# Estimation of active compounds in *Ganoderma* spp. and evaluation of its activity against leaf spot disease caused by *Alternaria tenuissima* on tomato

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## Abstract

This study aims to evaluat some active compounds of *Ganoderma* spp. to inhibit the growth of the pathogenic fungus *Alternaria tenuissima* in the laboratory and control leaves spot disease, inducing plant resistance and encouraging its growth in the field on tomato plants. The results showed the highest inhibition of the pathogenic fungus *A. tenuissima* growth by the filtrates of the *G. applanatum* Has.AA-6 , *G. lucidum* Has.AA-7, *G. resinaceum* 1049, and *G. resinaceum* Has.AA-8 growing in Malt Extract Broth medium, The diameters of the pathogenic fungus colonies were 2.79, 2.95, 3.17, and 3.19 cm, respectively.The highest chitinase and β-glucanase activities were in the interaction treatment with (extract + filtrate of G. lucidum) (1:1) in present of *A. tenuissima*, reached 2.532 and 1.774 units/ml, respectively in the Agro-Tip tomato variety. The same treatment achieved a high rate of reducing the percentage of infection severity against *A. tenuissima*, reaching 15.33, 17.07and 20.36% for the cultivars Agro-tip ,S25 and Rawaa ,respectively. Agro-tip cultivar treated with Extract + filtrate of G. lucidum in present of A. tenuissima also achieved the highest plant height, plant dry weight and yield reached to 148.56 cm, 112.38g and 7812.78 g.plant$^{-1}$ , respectively.

Keywords: *Alternaria tenuissima*, Tomato, *Ganoderma* spp. Biological control.

## Introduction

The mushroom, *Ganoderma* spp. one of the most important medicinal mushrooms known for a long time, which has been widely used as a healthy food in addition to traditional medicine in China, Japan, Taiwan and many Asian countries (34). Today, many medical drugs are manufactured in the form of pills and capsules that are included as supplements in treating many human diseases. There are 43 identified species belonging to the genus *Ganoderma*, among which five commonly cultured species are *G. lucidum*, *G. applanatum*, *G. australe*, *G. colossum*, and *G. subresinosum* (29). *Ganoderma* spp. contain many biologically active compounds, which include fibers, polysaccharides, oligosaccharides, triterpenoids, peptides, proteins, alcohols, and phenols, in addition to mineral elements (such as zinc, copper, iodine, selenium, germanium, and iron) and vitamins. It also has many other compounds such as Glycosides, triterpenoids, phenolic compounds, tannins, these compounds have many therapeutic properties for many diseases in addition to the antimicrobial activity (19, 22). (33) indicated that *G. lucidum* contains more than 100 different types of oxygenated terpenes that contain multiple pairs of functional stereoisomers. In the same context, (28) identified lots of terpenes, including Ganoderic acid which is used in the manufacture of medical drugs, in addition to polysaccharides such as (β_1_6, β_1_3, homo D_glucan), glucans acidic and polyglucan which play an important role in the medical importance in treating many diseases due to the

antifungal, antibacterial and antiviral activities (20).

Plant diseases caused by fungi are one of the serious problems on the tomato plant, which can cause significant losses and be one of the most important factors determining the cultivation of this important crop (36). The losses caused by the pathogenic fungus *Alternaria* sp. are estimated about 70-80% that affect the vegetative system, as the fungus destroys the host's tissues by reducing its ability to photosynthesis (38). Many fungal extracts and their active compounds of the macrofungi have been used to control plant diseases, as many studies have shown that the *Ganoderma lucidum* has an antifungal activity owing to it contains high concentrations of phenols (35). (43) showed that gandermin protein, isolated from the fruiting bodies of *Ganoderma* sp. had antifungal effects against *Botrytis cinerea*, *Fusarium oxysporium*, and *Physalo sporapiricola*. The hot aqueous extract of the *Ganoderma* sp. and its polysaccharides extracted by gas chromatography technique has an inhibitory effect against plant bacterial pathogens such as *Erwinia carotovora* and fungal pathogens such as *Penicillium digitatum* and *Botrytis cinerea* (6).

Due to the importance of *Ganoderma* spp. and its antifungal efficacy, the recent trend has been to use this mushroom in the biological control against the plant pathogens, including *Alternaria tenuissima.* Therefore, this study aimed to: estimation of some active compounds from *Ganoderma spp*. and evaluation of its efficiency for inhibiting the growth of the pathogenic fungus *Alternaria tenuissima* in the laboratory and control the leave spot disease in the field on three varieties of tomato.

## Materials and Methods

*Ganoderma spp*.

Four *Ganoderma* spp. cultivated in the mushroom farm in the College of Agriculture, Tikrit University, Iraq, including (*G. applanatum* strain Has.AA-6, *G. lucidum* strain Has.AA-7, and *G. resinaceum* strain Has.AA-8) were used, which were phenotypically and molecularly identified and registered in the International Genebank (NCBI) under the accession numbers ON834523.1, ON834541.1, and ON834527.1, respectively, (17) while the fourth fungus, *G. resinaceum* 1049 (MN448375) was diagnosed by (15) .

### Pathogenic fungus

The isolate of the highly pathogenic fungus *Alternaria tenuissima* strain Has.AA-4 was used, which was identified molecularly and phenotypically and registered in the International Genebank (NCBI) under the accession number KON834512.1 K (17).

### Preparation of alcoholic extracts

The alcoholic extracts were prepared by adding 10 g of fungal powder to 100 mL of 90% ethyl alcohol. The flasks were then left for 24 hours with continuous agitation. Subsequently, the extract was filtered using triple-layered medical gauze cloth and further filtered through Whatman No. 1 filter paper, utilizing a Buchner funnel connected to a vacuum apparatus. The resulting filtrate was concentrated using a rotary vacuum evaporator to eliminate the alcohol and obtain a concentrated liquid. This concentrated extract was subsequently spread onto polystyrene plates and dried at laboratory temperature. Upon complete drying, it was collected in glass bottles and stored in a refrigerator at 4°C until further use (31).

### Estimation of Total Alkaloids Content (TAC)

20 mg of the dried extract was dissolved in 10 mL of 2N HCl. The resulted solution was filtered using Whatman No. 1 filter paper. Then, 1 mL of the filtrate was transferred to a separating funnel and washed three times with 10 mL of chloroform. The pH was adjusted to 7 using 0.1 N NaOH. Subsequently, 5 mL of Bromocresol Green (BCG) dye and 5 mL of phosphate buffer (pH 4.7) were added to the 1 mL portion washed with chloroform. The mixture was vigorously shaken and extracted with 4 mL of chloroform. The extracted solution was collected in a 10 mL volumetric

flask and made up to the mark (10 mL) using chloroform. The absorbance was then measured at a wavelength of 470 nm using a spectrophotometer. The concentration of total alkaloids was determined using the standard tropine curve.

**Estimation of Flavonoids**

Flavonoids were quantified using the Aluminum chloride colorimetric assay method. In this method, 0.5 mL of the extract was placed in a test tube with 2 mL of distilled water. Then, 0.15 mL of sodium nitrate solution was added and left for 6 minutes at room temperature. Afterward, 0.15 mL of aluminum chloride solution was added, and the mixture was incubated for another 6 minutes at room temperature. Next, 2 mL of sodium hydroxide solution was added. The final volume of the reaction mixture was adjusted to 5 mL by adding distilled water, and it was left for 15 minutes. The absorbance was then measured at 510 nm against the blank. The concentration of flavonoids was determined using the standard rutin curve (44).

**Estimation of Tannins**

Tannins were estimated using the method described by (39). In this method, 1 mL of the extract was added to a test tube, followed by the addition of 0.5 mL of Folin's reagent. Then, 1 mL of sodium carbonate solution was added to the reaction mixture, and the final volume was adjusted to 10 mL using distilled water. The mixture was vigorously shaken and incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm. The tannin content was determined using the standard curve of tannic acid.

**Determination of Saponins**

Saponins were determined using the method described by (23). In this method, 0.5 mL of the extract was added to 400 µL of vanillin-acetic acid solution. Then, 1.6 mL of perchloric acid was added to the mixture, which was incubated in a water bath at 75°C for 5 minutes. Afterward, the mixture was cooled in an ice bath for 2 minutes, followed by the addition of 2.5 mL of ice-cold acetic acid. The absorbance was measured at 550 nm. The total saponin content was determined using the standard curve prepared from different concentrations of saponin solution.

**Determination of Glycosides**

To determine the glycosides content, 1 mL of the extract was added to 10 mL of pyridine solution. The solution was incubated at room temperature for one hour. Then, the reaction mixture was diluted by adding 20 mL of distilled water. The absorbance was measured at 495 nm. The glycosides content was determined using the standard curve of Securidaside (40) .

**Estimation of total phenols**

1ml of the extract was placed in a test tube and 1 ml of HCL (0.05 N) was added, with the addition of 1 ml of Arnault's solution, 10 ml of distilled water, and 2 ml of NaOH 4% solution.

The absorbance was measured at 515 nm against the blank (efficient preparation) which includes all the solutions included in the experiment except for the sample. The total phenols content was determined using the standard curve of catechol. (25 ).

**Prepration of *Ganoderma* spp. filtrate on liquid media.**

Five liquid media (Himedia- India) were used to grow Ganoderma spp. The media included (potato dextose broth (PDB), corn meal broth (CMB), malt extract broth (MEB), yeast extract peptone dextrose broth YPDB) and Czapek dox broth (CDB), which were grown by taking Part of a new colony of Ganoderma spp. at the age of 4 days, then incubated at a 25 $^{o}$C in a shaking incubator (120 rpm) for 7 days, after completion of growth, the biomass was separated from the filterate by Whatman No47 mm filter paper, then the filterate was passed through a 0.45 mm Milipore filter, and used in subsequent experiments.

**Estimation of the biological activity of filtrate and alcoholic extracts of *Ganoderma* spp. against *A. tenuissima***

The biological activity of filtrate and alcoholic extracts of *Ganoderma* spp. against *A. tenuissima* was assessed using the agar well diffusion method, following the protocol described by (24). Petri dishes with a diameter of 9 cm, containing Potato Dextrose Agar (PDA) medium were prepared. Five wells were made on the surface of each dish using a sterilized cork borer with a diameter of 0.5 cm, with equal distances between the wells. The plates were centrally inoculated with the disc of diameter 0.5 cm from the pathogenic fungus, then the plates were incubated at 25 ºC for 3 days. Subsequently, 0.1 of the alcoholic extracts and the fungal filtrate were separately added to the wells, while sterilized distilled water was added to other plate as a control. The dishes were further incubated at 25 ºC until the fungal full growth in the control, at that point, the fungal diameter was measured using a digital ruler.

**Field Experiment**

The experiment was conducted in a plastic greenhouse located in the agricultural department of the Ministry of Agriculture/Crop Protection Division, during the spring growing season. The experiment was designed using a randomized complete block design (RCBD) with three blocks, each containing six treatments. The soil was prepared by tilling, leveling, and smoothing it thoroughly. The house is provided with a drip irrigation system. The soil was sterilized using 5% formaldehyde for three days, followed by three days for ventilation. Tomato seeds of three varieties, namely "Roa'a," "Agro-Tip," and "S25," were sown in trays and transferred to the field at 30 days old. The seedlings were planted with a spacing of 20 cm between plants, and each experimental unit consisted of 10 seedlings. The treatments were applied to the seedlings 15 days after planting in the greenhouse soil. The treatments were distributed as follows for both experiments:

1- Healthy Plant (Control). 2- Pathogenic Fungus *A. tenuissima*. 3- Pathogenic Fungus + Chemical fungicide (Othilotop). 4- Pathogenic Fungus + extract of the fruit bodies and filtrate of *Ganoderma* spp. (1:1) V:V, 5- Pathogenic Fungus + Fruit Body Extract of *Ganoderma* spp. 6- Pathogenic Fungus + filtrate of *Ganoderma* spp.

The treatments were applied to the pathogenic fungus *A. tenuissima* by preparing a spore suspension ($10^8$ spores/ml) from a 7-day-old colony. The suspension was sprayed on the foliage of the plants until complete wetness, then the plants were covered with polyethylene bags for 3 days. After three days, treatments of the fungicide (0.1%) , extracts and filtrate of *Ganoderma* spp. were applied by spraying on the foliage of the plants until complete wetness.

**Inducing Resistance markers**

**Preparation of the crude enzymes**

The roots of three plants (from each replicate) were washed with tap water, then the roots were cut into small pieces and 1 g of roots was taken and placed in a mortar and 5 ml of acetate buffer solution pH 5.6 was added, then completely crushed and centrifuged at 5000 rpm for 5 minutes, the sediment was discarded, and the filtrate which represents the crude enzymes was sterilized via Millipore filter 22 µm.

**Estimation of Chitinase Activity**

The method described by (41) was followed to estimate the chitinase activity. The reaction mixture consisted of adding 0.5 mL of chitin solution (1%) and 0.5 mL of the enzyme extract for each treatment. The mixture was incubated in a water bath at 37ºC for two hours. Then, 1 mL of DNS (dinitrosalicylic acid) was added, and the mixture was placed in a water bath at 100ºC for 5 minutes. After cooling, the absorbance was measured using a spectrophotometer at 540 nm. The standard curve of N-acetyl glucosamine was used to

determine the enzyme activity. Enzyme activity was defined as the amount of enzyme required to release 1 micromole of N-acetyl glucosamine per minute under the reaction conditions.

**Estimation of β-1,3-Glucanase activity**

The reaction mixture for estimating β-glucanase enzyme consisted of adding 1 mL of B-glucan solution to 1 mL of the enzyme extract. The mixture was incubated in a water bath at 35ºC for 40 minutes. Then, 1 mL of the reaction mixture was taken after incubation and mixed with 1 mL of DNS solution. The mixture was heated in a water bath at 100ºC for 5 minutes. After rapid cooling, 2 mL of distilled water was added, then absorbance was measured using a spectrophotometer at 540 nm. Enzyme activity was defined as the amount of enzyme required to release 1 micromole of glucose per minute under the reaction conditions. (30).

**Estimation of Plant Infection Severity by *A. tenuissima***

The severity of infection was assessed for all treatments by evaluating disease symptoms on a five-point scale: 0 = no lesions, 1 = 1-5 lesions, 2 = 6-10 lesions, 3 = 11-15 lesions, 4 = leaf death. Disease severity was calculated using the equation provided by (26) as cited in (4).

Disease Severity (%) = (Sum of (Lesion Grade × Number of leaves)) / (Highest Grade × Total Number of leaves) × 100

**Estimation of Plant Height**

Plant height was measured by randomly selecting three plants from each replicate. The height of the above-ground biomass was measured using a measuring tape.

**Estimation of shoot system Dry Weights**

Three randomly selected plants from each treatment were harvested at the beginning of the flowering stage. The shoot system was dried in oven (50C). After achieving a constant content of 20.2 mg/g in *G. resinaceum 1049*. Furthermore, the estimation of flavonoid content in the fungi revealed that had the weight, the weights were recorded by a sensitive balance.

**Estimation of tomato yield**

The weight of fruits per plant (average three picks) was calculated by harvesting three plants from each experimental unit, and the average weight was recorded.

**Statistical Analysis**

Laboratory experiments were carried out in a completely randomized design while the field experiment was conducted using a randomized complete block design (RCBD). The data were analyzed using the Genestat software. Mean comparisons were performed using the least significant difference (L.S.D.) test at a significance level of 0.05 (3).

**Results and Discussion**

**Active Compounds in *Ganoderma* spp.**

The results presented in Table 1 showed the estimation of active compounds in four isolates of *Ganoderma* spp.. It was found that *G. resinaceum* strain Has.AA-8 exhibited the highest concentration of total alkaloids, reaching 35.5 mg/g, compared to *G. resinaceum* 1049, which had a total alkaloid concentration of 24.59 mg/g. Regarding the fungal content of tannins, the table indicates that *G. resinaceum* 1049 had the highest concentration of tannins at 31.13 mg/g, whereas *G. lucidum* had the lowest concentration at 23.08 mg/g. Furthermore, the highest saponin content was observed in *G. applanatum* at 31.13 mg/g, compared to the lowest content in *G. lucidum*, which was 16.42 mg/g. The highest concentration of total phenols was found in *G. resinaceum* strain Has.AA-8, reaching 33.71 mg/g, while *G. applanatum* exhibited the lowest concentration at 25.99 mg/g. Additionally, *G. lucidum* showed the highest content of glycosides, reaching 27.33 mg/g, compared to the lowest

highest value at 32.36 mg/g, whereas . *G. resinaceum 1049* had the lowest value at 19.17 mg/g.

**Table (1) The active compounds (mg/g) in *Ganoderma* spp**

| *Ganoderma* spp. | Total alkaloids | Tannins | Saponins | Total phenols | Glycosides | Flavonoid |
|---|---|---|---|---|---|---|
| *G. lucidum strain Has.AA-7* | 31.98 | 23.08 | 16.42 | 30.27 | 27.33 | 24.31 |
| *G. resinaceum* strain Has.AA-8 1 | 35.5 | 25.77 | 22.71 | 33.71 | 23.31 | 32.36 |
| *G. resinaceum 1049* | 24.59 | 31.13 | 19.32 | 27.36 | 20.2 | 19.17 |
| *G. applanatum strain Has.AA-6* | 30.19 | 27.54 | 31.13 | 25.99 | 22.06 | 27.28 |
| LSD 0.05 | 2.37 | 2.14 | 2.66 | 2.14 | 1.71 | 2.53 |

**Effect of filtrates and extracts of *Ganoderma* spp. on the inhibition of the pathogenic fungus *A. tenuissima*.**

Table 2 showed the highest inhibition of *A. tenuissima* growth by filtrates of *G. applanatum* Has.AA-6, *G. lucidum* Has.AA-7, *G. resinaceum* 1049, and *G. resinaceum* Has.AA-8, grown on Malt Extract Broth (MEB) medium, the colony diameters of the pathogenic fungus were recorded as 2.79 cm, 2.95 cm, 3.17 cm, and 3.19 cm, respectively. Followed by the alcohol extract of the biomass also showed inhibition, with colony diameters of 3.09 cm, 3.23 cm, 3.43 cm, and 3.85 cm for the four *Ganoderma* spp., respectively, compared to the control where *A. tenuissima* exhibited a diameter of 8.5 cm.

Table (2) Effect of filtrates and extracts of Ganoderma spp. on the inhibition of the pathogenic fungus *A. tenuissima*.

| Treatments | *Ganoderma* spp. | | | | | Average of treatments |
|---|---|---|---|---|---|---|
| | *G. resinaceum* 1049 | *G. applanatum* Has.AA-6 | *G. lucidum* Has.AA-7 | *G. resinaceum* Has.AA-8 | Control | |
| BDP-F | 3.52 | 3.24 | 3.15 | 3. 65 | 8.5 | 4.60 |
| MEB-F | 3.19 | 2.95 | 2.79 | 3.17 | 8.5 | 4.12 |
| CMB-F | 3.50 | 3. 16 | 3.12 | 3.33 | 8.5 | 4.613 |
| BDP-ME | 5.14 | 4.51 | 4.68 | 5. 44 | 8.5 | 5.71 |
| MEB-ME | 3. 43 | 3.09 | 3.23 | 3.85 | 8.5 | 4.67 |
| CMB-ME | 4.38 | 3.65 | 3.88 | 4.48 | 8.5 | 4.98 |
| Average of Ganoderma spp. | 3.95 | 3.49 | 3.48 | 3.71 | 8.5 | |
| LSD $_{0.05}$ | Ttreatments= 0.22 , *Ganoderma* spp.=0.13 , Treatments× *Ganoderma* spp.=0.34 | | | | | |

***Inhibition is estimated by the diameter of the pathogen colony (cm)BDP: potato dextrose broth, MEB: malt extract broth, CMB: corn meal broth, F: fungal filtrate, ME: alcoholic mycelium extract**

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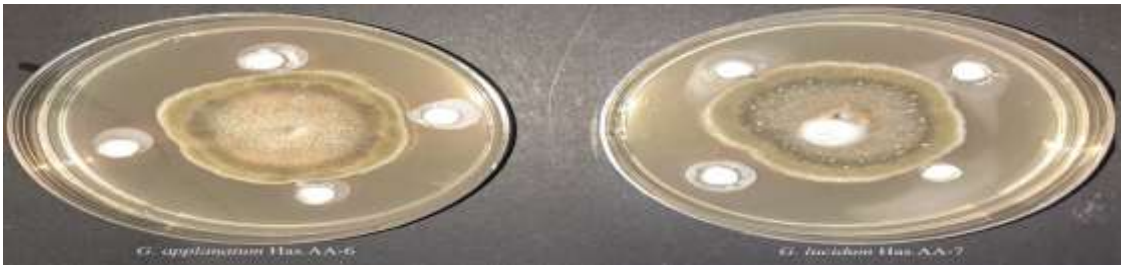

Figure (1) showed the highest inhibitory effect of the filtrate of *Ganoderma* spp. grown in MEB on the growth of the pathogenic fungus *A. tenuissima*

Figure (1) The highest inhibitory effect of the filtrate of *Ganoderma* spp. grown in MEB on the growth of the pathogenic fungus *A. tenuissima*

**Effect of alcohol extract of *Ganoderma* spp. fruit bodies on the growth of the pathogenic fungus *A. tenuissima*.**

Figure 2 illustrated the impact of alcohol extract from fruit bodies of *Ganoderma* spp. on the growth of *A. tenuissima*. The results revealed that extracts from all *Ganoderma* spp. exhibited significant superiority in inhibiting the growth of *A. tenuissima* compared to the control. Among them, the alcohol extract of *G. resinaceum* Has.AA-8 displayed the highest inhibition of the fungus *A. tenuissima*, with a colony diameter of 2.4 cm. On the other hand, *G. lucidum* Has.AA-7 achieved the lowest colony diameter of *A. tenuissima*, reaching 2.25 cm, compared to the con

trol where the colony diameter of the pathogenic fungus was 8.5 cm.

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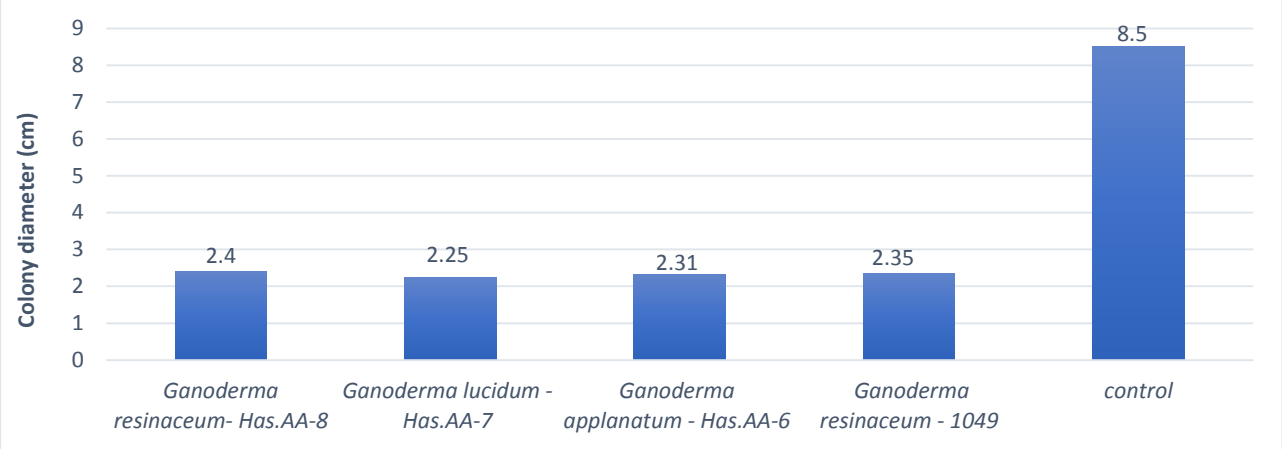


Figure (2). Effect of alcohol extract of *Ganoderma* spp. fruit bodies on the growth of the pathogenic fungus *A. tenuissima* (LSD 0.05= .13).

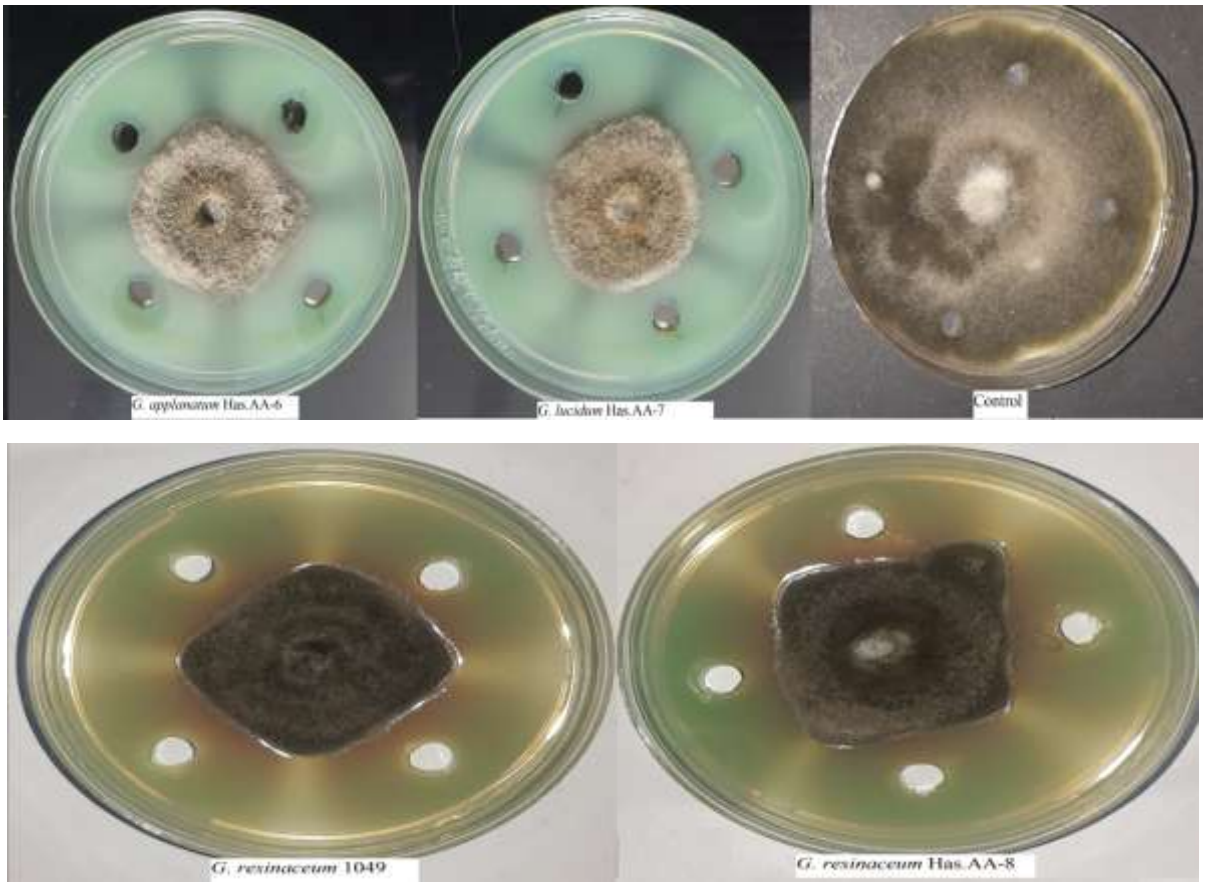


Figure (3) The highest inhibitory effect on the growth of the pathogenic fungus *A. tenuissima* by the alcoholic extract of the fruit bodies of *Ganoderma* spp. grown in MEB

**Field experiment**

**Effect of the *G. lucidum* extract and filtrate on the resistance induced markers in three tomato varieties under infection conditions with *A. tenuissima*.**

**Chitinase activity**

The results presented in Table 3 demonstrated the induction of chitinase activity in the treatments of the tomato varieties (Roa'a, Agro-Tip, and S25) under infection conditions

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with the pathogenic fungus *A. tenuissima*. The table revealed the efficacy of the treatments in increasing chitinase activity. The highest enzymatic activity was observed in the combining *G. lucidum* extract and filtrate (1:1) in present of *A. tenuissima*, with a value of 2.532 Units/mL in the Agro-Tip tomato variety, followed by *G. lucidum* extract (G.l) + A.t, which recorded an enzymatic activity of 2.173 Units/mL. In comparison, the control treatment of the healthy Agro-Tip variety exhibited the lowest chitinase activity of 0.034 Units/mL in Roa'a variety.

Table (3) Effect of *G. lucidum* extract and filtrate on the chitinase activity (unit.ml$^{-1}$) for three tomato cultivars under conditions of infection with A. tenuissima.

| **Treatments** | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro – Tip | S25 | |
| Control (healthy plants) | 0.034 | 0.043 | 0.039 | 0.039 |
| *A. tenuissima (A.t)* | 1.044 | 1.762 | 1.246 | 1.35 |
| Othilotop + *A.t* | 0.711 | 1.216 | 0.91 | 0.95 |
| Filtrate of *G. lucidum* + *A.t* | 1.458 | 2.173 | 1.657 | 1.76 |
| Extract of *G. lucidum* (*G.l*) + *A.t* | 1.643 | 2.358 | 1.842 | 1.95 |
| Filtrate and Extract of *G. lucidum* (1:1) +*A.t* | 1.837 | 2.532 | 2.016 | 2.13 |
| Average of varieties | 1.12 | 1.68 | 1.285 | |
| LSD, 0.05 | Treatments= 0.12 , Variety= 0.13, Treatments×variety=0.16 | | | |

**β-1,3 glucanase activity**

Table 4 revealed the induction of β-1,3 glucanase in the treatments of the three tomato varieties under infection conditions with *A. tenuissima*. The table demonstrated the efficacy of the treatments and their interactions in increasing β-1,3 glucanase activity. The highest enzymatic activity was 1.774 Units/mL observed in the combining *G. lucidum* extract and filtrate in the Agro-Tip variety, followed by 1.758 Units/mL in *G. lucidum* extract (G.l) + *A. tenuissima*. In comparison, the control treatment of the healthy Agro-Tip variety exhibited the lowest glucanase activity of 0.033 Units/mL in Roa'a variety.

Table (4) Effect of G. lucidum extract and filtrate on the -1,3 glucanaseβ activity (unit.ml$^{-1}$) for three tomato cultivars under conditions of infection with A. tenuissima.

| Treatments | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro-tip | S25 | |
| Control (healthy plants) | 0.033 | 0.035 | 0.033 | 0.03 |
| *A. tenuissima* (A.t) | 1.217 | 1.686 | 1.305 | 1.40 |
| Othilotop + A.t | 0.596 | 1.065 | 0.784 | 0.82 |
| Filtrate of *G. leucidum* + A.t | 1.258 | 1.727 | 1.346 | 1.44 |
| Extract of *G. lucidum* (G.l) + A.t | 1.289 | 1.758 | 1.477 | 1.51 |
| Filtrate and Extract of *G. lucidum* (1:1)+ A.t | 1.31 | 1.774 | 1.498 | 1.53 |
| Average of varieties | 0.95 | 1.34 | 1.074 | |
| LSD, 0.05 | Treatments= 0.07 , Variety=0.05 , Treatments×variety=0.09 | | | |

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**Disease severity**

The results in Table 5 demonstrated the efficacy of the treatments in reducing the percentage of disease severity in tomato plants infected with the pathogenic fungus *A. tenuissima* compared to the control treatment inoculated with *A. tenuissima* alone. Extract , and filtrate of *G.lucidum* in present of *A. tenuissima* achieved a high percentage of disease severity reduction, reaching 15.33%, 17.07%, and 20.36% for the Agro-Tip, S25, and Roa'a varieties, compared to the compared to 84.7%, 86.05%, and 87.82% in the pathogenic fungus treatment, respectively

Table (5) Effect of *G. lucidum* extract and filtrate on the disease severity (%) for three tomato cultivars under conditions of infection with *A. tenuissima*.

| Treatments | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro – Tip | S25 | |
| Control (healthy plants) | 0 | 0 | 0 | 0 |
| *A. tenuissima* (A.t) | 87.82 | 84.7 | 86.05 | 86.19 |
| Othilotop + A.t | 23.87 | 18.85 | 20.59 | 21.10 |
| Filtrate of *G. lucidum* + A.t | 25.29 | 20.25 | 21.96 | 22.5 |
| Extract of *G. lucidum* (G.l) + A.t | 22.13 | 17.09 | 18.83 | 19.35 |
| Filtrate and Extract of *G. lucidum* (1:1)+ A.t | 20.36 | 15.33 | 17.07 | 17.57 |
| Average of varieties | 29.91 | 26.04 | 27.42 | |
| LSD, 0.05 | Treatments= 2.11 , Variety= 1.43, Treatments×variety=2.62 | | | |

**Plant height**

Table 6 illustrates the effect of G. lucidum on plant height in three tomato varieties under infection conditions with *A. tenuissima*. The highest plant height was observed in the (Extract and filtrate of *G.lucidum*) in present of *A. tenuissima* reaching 148.56 cm in the Agro-Tip variety, followed by 144.93 cm in the treatment of *G. lucidum* extract (G.l) + *A. tenuissima*, , compared to the lowest plant height (71.6 cm) in Roa'a variety infected with *A. tenuissima*

Table (6) Effect of *G. lucidum* extract and filtrate on the Plant height (cm) for three tomato cultivars under conditions of infection with *A. tenuissima*.

| Treatments | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro – Tip | S25 | |
| Control (healthy plants) | 133.07 | 146.66 | 139.19 | 139.64 |
| *A. tenuissima* (A.t) | 71.6 | 85.14 | 76.97 | 77.90 |
| Othilotop + A.t | 126.94 | 140.47 | 132.31 | 133.24 |
| Filtrate of *G. lucidum* + A.t | 129.92 | 142.46 | 135.29 | 135.89 |
| Extract of *G. lucidum* (G.l) + A.t | 131.37 | 144.93 | 136.74 | 137.68 |
| Filtrate and Extract of *G. lucidum* (1:1)+ A.t | 135.02 | 148.56 | 140.39 | 141.32 |
| Average of varieties | 121.32 | 134.70 | 126.81 | |
| LSD, 0.05 | Treatments= 2.61 , Variety=2.16 , Treatments×variety=3.08 | | | |

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**Shoot system Dry weight**

Table 7 showed the (Extract and filtrate of *G.lucidum* in present of *A. tenuissima*) achieved the highest dry weight rate in the Agro-Tip variety, reaching 112.38 g. In comparison, the control treatment (healthy plants) exhibited a dry shoot system weight of 118.63 g for the Agro-Tip variety, while the lowest dry shoot system weight was 29.46 g in Roa'a variety infected with *A. tenuissima*.

Table (7) Effect of *G. lucidum* extract and filtrate on the Shoot system Dry weight (g) for three tomato cultivars under conditions of infection with *A. tenuissima*.

| Treatments | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro – Tip | S25 | |
| Control (healthy plants) | 98.33 | 118.63 | 107.88 | 108.28 |
| *A. tenuissima* (A.t) | 29.46 | 40.96 | 32.8 | 34.41 |
| Othilotop + A.t | 83.33 | 101.83 | 87.67 | 90.94 |
| Filtrate of *G. lucidum* + A.t | 84.35 | 103.85 | 89.69 | 92.63 |
| Extract of *G. lucidum* (G.l) + A.t | 85.91 | 105.41 | 91.25 | 94.19 |
| Filtrate and Extract of *G. lucidum* (1:1)+ A.t | 93.88 | 112.38 | 98.22 | 101.40 |
| Average of varieties | 79.21 | 97.18 | 84.59 | |
| LSD, 0.05 | Treatments=2.33, Variety=2.89, Treatments×variety=3.36 | | | |

**Crop yield**

Table 8 showed the effect of *Ganoderma lucidum* extract and filtrate on the yield weight of three tomato variety (Roaa, Agro-Tip, and S25) under infection conditions with the pathogenic fungus *A. tenuissima*. The combining *G. lucidum* extract and filtrate achieved the highest yield weight for the Agro-Tip variety , reaching 7812.78 g, followed by the treatment *of Ganoderma lucidum* extract (G.l) + *A. tenuissima*, which resulted in a yield weight of 7779.13 g for the same variety , while the lowest yield weight was 2365.16g in Roa'a varieties infected with *A. tenuissima*

Table (8) Effect of *G. lucidum* extract and filtrate on the Crop yield (g) for three tomato cultivars under conditions of infection with *A. tenuissima*.

| Treatments | Varieties of tomato | | | Average of treatments |
|---|---|---|---|---|
| | Roa'a | Agro-tip | S25 | |
| Control (healthy plants) | 7325.07 | 7808.83 | 7640.22 | 7591.373 |
| *A. tenuissima* (A.t) | 2365.16 | 2871.01 | 2711.77 | 2649.313 |
| Othilotop + A.t | 7230.87 | 7736.72 | 7577.48 | 7515.023 |
| Filtrate of *G. lucidum* + A.t | 7247.41 | 7753.26 | 7594.02 | 7531.563 |
| Extract of *G. lucidum* (G.l) + A.t | 7273.28 | 7779.13 | 7619.89 | 7557.433 |
| Filtrate and Extract of *G. lucidum* (1:1)+ A.t | 7306.93 | 7812.78 | 7653.54 | 7591.083 |
| Average of varieties | 6458.12 | 6960.288 | 6799.487 | |
| LSD, 0.05 | Treatments= 21.03 , Variety= 66.65, Treatments×variety=78.19 | | | |

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## Discussion

Fungi produce various biologically active compounds, such as phenols, benzoates, sugars, glucans, and lectins, which are reported to provide over 126 health benefits, including antimicrobial, immune-enhancing, antioxidant, antiviral, and other properties (5). Metabolites derived from the secondary metabolism of *Ganoderma lucidum* fungi possess antimicrobial, antitumor, and antioxidant properties, playing a significant role as control agents against plant diseases caused by viruses, fungi, and bacteria (37). Both *Ganoderma* spp. fungal extracts and their filtrates have been found to play a role in enhancing the activity of the enzymes chitinase and glucanase, activating these enzymes in tested tomato varieties. This effect may be attributed to the presence of secondary metabolites and enzymatic components in these extracts and exudates, which activate the genes responsible for producing these enzymes. This phenomenon is one of the mechanisms involved in the systemic resistance induction in plants, and it aligns with similar studies that have demonstrated the induction of systemic resistance in various plant species through the exudates of yeasts and filamentous fungi (10, 2, 13).

Numerous studies have identified 46 important enzymatic proteins in *Ganoderma* spp., including deoxyribonuclease, ribonuclease, protease, glucanase, and chitinase. Notably, the activities of protease, glucanase, and chitinase were recorded as 0.610, 6.5, and 0.053 units/mL, respectively, in *G. lucidum* (27). These enzymes are crucial compounds secreted by microorganisms as control agents to inhibit the growth of pathogens (21). The inhibitory effect can also be attributed to the non-enzymatic antifungal proteins produced by some organisms, such as antimicrobial peptides (AMPs), which play a vital role in suppressing viruses, bacteria, fungi, and parasites. AMPs have been found to stimulate microbial immunity and enhance plant resistance, serving as the first line of defense against pathogens (45). Ganoderma spp. fungal extracts and their exudates have been found to stimulate vegetative growth in tested tomato varieties. This effect can be attributed to the presence of growth promoters, such as certain hydrolytic enzymes, which help provide more readily available nutrients to the plants. Furthermore, there is a possibility of plant hormones production within the metabolic substances present in the *Ganoderma* spp. exudates, such as cytokinins. These findings are consistent with previous studies that have highlighted the role of fungi and their exudates in promoting plant growth (11 , 12 , 1). (43) reported the presence of ganodermine, an antifungal protein, in *Ganoderma* spp., which accounts for its inhibitory effect on fungi like *Botrytis cinerea*, *Fusarium oxysporium*, and *Pysalospora piricola*.

The inhibitory effect on pathogenic fungi may also be attributed to the presence of volatile oils, which are secondary metabolites of *Ganoderma* spp., known for their antifungal properties (46, 9). These findings are consistent with previous studies, such as (42), who demonstrated the high efficacy of alcohol extracts from *Ganoderma lucidum* against bacterial and fungal pathogens, including *Aspergillus niger* and *Aspergillus flavus*. Furthermore, (14) found that a mixture of enzymes and proteins from the edible fungus *Pleurotus ostreatus*, which possess anti-growth properties against the nematode Meloidiogyne javanica and pathogenic fungi *F. oxysporum* and *R. solani*, showed significant superiority in terms of dry weight of both shoot and root components, with the highest average dry weight of the shoot recorded in the presence of the pathogenic fungus *F. oxysporum* (3.72 g compared to the control treatment's 2.41 g).

(8) demonstrated that certain secondary metabolites produced by *Ganoderma* spp. exerted inhibitory effects against various phytopathogens. This inhibition was attributed to the presence of antifungal proteins and mycelial polysaccharides. Notably, extracts

from *G. lucidum* exhibited significant inhibitory effects against *Fusarium* spp. and the phytopathogenic fungus *Fusarium oxysporum*. . The decrease in the severity of tomato leaf spot disease due to *Ganoderma* spp. fungal extracts and their filtrates may be a result of inhibiting the pathogenic fungus *A. tenuissima*, due to the active components present in these extracts and exudates, such as alkaloids, phenolics, tannins, coumarins, and flavonoids. These compounds have been proven to possess antifungal properties in laboratory experiments conducted in this study (Table 1 and 2, Figure 1). These results are consistent with a study by (16 , 18 ) who demonstrated that alkaloids, phenolics, tannins, saponins, and flavonoids inhibited the growth of the pathogenic fungus Trichoderma on the edible mushroom *Agaricus bisporus*. These results are agree with the study of Shahad et al. (32), which achieved that alcoholic extracts derived from *Ganoderma lucidum* fungus demonstrated the capacity to inhibit *Alternaria alternate* fungus. The alcoholic extract from the fruiting bodies exhibited superior efficacy compared to the aqueous extract, showing an inhibition rate of 64%. *Ganoderma* spp. fungal extracts and their exudates have been found to contribute to increasing tomato productivity. This may be attributed to the effectiveness of these treatments in inducing plant resistance, promoting vegetative growth, and reducing disease severity, all of which ultimately lead to an increase in tomato yield

## Conclusion

From this study, it can be concluded that *Ganoderma* spp. fungal extracts and their exudates contain several active substances that inhibited the pathogenic fungus *A. tenuissima* . Additionally, these extracts and exudates induced systemic plant resistance and stimulated vegetative and productive growth in the studied tomato varieties. The tomato variety "Agro-tip" showed the best response to the treatments with Ganoderma spp. fungal extracts and their exudates, exhibiting lower disease severity caused by the pathogenic fungus A, superior growth parameters, and increased yield.

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