chemical fungicides and develop resistance to the variety of fungicides that are frequently used (**Zhang *et al.*, 2019**). It is important to find natural fungicides that are highly effective, less toxic, and leave no residues. Research has shown that bioactive proteins (**Abbas *et al*., 2023; Abbas *et al.*, 2020; Atallah *et al.*, 2021; Elshafie *et al.*, 2023; Osman *et al.*, 2016; Osman *et al.*, 2023a; Osman *et al.*, 2023b**), and plant extracts have the potential to inhibit fungal diseases, which makes the exploration of botanical fungicides and antifungal plant substances a promising area of study **(Bhandari *et al.*, 2021**). Phytochemicals are biologically active compounds found in plants. Interest in their potential health benefits and ability to prevent disease is increasing **(Pandey and Suttajit, 2022**). Ginger is also a rich source of phytochemicals **(Fahmi *et al.*, 2019**). Ginger is a member of the *Zingiberacea*e family. It is extensively grown in numerous countries and distributed in various regions across the globe. Ginger plant rhizomes, after being dried, are utilized as a spice and additive for enhancing flavor. Additionally, they are reputed to possess numerous therapeutic benefits (**Kumar *et al.*, 2011**). In recent times, ginger has been experiencing an increase in popularity because of its possible antioxidant, antibacterial, antifungal, anti-inflammatory, and antidiabetic effects (**Adom *et al.*, 2019; Avci *et al.*, 2020; Unuofin *et al.*, 2021**). Oxidative stress contributes to the pathogenesis and progression of many diseases. People are increasingly practicing the use of natural antioxidants as a therapeutic option **(Morakinyo *et al.*, 2011**). The goals of this study are to show that the phytochemical components of ethanolic crude extracts from ginger rhizomes have antifungal and antioxidant effects.

MATERIALS AND METHODS

Ethanolic Extract Preparation

Rhizomes of ginger were purchased from the local market in Zagazig City, Egypt. The plant was peeled, chopped into tiny bits, air-dried for 2 weeks, and ground with a mechanical grinder. To extract the active compounds, 50 g of the ground plant was macerated in one L of ethanol 70% v/v for 24 hours. The solution was filtered through Whatman filter paper No.1. The remains were re-extracted twice more under identical conditions. The ethanol was filtered through a vacuum rotary evaporator (BüCHI-water bath-B-480), and the residual water was lyophilized using a freeze-drier (Thermo-electron Corporation-Heto power dry LL 300) **(Anosike *et al.*, 2009**).

Phytochemical Screening

We conducted preliminary phytochemical testing on the ethanol extract of ginger using the methods described by **Harborne (1984) and Trease and Evans (1996)**. Using the aforementioned methods, tests for the presence or absence of phytochemical substances entail introducing a suitable chemical reagent into the plant extract within a test tube. Depending on the circumstances, we agitated the mixture either vigorously or softly. We noted the presence or absence of phytochemicals such as saponins, flavonoids, alkaloids, tannins, terpenoids, steroids, etc.

Total Phenolic Compounds (TPCs) Estimation

The Folin-Ciocalteu reagent method was used to assess the total phenolic compounds (TPCs) according to **Singleton *et al.* (1999)**. To conduct the test, combine one mL of the sample, which has a concentration of 1000 µg/mL, with 5 mL of Folin-Ciocalteu reagent that has been diluted with water at a ratio of 1:10 (v/v), in a test tube. Next, introduce 4 mL of sodium carbonate solution at a concentration of 75 g/L into the identical test tube. Vortex the contents of the tube well for 15 sec, then allow it to rest at a temperature of 40 °C for 30 min to allow the color to develop. Finally, use a spectrophotometer to quantify the sample's absorbance at 765 nm. A calibration curve was generated using gallic acid at values ranging from 0 to 500 µg/mL. We measured the total quantity of phenolic compounds in mg / 100g (GAE).

Total Flavonoids (TFs) Estimation

The estimation of total flavonoids (TFs) was conducted following the procedure established by **Ordonez *et al.* (2006)**. A volume of one mL

of extract with a concentration of 1000 µg/mL was mixed with 2 mL of an ethanol solution containing 20 g/L of $AlCl_3$. The absorbance at a wavelength of 420 nm was measured after 60 min. A calibration curve was generated using quercetin at values ranging from 0 to 500 µg/L. We quantified the total flavonoid content in mg of quercetin equivalents (QE) per 100 g of extract.

Antioxidant Activity (DPPH-assay)

Hatano *et al.* (1988) described the DPPH (2,2-diphenyl-1-picrylhydrazyl) test to determine the antioxidant activity of the ethanolic extract of ginger rhizomes. 500 µL of extract (20, 40, 80, 160, 320, and 640 µg extract/1 mL ethanol) was mixed with 2.5 mL DPPH solution (0.1 mM dissolved in ethanol). We measured the absorbance at a wavelength of 517 nm alongside the control sample after incubating for 30 min at a temperature between 27 °C and 3°C (**Gülçin *et al.*, 2004**).

The antioxidant potential of DPPH radicals (%) was studied as follow:

$$Inhibition\ (\%) = [(Abs\ control - Abs\ sample)/Abs\ control]x\ 100$$

The absorbance of the control, known as Abs. control, and the absorbance of the sample, known as Abs. sample, are measured in the presence of a ethanolic extract of ginger rhizomes.

In vitro Antifungal Activity

We tested how different amounts of ethanolic ginger rhizomes extract (0, 100, 200, 400, and 800 µg/mL) affected the growth of *F. solani* and *R. solani* in a potato dextrose agar (PDA) medium. We incubated the plates at 25°C in an incubator. We measured the colony diameters daily until the fungal growth completely covered the control Petri plates. We calculated linear growth using the following equation.

$$\boldsymbol{Linear\ growth\ reduction}\ (\%) = ((\boldsymbol{Control\ growth} - \boldsymbol{Treatment\ growth}))/(\boldsymbol{Control\ growth})\ \boldsymbol{x\ 100}$$

Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) was used to observe *F. solani* and *R. solani* that had been treated with an ethanolic extract of ginger rhizomes at a concentration of 200 µg/mL for 7 days at 25°C to see how the hyphae changed shape compared to the control treatment (**Sitohy *et al.*, 2013**).

Statistical Analysis

The experimental design employed a factorial design and utilized a completely randomized design (CRD). We conducted a comprehensive analysis of variance (ANOVA) on each individual data point for a fully randomized experimental design. The concentration differences were determined using Tukey's range test with a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

The ginger rhizome ethanolic extract was subjected to a phytochemical examination, and the findings are displayed in Table 1. The ginger rhizome ethanolic extract was subjected to phytochemical analysis, which revealed the presence of flavonoids, alkaloids, terpenoids, tannins, carbohydrates, saponins, and glycosides. There was agreement between this study's results and those of **Umeh *et al.* (2013)**. They said that phytochemical analysis of the plant extract showed that the crude extract has flavonoids, terpenoids, alkaloids, and saponins.

Total Phenolics and Total Flafonoids Estimation

Phenolics, in addition to their antioxidant properties, have been acknowledged as a crucial component of a daily diet due to their beneficial health-enhancing capacity **(Singh *et al.*, 2020)**. Fig. 1 presents the TPCs and TFs in the ginger rhizome ethanolic extract. The ethanolic extract of ginger rhizome contained 573.87 mg GAE/ 100 g extract of total phenols (TPCs) and 232.43 mg QE/100 g extract of total flavonoids (TFCs). The results of the present study were consistent with the findings of **Tanweer *et al.* (2020)**, who assessed the TPC and TFCs level of ginger rhizome in ethanolic extract and recorded that the value for the extract was 650.44 mg GAE/ 100 g extract, and 239.52 QE/100g extract. According to **Cai *et al.* (2004)**, the ethanol extract of the ginger components (rhizomes, leaves, and flowers) had the highest total phenolic content (TPC), followed by the methanol and water extracts.

Table 1. Phytochemical screening of ginger rhizomes ethanolic extract

Parameter	Present/absent
Flavonoids	+
Alkaloids	+
Terpenoids	+
Saponins	+
Glycosides	+
Tannins	+
Carbohydrates	+

+: Present; -: absent

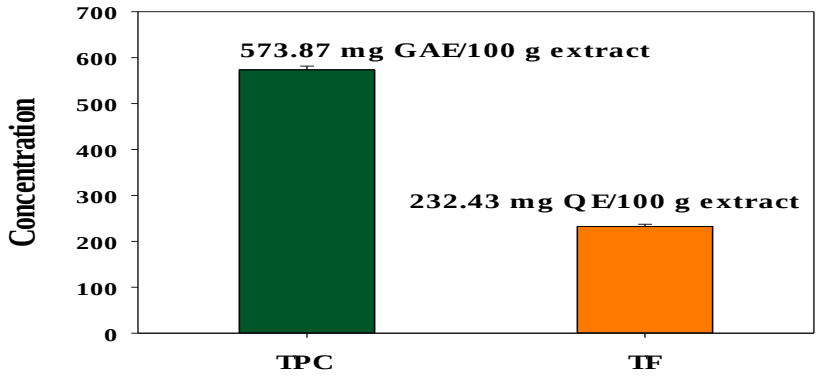

Fig. 1. Total phenolic compounds (TPC) expressed as GAE 100 g^{-1} dry extract), and total flavonoids (TFs) expressed as QE 100 g^{-1} dry extract

Antioxidant Activity (DPPH- assay)

Using the DPPH assay, we estimated the antioxidant activity (% inhibition) of the ginger rhizome ethanolic extract, and Fig. 2 presents the results. The ginger rhizome extract concentration and DPPH radical scavenging activity generally showed a linear trend, meaning that an increase in plant extract concentration led to an increase in percentage inhibition. The DPPH radical scavenging activity increased from 13% to 89% when the extract's concentration increased from 100 to 600 µg /mL. The results of the present study were consistent with the findings of **Barbosa and Peteros (2018)**.

Antifungal Activity

Figs. 3 and 4 present the estimated antifungal activity of ginger rhizome ethanolic extract against *F. solani* and *R. solani*. We observed that the ethanolic extract of ginger rhizome inhibited the growth of *F. solani* mycelium in solid agar medium Petri dishes, with the extent of inhibition dependent on the concentration. The observed effect occurred after a period of 7 days of incubation at a temperature of 24°C, as depicted in Figure 3. The greatest decrease in the growth of *F. solani* (84.44%) occurred when the concentration was 800 µg/mL. Similarly, during a 7-day incubation period at a temperature of 24°C, the ethanolic extract derived from ginger rhizome exhibited a decelerating effect on the growth of *R. solani* mycelial cells on solid agar medium Petri plates. The extent of this effect was found to be contingent upon the concentration of the extract, as depicted in Fig. 4. The highest inhibition of *R. solani* growth (77.77%) was seen at a concentration of 800 µg/mL. The results of the present study were

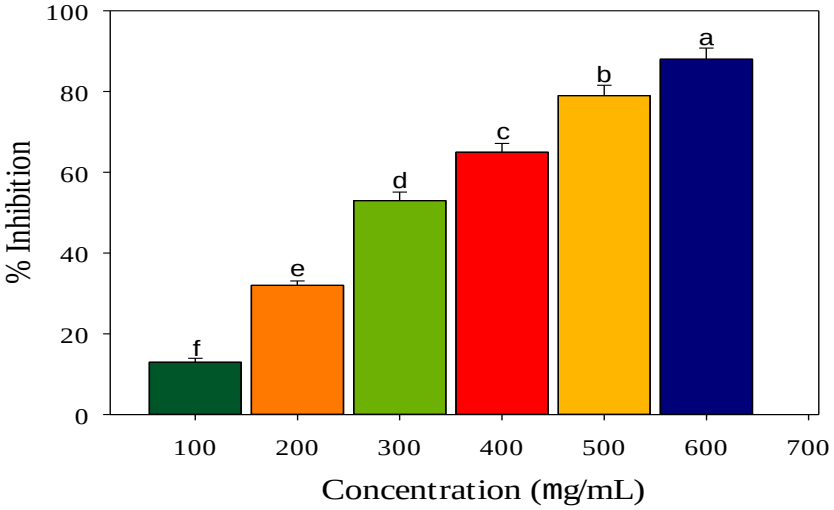

Fig. 2. Antioxidant activity (% inhibition) of ginger rhizomes ethanolic extract at different concentration (100, 200, 300, 400, 500, and 600 µg/mL). Bars with different letters are significantly different at p < 0.05. Data for each bar are expressed as the mean of three replicates ± SDs

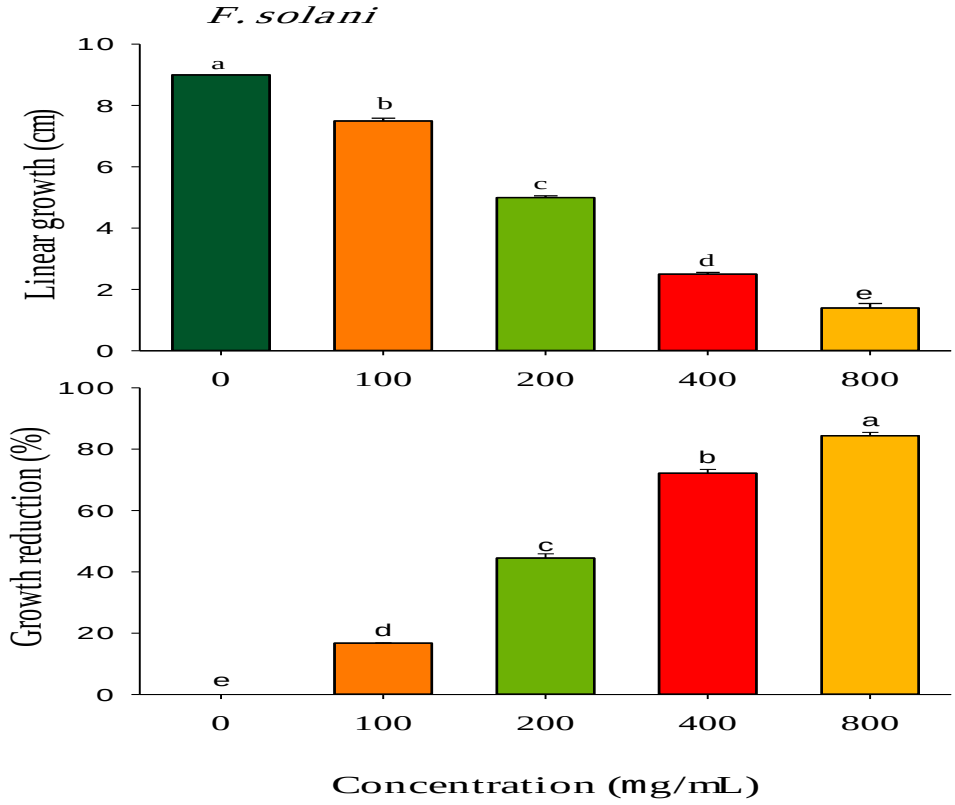

Fig. 3. Antifungal activity of ginger ethanolic extract against *F. solani* at different concentrations (100, 200, 400, and 800 µg/mL) compared to control (0 µg/mL). Bars with different letters are significantly different at p < 0.05. Data for each bar are expressed as the mean of three replicates ± SDs

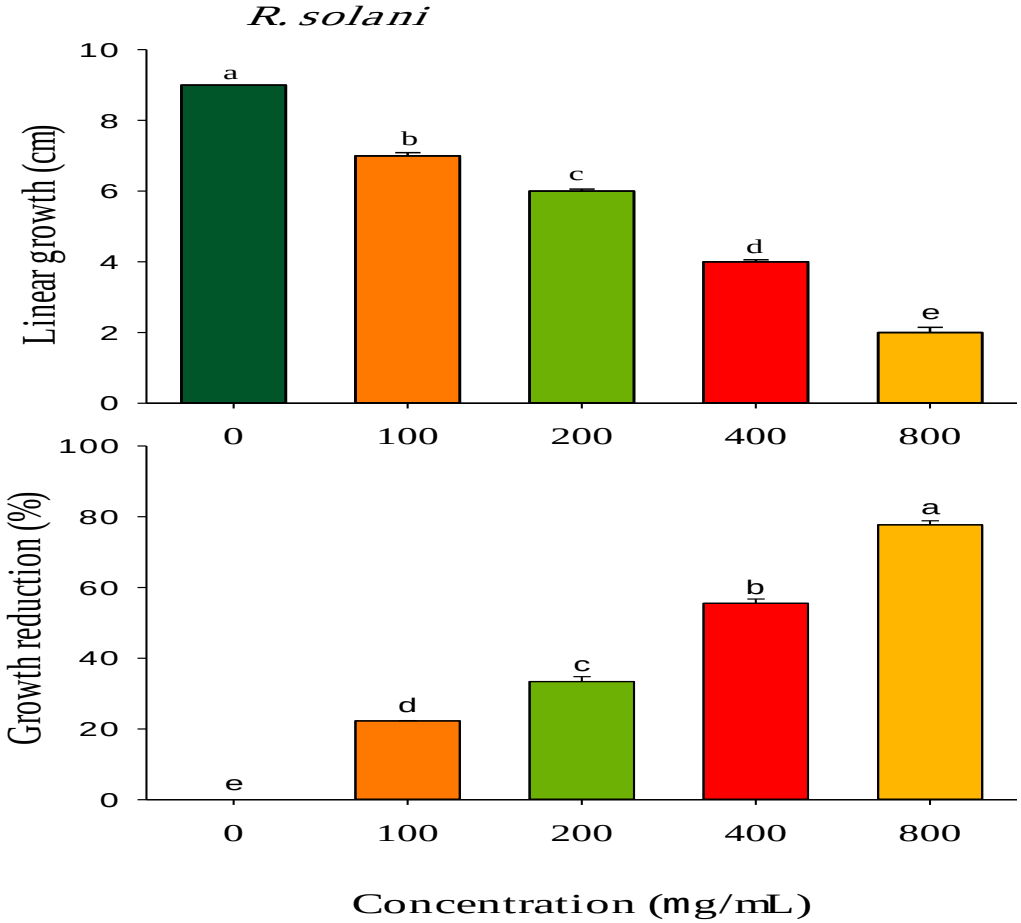

Fig. 4. Antifungal activity of ginger ethanolic extract against *R. solani* at different concentrations (100, 200, 400, and 800 µg/mL) compared to control (0 µg/mL). Bars with different letters are significantly different at $p < 0.05$. Data for each bar are expressed as the mean of three replicates ± SDs.

consistent with the findings of **Xi *et al*. (2022)**, who found that 20 mg/mL of ginger rhizome extract stopped the growth of *F. solani* in an antifungal assay.

Morphological Changes

To further examine the impact of an ethanolic extract derived from ginger rhizome on the growth of *F. solani* and *R. solani*, scanning electron microscopy (SEM) was utilized to evaluate any alterations in the physical structure of the fungal mycelia following treatment with a 200 µg/mL concentration of the extract for a duration of 7 days. The results demonstrated that the application of 200 µg/mL of extract had an impact on the morphology of *F. solani* and *R. solani* mycelia. The control mycelia exhibited a robust and unblemished morphology, with a well-developed structure and a sleek surface (Figs. 5A and 6A). Following exposure to a concentration of 200 µg/mL of ginger rhizome ethanolic extract, the hyphae underwent changes in their surface texture, becoming rough, twisted, and deformed (Figs. 5B and 6B).

Conclusions

The ginger rhizome extract has been discovered to have inhibitory properties against fungal strains, indicating its potential as a candidate for the development of novel systemic and topical antifungal medications. Furthermore, this extract also exhibits inherent antioxidant capabilities.

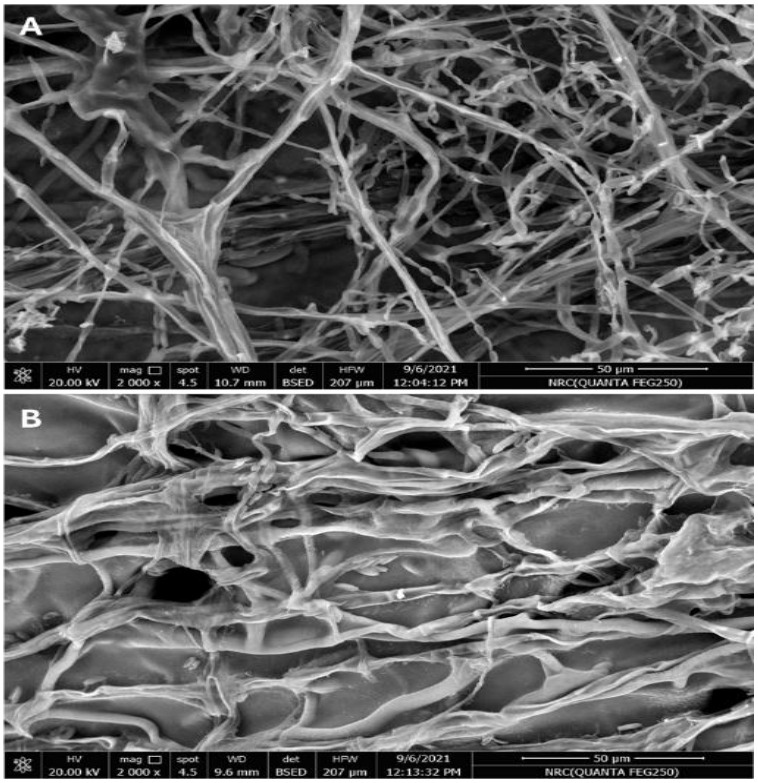

Fig. 5. SEM images of *F. solani* (A) Control (B) Treatment with 200 µg/mL of ginger rhizome ethanolic extract

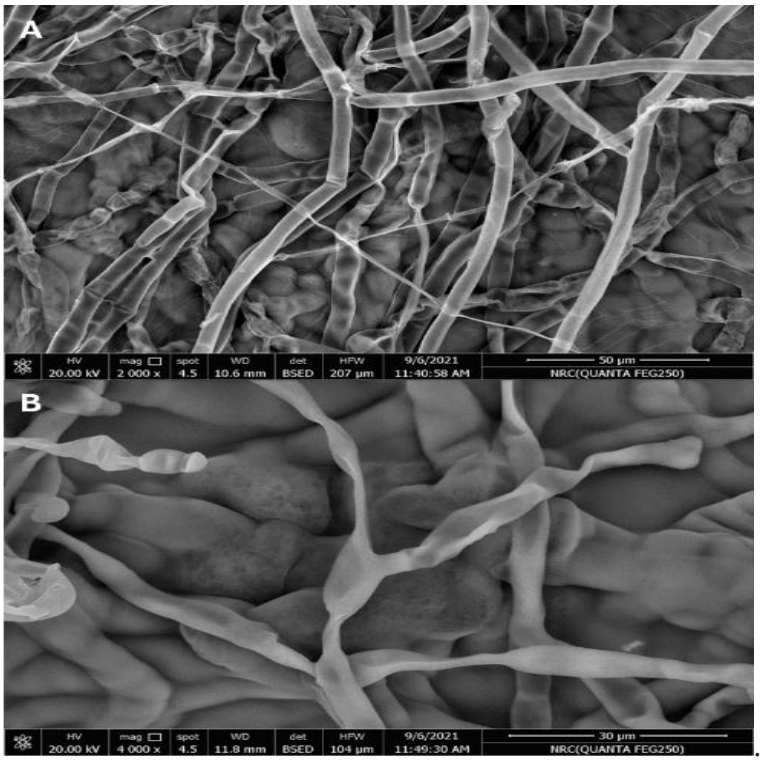

Fig. 6. SEM images of *R. solani* (A) Control (B) Treatment with 200 µg/mL of ginger rhizome ethanolic extract

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النشاط المضاد للأكسدة والمضاد للفطريات لمستخلص الزنجبيل ضد فطريات *Fusarium solani* و*Rhizoctonia solani*

ايمان الدسوقى – انتصار عباس – على عثمان – محمود سطوحى

قسم الكيمياء الحيوية - كلية الزراعة - جامعة الزقازيق – مصر

تؤثر فطريات *F. solani* و *R. solani* والتى تنقلها التربه على كمية وجوده الانتاج الزراعى. يستخدم الزنجبيل كمضاد للاكسدة والفطريات نظرا لمحتواه العالى من المركبات الفعاله. يهدف هذا العمل إلى إظهار التأثيرات المضادة للفطريات ومضادات الأكسدة للمكونات الكيميائية النباتية الموجودة في المستخلص الايثانولى للزنجبيل. خضع المستخلص الإيثانولي للزنجبيل للفحص الكيميائي النباتي، الذي كشف عن وجود مركبات الفلافونويد، القلويدات، التربينويدات، التانينات، السكريات، الصابونين، والجليكوسيدات. كمية المركبات الفينولية الكلية 573.87 ملليجرام مكافئ من حامض الجاليك لكل 100جرام مستخلص بينما كمية مركبات الفلافونويدات الكلية 232.87 ملليجرام مكافئ من الكيريستين لكل 100جرام مستخلص. يمتلك المستخلص الايثانولى نشاطا مضادا للأكسدة حيث يزداد النشاط من 13% الى 89% بزيادة تركيز المستخلص من 100 إلى 600 ميكروجرام/ملل. كما أثبتت النتائج تثبيط نمو فطريات *F. solani* و *R. solani* نتيجه المعامله بمستخلص الايثانول ويزداد التأثير بزيادة التركيز. تشير النتائج إلى أن الزنجبيل لديه القدرة على أن يكون مصدرًا قيمًا لمضادات الأكسدة الطبيعية والمواد المضادة للفطريات.

المحكمـــون:

1- أ.د. عبدالله السيد عبدالله حسن أستاذ الكيمياء - كلية الزراعة بمشتهر - مشتهر بنها.

2- أ.د. خالد محمد محمد وهـــدان استاذ الكيمياء الحيوية ووكيل كلية الزراعة - جامعة الزقازيق.