Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (10) Issue (3) (2025), (57-68) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg
Applied Agriculture Science

Studying the effect of some abiotic factors on managing root and crown rot diseases of strawberries and their effectiveness on their contents from phenols and defense enzyme activity

Heba A.Abdelhalem, Faten M.Abd ellatif, Khaled E.Eid and Ahmed A.Elsisi
Plant Pathology Dept., Faculty of Agriculture, Benha University, Egypt
E-mail: hebaslama808@gmail.com

Abstract
Crown and root rot in strawberries (*Fragaria ananassa*), instigated by the fungal pathogen *Macrophomina phaseolina*, poses a formidable challenge to global strawberry agriculture. This investigation scrutinizes the effectiveness of various chemical and natural inducers against *Macrophomina phaseolina* strain AUMC 16307 with accession number PP178224, a soilborne menace to strawberry vitality. Among the treatments assessed, oxalic acid and Rizolex-T 50% exhibited remarkable efficacy, achieving complete inhibition of mycelial growth at concentrations of 8 mM and 3 g/L, respectively. Oxalic acid compromised fungal cell membranes and chelated vital metal ions, while Rizolex-T 50% impeded lipid biosynthesis, undermining cell membrane integrity. Conversely, chitosan, despite its recognized antifungal attributes, proved ineffective at concentrations ranging from 1 to 3 g/L, potentially due to inadequate molecular weight or deacetylation levels. Zinc oxide nanoparticles (ZnO. NPs) demonstrated promising results, achieving 88.1% efficacy at 3%, by generating reactive oxygen species that inflicted damage on fungal cells. Camphor oil, a natural terpenoid, displayed moderate efficacy with 59.6% inhibition at 3%, disrupting fungal membranes and mitochondrial functions. Rizolex-T 50% emerged as the most potent treatment, significantly curtailing disease incidence and severity, although its chemical nature raises environmental and resistance concerns. Natural inducers like oxalic acid, chitosan, ZnO. NPs and camphor oil exhibited moderate effectiveness, underscoring their potential as sustainable alternatives. These treatments bolster plant growth and enhance phenolic content and defense enzyme activities, aligning with their roles in systemic resistance and oxidative defense.

Keywords: Zinc oxide nanoparticles, oxalic acid, camphor oil, Chitosan, Rizolex-T 50% *Macrophomina phaseolina*, crown, root rot, and strawberry.

1. Introduction

Crown and root rot disease, caused by soil-borne pathogens such as *Phytophthora cactorum* and *Macrophomina phaseolina,* is a major constraint in strawberry production, leading to significant economic losses due to reduced yield and fruit quality.

Conventional control methods, such as chemical fungicides, have been widely used but are increasingly scrutinized for their environmental impact, potential health risks, and the emergence of resistant pathogen strains. As a result, there is a growing interest in sustainable and eco-friendly alternatives for disease management. Inducer factors such as zinc oxide nanoparticles (ZnO. NPs), oxalic acid, camphor oil, and chitosan have emerged as promising tools for controlling crown and root rot disease in strawberries. These inducers work through multiple mechanisms, including the activation of systemic acquired resistance (SAR), direct antimicrobial activity, and the enhancement of plant physiological responses. For example, ZnO. NPs have demonstrated strong antifungal properties and the ability to stimulate plant growth (10). Oxalic acid has been shown to induce defense-related enzymes and phytoalexin production, enhancing plant resistance to pathogens (39). Camphor oil, with its natural antifungal and antibacterial properties, provides a biodegradable option for disease control (1). Chitosan, a biopolymer derived from chitin, is widely recognized for its ability to boost plant immunity and inhibit pathogen growth (23). This study aims to highlight scientific evidence supporting the importance of these induced factors in managing crown and root rot disease in strawberries and underscore their potential as sustainable components of integrated disease management strategies.

2. Materials and Methods

1. Pathogen isolation, purification, and identification:

Samples of strawberry plants showing root and crown rot symptoms collected from different strawberry fields were subjected to isolation trials for the causal fungi. The infected roots and crowns were thoroughly washed under running tap water to remove soil particles, then cut into small pieces before being surface treated in 1% sodium hypochlorite solution for 1 minute, rinsed twice in sterile distilled water, and drained between two sterilized filter papers, then placed onto PDA medium in Petri dishes and incubated at 24° C for 7 days. Fungi that developed on the medium were purified using the hyphal tip technique or single spore method suggested by (9) and then transferred to Petri dishes containing PDA for 10 days before identification. Inoculum of each purified culture was transferred to PDA slants and incubated at 25°C. All fungal isolates were cultured on PDA for identification. The various purified isolated fungi were identified based on their morphological characteristics. According to (17) ,(5), (6), and (12). The identification of all fungus isolates was confirmed by The Assuit University Mycological Center (AUMC).

1.2. Molecular identification of fungal isolate:
Fungal isolate was grown on Czapek`s yeast extract agar (CYA) medium and incubated at 28°C for 5 days (27). DNA extraction was performed at the Molecular Biology Research Unit, Assiut University using a Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). Polymerase chain reaction (PCR) and gene sequencing were done with the help of SolGent Company, Daejeon, South Korea. The internal transcribed spacer (ITS) region of the ribosomal ribonucleic acid (rRNA) gene was amplified using the universal primers ITS1 (forward) and ITS4 (reverse), which were incorporated into the reaction mixture. Primers have the following composition: ITS1 (5' - TCCGTAGGTGAA CCTGCGG - 3'), and ITS4 (5'- TCCTCCGCTTATTGATATGC -3'). The purified PCR product was sequenced with the same primers with the addition of dideoxynucleotides (ddNTPs) in the reaction mixture (38). The sequenced region covers the small subunit 18S (partial sequencing), ITS1, 5.8S, ITS2 (complete sequencing), and the large subunit 28S (partial sequencing). The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Analysis of sequences and establishment of phylogenetic trees were done using MegAlign (DNA Star) software version 5.05.

2. *In Vitro* Assessment of Abiotic Influences on the Linear Proliferation of *Macrophomina phaseolina:*

The etiological agent responsible for crown and root rot infection in strawberries, *M. phaseolina*, underwent *in vitro* scrutiny to elucidate the ramifications of abiotic factors on its linear growth. Inocula from the most virulent strains of *M. phaseolina* were cultivated on Potato Dextrose Agar (PDA) medium. Petri dishes containing 15 mL of PDA were inoculated with 5 mm diameter discs extracted from the periphery of 5-day-old cultures of *M. phaseolina*, which had been nurtured at a temperature of 25 ± 2°C.

2.2. Assessment of Diverse Concentrations of Commercial Camphor Oil on the Linear Growth of M. phaseolina:

Camphor oil (*Cinnamomum camphora*) was procured from Al-Qus Company, esteemed for its cold oil extraction methods, situated in Qena Governorate, Egypt. The commercial camphor oil was meticulously evaluated for its capacity to impede the growth of *M. phaseolina*. Specific volumes of the oil were amalgamated into PDA media flasks to attain the desired concentrations of 1, 1.5, and 2%, supplemented with 0.1% Tween-80 (28). The treated and untreated (control) media were allocated into four Petri dishes for each concentration. Upon solidification of the media, the Petri dishes were inoculated with a 5 mm disc from a 7-day-old culture of *M. phaseolina*. The plates were subsequently incubated at a regulated temperature of 25 ± 2°C for 7 days. Four plates for each treatment served as replicates. The linear growth of the pathogens was gauged once the fungal mycelium had entirely enveloped the control plates, and the percentage reduction in mycelial growth was computed using the formula delineated subsequently.

2.3. Evaluation of Chemical Inducers:

The investigation into the effects of various chemical inducers on the growth of *M. phaseolina in vitro*. The chemicals scrutinized included oxalic acid (ASIN: BOC1X164FY) was sourced from ACG EGYPT, characterized by a molecular weight of 126.07 (C2H2O4.2H2O), at three concentrations: 2, 4, and 8 mM and alongside chitosan at concentrations of 1, 2, and 3 g/L.

2.3.1. Preparation of Chitosan Stock Solution:

Chitosan (CAS number: 9012-76-4) was sourced from Sigma-Aldrich, distinguished by a molecular weight of 161.16 (C6H11NO4X2) and a degree of deacetylation of ≥80%. A stock solution of chitosan was concocted by weighing 2 g of chitosan and dissolving it in 100 mL of distilled water, augmented with 1 mL of acetic acid. The mixture was subjected to heating while being continuously agitated for 24 hours. The pH of the solution was subsequently adjusted to 5.6 through the addition of 1 N sodium hydroxide (0.1 mL). This chitosan solution was then employed to derive various concentrations of 1, 2, and 3 g/L for *in vitro* studies (33)

2.4. Evaluation of Zinc Oxide Nanoparticles (ZnO NPs):

Zinc oxide nanoparticles (ZnO NPs) were graciously *provided* by the Nanotechnology & Advanced Nano-Materials Laboratory (NANML), Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, and were synthesized utilizing methodologies delineated by (15). The nanoparticles were prepared at concentrations of 0.25, 0.50, and 1 mM.

2.4.1. Transmission Electron Microscopy (TEM) Analysis of Zinc Oxide Nanoparticles (ZnO NPs):

For TEM analysis, a droplet of the ZnO. NPs solution was deposited on carbon-coated copper grids (CCG) and allowed to dry at ambient temperature to facilitate water evaporation. Electron micrographs were captured using a JEOL GEM-2100 PLUS transmission electron microscope at an accelerating voltage of 200 kV, conducted at the Department of Genetic Engineering, Faculty of Science, Al-Azhar University (35). The efficacy of the chemical inducers—oxalic acid (2, 4, and 8 mM), chitosan (1, 2, and 3 g/L), and zinc oxide nanoparticles (ZnO. NPs; 0.25, 0.50, and 1 mM) was evaluated employing the poisoned food technique on PDA medium, adhering to the modified method of (26). The chemical inducers were aseptically incorporated into warm, sterilized PDA medium at the specified concentrations and dispensed into Petri dishes (90 mm diameter) at a rate of 15 mL per plate. Each plate was inoculated at the center with a 5 mm disc from a 7-day-old culture of *M. phaseolina*. Control plates contained PDA medium devoid of chemical inducers. Four replicates were utilized for each concentration, and all plates were incubated at 25 ± 2°C.

2.5. Evaluation of Rizolex-T 50% WP Fungicide:

The inhibitory efficacy of Rizolex-T 50% WP (comprising tolclofos-methyl and thiram) on *M. phaseolina* was appraised *in vitro* at concentrations of 1, 2, and 3 g/L. The active ingredients, chemical formulas, and manufacturer details are delineated in Table 1

Table 1. List of tested fungicide, active ingredients, chemical formula, manufacturer, and application rate.

Trade name	Common name & Active ingredient	Manufacture	Chemical formula	Used dose
Rizolex-T 50% WP	Tolclofos methyl +Thiram	Sumitomo Chemical Co., Ltd., Japan	20%Tolclofos methyl(0,2,6-dichloro-4-methyl phenyl-0,0-dimethyl phosphorothioate) and 30% Thiram (tetramethylthiram disulfide)	3 g/L of water

Fifty milliliters of PDA medium were poured into 250 mL sterilized glass conical flasks, to which the specified concentrations of the fungicide were added and thoroughly mixed. The poisoned PDA medium was then dispensed into sterilized Petri dishes (90 mm diameter) at 15 mL per plate and allowed to solidify. Each plate was inoculated with a 5 mm disc from a 7-day-old culture of *M. phaseolina*. Control plates contained PDA medium without fungicide. Four replicates were employed for each concentration, and all plates were incubated at 25 ± 2°C. The experiment concluded when the mycelial mats covered the medium surface in the control treatment. Fungal growth was measured, and the growth inhibition percentage (GIP %) was calculated.

2.6. Measurement of Fungal Growth Inhibition:

by (4) computed the growth inhibition percentage (GIP) of fungl mycelial growth using the formula: GIP = (C−T / C) × 100

Where:

- GIP = growth inhibition percentage over control,
- C = Growth of the pathogen in the absence of the antagonist (mm),
- T = Growth of the pathogen in the presence of the antagonist (mm).

Four replicates were utilized for each treatment, and the plates were incubated at 25 ± 2°C for seven days. The colony diameter of *M. phaseolina* on the control plates was recorded, and the inhibition percentages were calculated.

2. *In Vivo* Evaluation of Abiotic Factors for Mitigating

Strawberry Crown and Root Rot Infection:

The *in vivo* experiment was conducted on strawberry plants (cv. Fortuna) Nurseries of Green Point Company for Import, Export and Commercial Agencies (13.08.47.26.STN) Two weeks old seedlings in pots (20 cm diameter) under pots experimental conditions at plant pathology dep., Faculty of Agriculture, Banha University, Egypt, during the 2023 growing season. Two strawberry seedlings were cultivated in each pot, with four pots designated for each treatment.

2.1. Preparation of Pots and Soil:

Pots and soil were prepared as delineated in the pathogenicity test. The soil was sterilized and utilized for the planting of strawberry seedlings.

2.2. Preparation of Pathogen Inocula:

Inocula of *M. phaseolina* were cultivated on Potato Dextrose Agar (PDA) plates for aduration of 10 days at a controlled temperature of 25 ± 2°C. Following this incubation period, the inocula were transferred to a sand-barley medium, where they were allowed to proliferate for an additional two weeks. The resultant inocula were then amalgamated with the potted soil at a concentration of 3.0% (w/w), ensuring thorough integration with the soil surface. This mixture was allowed to rest for one week to facilitate uniform distribution throughout the substrate. Control pots, which were infested with the pathogenic fungi and planted with untreated strawberry seedlings subjected to the abiotic factors, were maintained under experimental conditions at 25 °C ± 2. The incidence and severity of disease affecting both crown and root rot were meticulously evaluated at intervals of 21 and 45 days post-planting, respectively.

2.3. Evaluation of Plant Camphor Oil:

The efficacy of plant-derived camphor oil at a concentration of 2% was scrutinized as a root-dipping treatment to control *M. phaseolina*, responsible for root and crown rot diseases. Before application, the camphor oil was emulsified with 0.05% Tween 20, following the methodology outlined by (37) This emulsion was utilized for a root-dipping procedure lasting 2 hours before the transplantation of seedlings. Subsequently, the camphor oil was administered to the soil at the same concentration as a soil drench, applied after 7 and 15 days post-planting.

2.4. Evaluation of Chemical Resistance Inducers:

The effectiveness of various chemical inducers, specifically oxalic acid (OX) at a concentration of 8 mM and chitosan at 3 g/L, was assessed as root-dipping treatments for the control of *M. phaseolina-induced* root and crown rot diseases. These chemical agents were applied as a root-dipping treatment for 2 hours before planting, as per the protocol established by (2). The same concentrations of the chemical inducers were subsequently utilized as a soil drench after 7 and 15 days of planting.

2.5. Evaluation of Zinc Oxide Nanoparticles (ZnO. NPs):

The efficacy of zinc oxide nanoparticles (ZnO. NPs) at a concentration of 1 mM was evaluated as a root-dipping treatment for the control of *M. phaseolina* root and crown rot diseases. The application involved a 2-hour root-dipping procedure prior to planting. A

separate group of pots containing un-inoculated medium served as a control. Following the sowing of seedlings in soil inoculated with *M. phaseolina*. The resultant inocula were then amalgamated with the potted soil at a concentration of 3.0% (w/w), the seedlings were sprayed with 15 mL of ZnO. NPs after 7 and 15 days.

2.6. Evaluation of Rizolex-T 50% WP Fungicide:

Rizolex-T 50% WP, fungicide at a concentration of 3 g/L in water, was employed as a root-dipping treatment. Healthy strawberry seedlings (15 days old, cv. Fortuna) were immersed in the fungicide solution for 5 minutes, then removed and allowed to air dry before planting. Following this, the fungicide was incorporated into the soil at the same concentration as a soil drench after 7 and 15 days of planting.

2.7. Biochemical Analysis

To assess plant defense responses, key enzymatic activities were investigated. Peroxidase (PO), polyphenol oxidase (PPO), and chitinase activities were measured using spectrophotometric methods as described by (19) and (32). Total phenol analysis was determined by spectrometry method (24) 100 µL sample was added with 1 mL Folin-Ciocalteu 10%- and 2-mL Sodium Carbonate 7.5%. The mixture was added with water in a 10 mL volumetric flask and shaken. The solution was incubated at an ambient temperature for 30 min, and the absorbance of the sample was measured at λ 760 nm. The total phenolic content of the sample was stated by gallic acid equivalence (GAE)/g sample dry weight.

3. Statistical analysis

Data from the experiments were analyzed as per the procedure of the Randomized Complete Block Design with three replicates according to (36). Data were subjected to analysis of (ANOVA) with a p-value of < 0.05. The least significant difference (LSD 0.05) was used as a post hoc. All statistical analyses were carried out using the Software program, COSTAT version 6.311 was used to perform the analysis, and means were compared using Duncan`s test at a significant level of 0.05.

3. Results

1. Molecular identification of *M. phaseolina* was isolated from strawberry

The identification of the most aggressive isolates, *M. phaseolina* was further confirmed based on the sequence of large subunit ribosomal RNA of the internal transcribed spacer (ITS) region (Figure 1). Briefly, the query sequence showed a high similarity with the large subunit ribosomal RNA gene of *M. phaseolina*, strains. Figure 1 showed that the Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*M. phaseolina* AUMC16307, arrowed) aligned with closely related strains accessed from the GenBank. This strain showed 99.45% - 100% identity and 95% - 100% coverage with several strains of the same species including the type material *M. phaseolina* strain CBS205.47T with accession no. KF951622.

2. *In vitro* evaluation of Zinc oxide nanoparticles (ZnO. NPs), Chitosan, Oxalic acid, camphor oil, and Rizolex-T 50% WP. With different concentrations on the linear growth of mycelium inhibition of *M. phaseolina*:

2.1. Characterization of Zinc oxide nanoparticles (ZnO NPs):

Zinc oxide nanoparticles (ZnO NPs) were graciously provided by the Nanotechnology & Advanced Nano-Materials Laboratory (NANML), Plant Pathology Research Institute, Agricultural Research Center, Giza 12619, and were synthesized utilizing methodologies delineated by (15). The morphological characteristics of nanoparticles were investigated by transmission electron microscope (TEM). Zinc oxide nanoparticles (ZnO NPs) were uniform and spherical with an average particle size of 13.8 -42.61 nm (Fig. 2).

2.2. Antifungal activity of zinc oxide nanoparticles (ZnO. NPs), chitosan, oxalic acid, camphor oil, and Rizolex-T 50% W. with different concentrations on linear growth and growth reduction of *M. phaseolina*:

Table 2 presents the evaluation of various chemical inducers on the linear of mycelial growth inhibition of *M. phaseolina*, the causative agent of root and crown rot diseases in strawberries. In conclusion, Fig. 3 provides valuable insights into the efficacy of different chemical inducers against M. *phaseolina*. The control group (untreated) showed 90.0 mm mycelial growth and 0.0% efficacy, serving as a baseline for comparison. Oxalic acid and Rizolex-T stand out as the most effective treatments. Higher concentrations of most treatments (except chitosan) generally led to increased inhibition of mycelial growth. This suggests a dose-dependent response for oxalic acid, camphor oil, zinc oxide nanoparticles (ZnO NPs), and Rizolex-T50% WP. Oxalic acid and Rizolex-T were the most effective treatments, achieving complete inhibition (100% efficacy) at their highest concentrations. Chitosan at all tested concentrations (1g/L, 2g/L, and 3g/L) showed no efficacy (0.0%) in inhibiting the mycelial growth of M. *phaseolina*, as the mycelial growth remained at 90.0 mm, identical to the control.

This suggests that chitosan, at these concentrations, is ineffective against this pathogen. Oxalic acid demonstrated significant efficacy, with increasing concentrations leading to higher inhibition. At 8 mM, it achieved 100% efficacy, completely inhibiting mycelial growth (0.0 mm). This indicates that oxalic acid is highly effective against *M. phaseolina.* Camphor oil showed moderate efficacy, with the highest concentration (3%) achieving 59.6% inhibition. However, its effectiveness was lower compared with oxalic acid and nano zinc oxide. Zinc Oxide NPs, this treatment showed promising results, with 3% concentration achieving 88.1% efficacy. The inhibition increased with higher concentrations, suggesting its

potential as a biocontrol agent. Similar to oxalic acid, Rizolex-T 50% was highly effective, with 3 g/L achieving 100% efficacy. This indicates its strong antifungal activity against *M. phaseolina*.

3. *In vivo* evaluation of some abiotic factors for controlling strawberry crown and root rot infection under pot experimental conditions:

3.1. Effects of various treatments on disease incidence and severity of root and crown rot:

Table 3 evaluated the efficacy of different treatments on disease incidence and severity of root and crown rot in strawberry plants under pot experimental conditions. The treatments included oxalic acid, chitosan, zinc oxide nanoparticles (ZnO NPs), camphor oil, rizolex-T 50% (chemical fungicide), and an untreated control.

Disease incidence and severity were assessed at 21 and 45 days post-treatment. The untreated control exhibited the highest disease incidence at both time points, with values of 90.00% on 21 days and 96.66% on 45 days. Similarly, the untreated control showed the highest disease severity for both root rot (93.33%) and crown rot (90.00%), underscoring the aggressive nature of the disease in the absence of intervention. Among the treatments, Rizolex-T 50% demonstrated the highest efficacy, with the lowest disease incidence on 21 days (8.33%) and 45 days (11.66%). It also exhibited the lowest disease severity for both root rot (6.00%) and crown rot (6.33%), highlighting its superior ability to suppress disease progression. Oxalic acid, chitosan, ZnO NPs, and camphor oil showed moderate efficacy in reducing disease incidence and severity compared to the untreated control. Disease incidence for these treatments ranged from 46.33% to 59.43% on 21 days and 61.66% to 68.33% on 45 days. Disease severity for root rot ranged from 41.33% (camphor oil) to 47.00% (oxalic acid), while for crown rot, severity ranged from 46.66% (camphor oil) to 60.00% (oxalic acid). Although these treatments were significantly more effective than the untreated control, they were less effective than Rizolex-T 50%. Notably, camphor oil showed the lowest disease severity among the natural inducers for both root rot (41.33%) and crown rot (46.66%), suggesting it may be the most promising natural alternative. However, no significant differences were observed among oxalic acid, chitosan, ZnO NPs, and camphor oil in terms of disease incidence or severity. All natural inducers were significantly less effective than Rizolex-T 50% but significantly better than the untreated control.

3.2. Effects of different treatments on plant growth parameters:

The effects of various treatments, i.e., oxalic acid, chitosan, zinc oxide nanoparticles (ZnO NPs), camphor oil, Rizolex-T 50%, and an untreated control, on plant growth parameters, including fresh and dry weights of shoots and roots, plant height, and root length, were evaluated.

Table 4 shows that untreated control group consistently recorded the lowest values across all parameters, as expected. For example, fresh shoot weight (8.33g) and dry shoot weight (1.66g) were significantly lower than the other treatments. Rizolex-T 50% emerged as the most effective treatment, significantly enhancing all measured plant growth parameters compared to other treatments and the control. Oxalic acid, chitosan, ZnO. NPs. and camphor oil showed moderate growth-promoting effects but were statistically comparable to one another in most cases.

Rizolex-T 50% demonstrated the most pronounced positive effects on all measured growth parameters. It significantly outperformed all other treatments and the control group, as evidenced by the highest values for fresh shoot weight (33.66g), fresh root weight (10.36g), dry shoot weight (9.63g), dry root weight (5.66g), plant height (25.13cm), and root length (15.66cm). The "a" superscript assigned to Rizolex-T 50% across all parameters indicates its statistical superiority, placing it in the highest grouping.

Oxalic acid, chitosan, ZnO. NPs and camphor oil exhibited moderate effects on plant growth, with no significant differences observed among them in most cases, as indicated by their grouping under "b" or "bc" superscripts. For instance, fresh shoot weights ranged from 11.66g (chitosan) to 15.00g (camphor oil), while dry shoot weights varied between 2.33g (oxalic acid) and 3.66g (camphor oil). Although these treatments were more effective than the control, their performance was significantly inferior to Rizolex-T 50%.

3.3. Effect of various treatments on total phenol content:

The results in Table 5 investigate the impact of different treatments on the total phenol content (mg/1g fresh weight) in strawberry plants grown under experimental pot conditions. Phenolic compounds, which play a critical role in plant defense mechanisms by contributing to the synthesis of phytoalexins, lignin, and other pathogen-resistant compounds, were quantified. The efficacy percentage of total phenol production relative to the untreated control was also evaluated.

The results highlight the potential of both chemical and natural inducers to enhance phenolic compound synthesis. The untreated control exhibited the lowest total phenol content (10.62 mg/1g fresh weight), reflecting minimal activation of defense mechanisms in the absence of treatment. The chemical fungicide Rizolex-T 50% treatment yielded the highest total phenol content (28.21 mg/1g fresh weight). Oxalic acid, chitosan, zinc oxide nanoparticles (ZnO NPs), and camphor oil treatments significantly increased total phenol content compared to the control, with values ranging from 22.93 mg/1g (oxalic acid) to 24.17 mg/1g (chitosan). The efficacy percentage of control

(untreated) was 0%, serving as the baseline for comparison. Rizolex-T 50% treatment exhibited the highest efficacy percentage (165%). The efficacy percentages for Oxalic acid, chitosan, zinc oxide nanoparticles (ZnO. NPs), and camphor oil treatments ranged from 116% (oxalic acid) to 128% (chitosan), reflecting increases in phenol content of 16% to 28% relative to the control. Among the treatments, chitosan was the most effective, followed by zinc oxide nanoparticles (ZnO. NPs; 126%) and camphor oil (121%). Oxalic acid was the least effective inducer, still demonstrating a significant increase over the control. The treatments (oxalic acid, chitosan, ZnO NPs, and camphor oil) significantly enhanced phenol content compared to the untreated control, confirming their ability to activate plant defense mechanisms. However, their efficacy was lower than that chemical fungicide Rizolex-T 50%. Among the natural inducers, chitosan achieved the highest phenol content (24.17 mg/1g) and efficacy percentage (128%), positioning it as the most effective inducer for stimulating phenolic compound synthesis.

3.4. Effects of different treatments on defense-related enzyme activities in strawberry plants:

The effects of various treatments like oxalic acid, chitosan, zinc oxide nanoparticles (ZnO NPs), camphor oil, Rizolex-T50%, and untreated control on the activities of defense-related enzymes (peroxidase (PO), polyphenoloxidase (PPO), and chitinase in strawberry roots were investigated under pot experimental conditions.

Table 6 shows that Rizolex-T 50% demonstrated the most significant enhancement of enzyme activities, followed by oxalic acid, chitosan, ZnO. NPs, and camphor oil. The untreated control exhibited the lowest enzyme activities with PO at 5.69, PPO at 3.79, and chitinase at 4.04. Underscoring the importance of treatments in activating plant defense mechanisms.

Rizolex-T 50% significantly increased the activities of all defense-related enzymes compared to other treatments and the control. Specifically, peroxidase (PO) activity reached 20.05, polyphenoloxidase (PPO) activity was 18.94, and chitinase activity was 18.58, representing the highest values among all treatments. These results indicate that Rizolex-T50% is highly effective in stimulating the plant's defense mechanisms.

Oxalic acid, chitosan, ZnONPs, and camphor oil induced moderate increases in enzyme activities, indicating their potential to enhance plant defense mechanisms, albeit to a lesser extent than Rizolex-T 50%. Among these, oxalic acid was the most effective, followed by chitosan and ZnO. NPs. Camphor oil, while showing the lowest PO activity, exhibited relatively higher chitinase activity.

Oxalic Acid treatment resulted in moderate increases in enzyme activities, with PO at 18.83, PPO at 16.19, and chitinase at 17.89. Oxalic acid showed the highest enzyme activities among the non-Rizolex-T 50%. treatments. Chitosan was less effective than oxalic acid but still demonstrated a significant increase compared to the control with PO at 17.31, PPO at 12.59, and chitinase at 15.66. Zinc oxide nanoparticles (ZnO NPs) treatment showed moderate enzyme activity increases, like chitosan with PO at 17.76, PPO at 14.75, and chitinase at 14.66. Camphor oil exhibited the lowest PO activity (12.48) among the treatments but showed relatively higher chitinase activity (16.89), with PPO activity at 13.85.

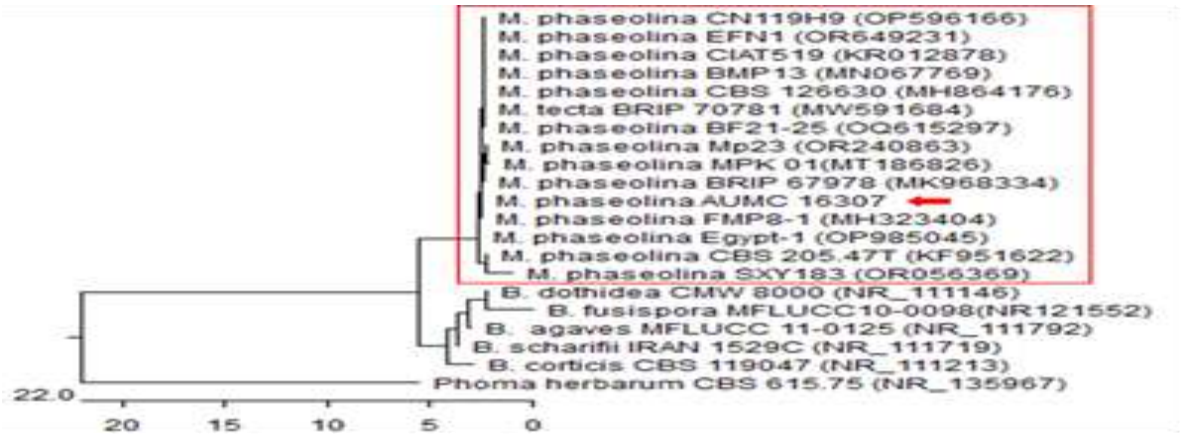

Fig. (1) Inference phylogenetic tree of the *M. phaseolina* strain AUMC 16307 with accession number PP178224, isolated from strawberry. Some species of the genus *Botryosphaeria* are included in the tree because this genus and *M.* are members of the family *Botryosphaeriaceae. Phoma herbarum* is included in the tree as an outgroup strain. B. = *Botryosphaeria*, M. = *Macrophomina.*

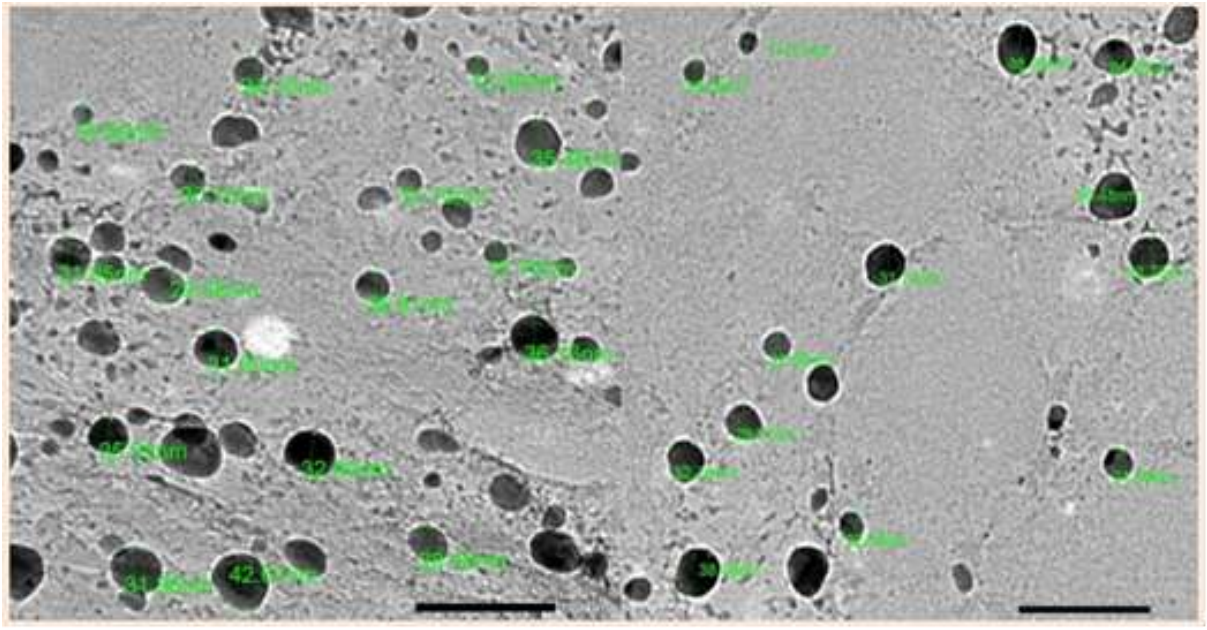

Fig. (2) Transmission electron microscope (TEM) image of Zinc oxide nanoparticles (ZnO NPs).

Table 2. Effect of some chemical inducers on the linear of mycelial growth inhibition of *Macrophomina phaseolina*, the causal organism of root and crown rot diseases in strawberry.

Treatment	Concentration	linear of mycelial growth (mm)	% Efficacy
Chitosan	1g /L	90.0a*	0.0e
	2g /L	90.0a	0.0e
	3g/L	90.0a	0.0e
Oxalic acid	2mM	40.0abc	55.5bc
	4mM	10.0e	88.9ab
	8mM	0.0f	100.0f
Camphor Oil	1%	80.8b	10.2a
	1.5%	74.0ab	17.8a
	2%	36.3c	59.6bc
Zinc oxide nanoparticles (ZnO NPs.)	0.25mM	37.33c	58.5bc
	0.5mM	12.66e	85.9ab
	1mM	10.66e	88.1ab
Rizolex-T50% WP	1g/L	11.8e	86. 8ab
	2g/L	9.0e	90.0d
	3 g/L	0.0f	100.0f
Control		90.0a	0.0e
LSD at 0.05	2.35		3.62

***Values marked with different letters (a, b, c) indicate significant differences at the 0.05 significance level.**

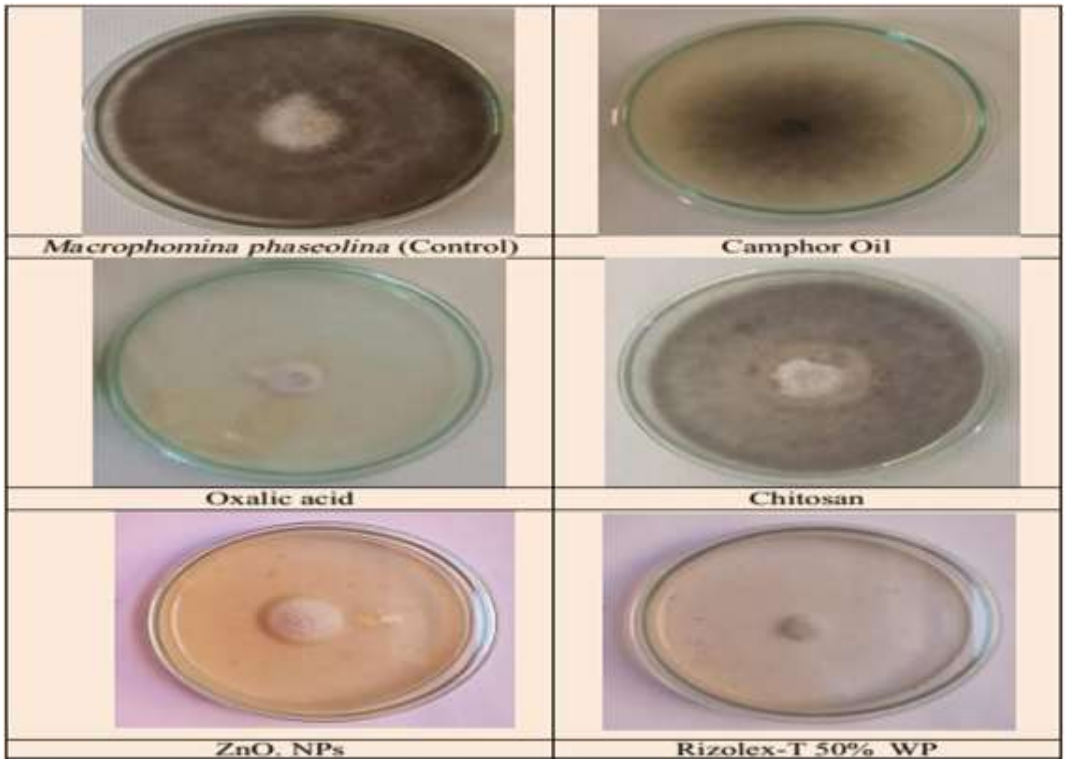

Fig.(3) Effect of some chemical inducers on the linear growth inhibition of *M.phaseolina.*

Table 3. Effects of various treatments on disease incidence and severity of root and crown rot in strawberry plants under pot experimental conditions.

Treatment	Disease incidence(21 day) %	Disease incidence(45 day)%	Disease severity for root rot diseases	Disease severity for crown rot diseases
Oxalic acid (8mM)	55.53ab*	63.33ab	47.00b	60ab
Chitosan (3g/L)	46.33ab	64.43ab	43.33b	53.33ab
Zinc oxide nanoparticles (ZnO. NPs) (1mM)	46.63ab	68.33ab	42.00b	58.66ab
Camphor Oil (2%)	59.43ab	61.66bab	41.33b	46.66b
Rizolex-T50%(3g/L)	8.33b	11.66c	6.00c	6.33c
Control (untreated)	90a	96.66a	93.33a	90.00a
LSD 0.05	38.07	33.11	21.14	28.19

*Values marked with different letters (a, b, c) indicate significant differences at the 0.05 significance level.

Table 4. Effects of different treatments on plant growth parameters of strawberry plants under pot experimental conditions.

Treatment	Fresh weight of shoot (g)	Fresh weight of Root (g)	Dry weight of Shoot (g)	Dry weight of Root(g)	Plant height (cm)	Root length (cm)
Oxalic acid (8mM)	12.33bc*	5.00b	2.33bc	1.33b	9.33b	4.00b
Chitosan(3g/L)	11.66bc	5.16b	3.00bc	2.00b	10.66b	4.33b
Zinc oxide nanoparticles (ZnO. NPs)(1mM)	13.33bc	4.00b	2.66bc	1.66b	9.66b	3.00b
Camphor Oil(2%)	15.00b	5.50b	3.66b	2.00b	9.33b	4.00b
Rizolex-T50%(3g/L)	33.66a	10.36a	9.63a	5.66a	25.13a	15.66a
Control (untreated)	8.33c	3.66b	1.66c	1.00c	3.33c	1.36b
LSD 0.05	4.60	1.63	1.40	1.25	3.03	2.64

*Values marked with different letters (a, b, c) indicate significant differences at the 0.05 significance level.

Table 5. Effect of various treatments on total phenol content (mg/1g fresh weight) in strawberry plants under pot experimental conditions.

Treatment	Total Phenol	Efficacy% % of the total Phenol
Oxalic acid (8mM)	22.93	116
Chitosan(3g/L)	24.17	128
Zinc oxide nanoparticles (ZnO. NPs)(1mM)	23.98	126
Camphor Oil(2%)	23.46	121
Rizolex-T50%(3g/L)	28.21	165
Control (untreated)	10.62	0.0

Table 6. Effects of different treatments on defense-related enzyme activities in strawberry plants under pot experimental conditions.

Treatment	Peroxidase enzyme (PO)	Polyphenoloxidase enzyme (PPO)	Chitinase enzyme
Oxalic acid (8mM)	18.83	16.19	17.89
Chitosan(3g/L)	17.31	12.59	15.66
Zinc oxide nanoparticles (ZnO. NPs)(1mM)	17.76	14.75	14.66
Camphor Oil(2%)	12.48	13.85	16.89
Rizolex-T50%(3g/L)	20.05	18.94	18.58
Control (untreated)	5.69	3.79	4.04

4. Discussion

Strawberry (*Fragaria* spp) is much prized for its flavor and delicacy, as well as being consumed in a fresh state. It is also important in the manufacture of conservers. Yields and quality of fruits are usually reduced when fungi infect strawberry plants. Fungal diseases of strawberry are important worldwide and occur in all parts of the plant, including fruit, leaves, crowns, and roots. The isolation and identification of fungi from infected strawberry plants in Egypt follow standard mycological practices. The use of morphological characteristics and molecular identification techniques, particularly ITS region sequencing, aligns with the methods used in similar studies globally. Previous research supports the accuracy of these methods in identifying fungal species involved in root rot diseases. For example, (16) and (22) have used similar approaches to identify *Fusarium* spp., *M. phaseolina*, and *R. solani* in various crops, including strawberries. Using similar molecular techniques in Jordanian strawberries confirms that the methods used in the current study are robust and widely accepted in the scientific community. Similarly, research by (29) showed that *M. phaseolina* is commonly associated with root rot in strawberries, particularly in regions with warm climates. The molecular identification of *M. phaseolina*, *R. solani*, and *F. fujikuroi* isolated from strawberry plants is validated using the Internal Transcribed Spacer (ITS) region sequence. This method is widely recognized for its accuracy in identifying fungal species at the molecular level, as the ITS region is highly conserved and commonly used as a DNA barcode for fungi. The use of ITS sequencing is a standard method in mycology for the identification and phylogenetic analysis of fungi. Studies such as (34) have established ITS as the universal DNA barcode for fungi due to its reliability in distinguishing between closely related species. The high similarity (99.45% - 100%) observed between the isolated strains and reference sequences from the GenBank database supports the validity of the identification process. This high degree of identity confirms that the sequences obtained in this study accurately represent *M. phaseolina*.

This study provides scientifically validated insights into the efficacy of various chemical inducers against *M. phaseolina*, a soil-borne fungal pathogen. The findings demonstrate that oxalic acid and Rizolex-T are the most effective treatments, while chitosan, at the tested concentrations, exhibited no significant efficacy. The control group, with 90.0 mm mycelial growth and 0.0% efficacy, served as a baseline, confirming the aggressive nature of the pathogen in the absence of intervention. This experimental design ensures that observed effects are attributable to the treatments rather than extraneous variables. Oxalic acid achieved 100% efficacy at 8 mM, completely inhibiting mycelial growth (0.0 mm). As a dicarboxylic acid, oxalic acid disrupts fungal cell membranes, interferes with enzymatic activity, and chelates essential metal ions, leading to cell death. This dose-dependent response aligns with previous studies, such as (40), which highlighted its ability to inhibit fungal pathogens by compromising cellular integrity and metabolic processes. In contrast, chitosan, a polysaccharide derived from chitin, showed 0.0% efficacy across all tested concentrations (1 g/L, 2 g/L, and 3 g/L), with mycelial growth identical to the control. Despite its

known antifungal properties, which typically involve membrane disruption and induction of plant defense responses, the lack of efficacy in this study suggests that factors such as molecular weight, degree of deacetylation, and pathogen specificity may influence its performance. For instance, (14) reported chitosan's effectiveness in inducing oxidative stress and membrane damage in other fungal species, underscoring the need for optimized formulations. Camphor oil demonstrated moderate efficacy, with the highest concentration (3%) achieving 59.6% inhibition. As a terpenoid, camphor oil disrupts fungal cell membranes and mitochondrial function, leading to energy depletion and cell death. Its variable efficacy aligns with studies on essential oils, which exhibit concentration-dependent antifungal activity (25). Zinc oxide nanoparticles (ZnO. NPs) showed promising results, with a 3% concentration achieving 88.1% efficacy. ZnO. NPs generate reactive oxygen species (ROS) that damage fungal cell membranes and DNA, inhibit enzyme activity, and disrupt cellular homeostasis. This dose-dependent response is consistent with findings by (20), who demonstrated ZnO. NPs' ability to induce oxidative stress and membrane damage in fungal pathogens. Rizolex-T 50%, containing tolclofos-methyl, achieved 100% efficacy at 3 g/L, highlighting its potent antifungal activity. Tolclofos-methyl inhibits lipid biosynthesis, disrupting fungal cell membrane integrity. Its efficacy is supported by its widespread use against soilborne pathogens, as demonstrated by (13) in controlling *M. phaseolina* in various crops. Rizolex-T 50% emerged as the most effective treatment for controlling root and crown rot in strawberry plants, significantly reducing disease incidence and severity. Its mode of action, targeting lipid metabolism and cell membrane biosynthesis, aligns with its superior performance (21). However, the reliance on chemical fungicides raises concerns about environmental impact and pathogen resistance, necessitating the exploration of sustainable alternatives. Oxalic acid exhibited moderate efficacy, consistent with its role as a resistance inducer rather than a direct antifungal agent. It activates systemic acquired resistance (SAR) by inducing defense-related enzymes and chelating metal ions essential for fungal growth (8). Chitosan, despite its moderate efficacy, demonstrated potential as a biopolymer capable of inducing plant defense responses and forming physical barriers against pathogens (14). However, its performance was inferior to Rizolex-T 50%, likely due to its reliance on host plant responses rather than direct pathogen inhibition. ZnO. NPs showed moderate efficacy, consistent with their antimicrobial properties and ability to enhance plant immunity by upregulating defense-related genes (30). Camphor oil, with the lowest disease severity among natural inducers, demonstrated potential as a sustainable alternative, though further optimization is required for field applications (25). The untreated control exhibited the lowest growth parameters, underscoring the importance of treatments in mitigating stress and enhancing plant growth. Rizolex-T 50% significantly improved plant growth by suppressing pathogenic fungi, reducing stress, and enhancing nutrient uptake (3). Oxalic acid and chitosan also promoted growth, albeit to a lesser extent, by modulating nutrient availability and inducing systemic resistance (31; 23). ZnO. NPs and camphor oil showed moderate growth-promoting effects, consistent with their antimicrobial properties and ability to reduce pathogen load. The study revealed significant variations in total phenol content and defense-related enzyme activities among treated plants. Rizolex-T 50% yielded the highest total phenol content (38.21 mg/1g fresh weight), attributed to its systemic action and induction of stress responses (11). Oxalic acid and chitosan also increased phenol content, consistent with their roles in activating phenylpropanoid pathways and inducing systemic resistance (7; 14). ZnO. NPs and camphor oil exhibited moderate increases, aligning with their mechanisms as nano-elicitors and terpenoids, respectively (30; 25). Defense enzyme activities, including peroxidase (PO), polyphenoloxidase (PPO), and chitinase, were significantly enhanced by Rizolex-T50%, oxalic acid, and chitosan, consistent with their roles in lignin biosynthesis, oxidative defense, and fungal resistance (18). ZnO. NPs and camphor oil also induced moderate increases, though their effects were less pronounced.

References

[1] Abd-El-Khair, H., El-Gamal, N. G., & Abdel-Kader, M. M. (2019). Antifungal activity of camphor oil against *Fusarium oxysporum* and its role in controlling tomato wilt disease. Journal of Plant Pathology, 101(3), 597-605. https://doi.org/10.1007/s42161-019-00255-2

[2] Abdel-Monaim, M. F., Abdel-Gaid, M. A., & El-Morsy, M. E. M. A. (2012). Efficacy of rhizobacteria and humic acid for controlling Fusarium wilt disease and improvement of plant growth, quantitative and qualitative parameters in tomato. International Journal of Phytopathology, 1(1), 39-48.

[3] Agrios, G. N. (2005). Plant Pathology (5th ed.). Elsevier Academic Press.

[4] Arora, D. K., & Upadhyay, R. K. (1978). Effect of fungal staling growth substances on colony interaction. Plant and soil, 49, 685-690.

[5] Barnett, H. L., & Hunter, B. B. (1972). Illustrated genera of imperfect fungi.

[6] Booth, C. (1971). Methods in microbiology (Vol. 4). Academic Press.

[7] Camejo, D., Guzmán-Cedeño, Á., & Moreno, A. (2016). Reactive oxygen species, essential molecules, during plant-pathogen interactions. Plant Physiology and Biochemistry, 103, 10-23. https://doi.org/10.1016/j.plaphy.2016.02.035.

[8] Cessna, S. G., Sears, V. E., Dickman, M. B., & Low, P. S. (2000). Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses host

oxidative burst. The Plant Cell, 12(11), 2191–2200. https://doi.org/10.1105/tpc.12.11.2191.

[9] Dhingra, O. D., & Sinclair, J. B. (1985). Basic plant pathology methods (pp. 341-pp).

[10] Dimkpa, C. O., McLean, J. E., Britt, D. W., & Anderson, A. J. (2013). Zinc oxide nanoparticles alter wheat physiological and biochemical responses to *Fusarium graminearum* infection. Journal of Nanoparticle Research, 15(8), 1-14. https://doi.org/10.1007/s11051-013-1798-8

[11] Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. The Plant Cell, 7(7), 1085-1097. https://doi.org/10.1105/tpc.7.7.1085.

[12] Domsch, K. H., Gams, W., & Anderson, T. H. (1980). Compendium of soil fungi. Volume 1.

[13] Dutta, P., Das, B. C., & Sen, S. (2014). Efficacy of tolclofos-methyl against *Macrophomina phaseolina* in groundnut. Journal of Plant Pathology, 96(2), 345-350. https://doi.org/10.4454/JPP.V96I2.032.

[14] El Hadrami, A., Adam, L. R., El Hadrami, I., & Daayf, F. (2010). Chitosan in plant protection. Marine Drugs, 8(4), 968-987. https://doi.org/10.3390/md8040968.

[15] El-Abeid, S. E., Mosa, M. A., El-Tabakh, M. A., Saleh, A. M., El-Khateeb, M. A., & Haridy, M. S. (2024). Antifungal activity of copper oxide nanoparticles derived from *Zizyphus spina* leaf extract against Fusarium root rot disease in tomato plants. Journal of Nanobiotechnology, 22(1), 28.

[16] Fang, X., Kuo, J., You, M. P., Finnegan, P. M., & Barbetti, M. J. (2012). Comparative root colonisation of strawberry cultivars Camarosa and Festival by Fusarium oxysporum f. sp. fragariae. *Plant and soil*, *358*, 75-89.

[17] Gillman, T. (1957). Venous Obstruction in the Pathogenesis of Hepatic Bilharziasis a Preliminary Report of Comparative Findings in Rats, Monkeys and Man. Annals of Tropical Medicine & Parasitology, 51(4), 409-416.

[18] Hammerschmidt, R. (1999). Phytoalexins: What have we learned after 60 years? Annual Review of Phytopathology, 37(1), 285-306.

[19] Hammerschmidt, R.; Nuckles, E.M. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. Physiological Plant Pathology, 20, 73-82.

[20] He, Y. F., Li, B. Z., Li, Z., Liu, P., Wang, Y., Tang, Q., ... & Xu, G. L. (2011). Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science, 333(6047), 1303-1307.

[21] Kato, T., Tanaka, S., Ueda, M., & Kawase, Y. (1984). Mechanism of action of tolclofos-methyl as a fungicide. Journal of Pesticide Science, 9(3), 489–495. https://doi.org/10.1584/jpestics.9.489.

[22] Mahasneh, A. M., Al-Mazra'awi, M. S., & Al-Momani, F. (2015). Identification and pathogenicity of *M. phaseolina* and *Fusarium* spp. isolated from strawberries in Jordan. *Jordan Journal of Agricultural Sciences*, 11(3), 635-648.

[23] Malerba, M., & Cerana, R. (2016). Chitosan effects on plant systems. International Journal of Molecular Sciences, 17(7), 1-15. https://doi.org/10.3390/ijms17070996.

[24] Muntana, N., and Prasong, S. (2010). Study on total phenolic contents and their antioxidant activities of Thai white, red, and black rice bran extracts. Pakistan Journal of Biological Sciences: PJBS, 13(4), 170-174.

[25] Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2017). Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6(12), 1451-1474. https://doi.org/10.3390/ph6121451.

[26] Nene, Y. L. and Thapliyal, P. N. (1993). Fungicides in plant disease control. Oxford and IBH Publication Company. New Delhi. 507 p.

[27] Pitt, J. I.and Hocking, A. D. (2009). Fungi and food spoilage (Vol. 519, p. 388). New York: Springer.

[28] Porcino, D. D., Tomasello, G., Mauriello, F., & Malara, A. (2023). Environmental and geotechnical properties of lightweight aggregates made of reused solid wastes. Environmental Geotechnics, 11(9), 735-752.

[29] Porras-Alfaro, A., Bayman, P., Wilkinson, H. H., & Arnold, A. E. (2011). Fungal endophytes in roots and leaves of native and cultivated tropical grasses in Puerto Rico. *Microbial Ecology*, 62(2), 448-457. doi:10.1007/s00248-011-9842-6

[30] Raliya, R., Tarafdar, J. C., & Biswas, P. (2015). Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using ZnO nanoparticles synthesized by soil fungi. Journal of Agricultural and Food Chemistry, 64(16), 3111–3118. https://doi.org/10.1021/acs.jafc.5b05224.

[31] Rangel, W. M., Schneider, J., Costa, E. T. S., & Moreira, F. M. S. (2016). Phytostimulation and phytoprotection by plant growth-promoting rhizobacteria in the presence of oxalic acid. Plant and Soil, 406(1-2), 99–116. https://doi.org/10.1007/s11104-016-2877-2.

[32] Reissig, J. L.; Strominger, J. L. and Leloir, L. F. (1955). A modified colorimetric method for the estimation of N-acetylamino sugars.

[33] Sánchez-Domínguez, M. (2011). Exogamia matrimonial de los inmigrantes latinoamericanos con españoles: integración o estrategia migratoria. Revista Latinoamericana de Población, 5(8), 33-62.

[34] Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & White, M. M. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the national academy of Sciences*, *109*(16), 6241-6246. doi:10.1073/pnas.1117018109.

[35] Shoala, T., Al-Karmalawy, A. A., Germoush, M. O., ALshamrani, S. M., Abdein, M. A., & Awad, N. S. (2021). Nanobiotechnological approaches to enhance potato resistance against potato leafroll virus (PLRV) using glycyrrhizic acid ammonium salt and salicylic acid nanoparticles. Horticulturae, 7(10), 402.

[36] Steel, R.G.D., Torrie, J.H. (1980). Principles and procedures of statistics. A biometrical approach. 2nd edition. McGraw-Hill, New York.

[37] Terzi, V., Morcia, C., Faccioli, P., Vale, G., Tacconi, G., & Malnati, M. (2007). *In vitro* antifungal activity of the tea tree (Melaleuca alternifolia) essential oil and its major components against plant pathogens. Letters in applied microbiology, 44(6), 613-618.

[38] White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, 18(1), 315-322.

[39] Zhang, Y., Li, X., Wang, Z., & Zhang, H. (2019). Oxalic acid enhances resistance to *Phytophthora capsici* in pepper by modulating the antioxidant system and phenylpropanoid pathway. Frontiers in Plant Science, 10, 1-12. https://doi.org/10.3389/fpls.2019.00911.

[40] Zhang, Y., Li, X., Zhang, F., Yang, J., & Li, H. (2013). Oxalic acid enhances resistance to *Phytophthora capsici* in pepper (*Capsicum annuum L.)* by modulating the phenylpropanoid pathway. Plant Physiology and Biochemistry, 73, 252-260.