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This study investigated the application of biocontrol agents to enhance the physiological and genetic traits of maize in the context of mycoflora management. The mycoflora, which is symbiotically linked with plants, presents a substantial worldwide menace to maize cultivation. The use of chemical fungicides for controlling mycoflora has created environmental concerns and potential risks to human health, prompting the need for alternative techniques. In this study, we used plant extracts dissolved in water as a method of biological control against maize seeds infected with *Fusarium verticillioides*. This study incorporated four plant extracts, namely, neem (*Azadirachta indica*), rosemary (*Rosmarinus officinalis*), *Nerium oleander*, and garlic (*Allium sativum*) bulbs. Each extract was utilized at two distinct concentrations: 10% and 20%. The results indicated that garlic extract was highly effective against *F. verticillioides*, as demonstrated by the substantial effects of garlic extract at two distinct concentrations. Compared with the other extracts, the rosemary extract also exhibited a degree of efficacy. Following the therapy, the infected maize exhibited a significant enhancement in multiple dimensions. There was a considerable increase in the dry and fresh weights of the roots and shoots, as well as an increase in plant height. The utilization of garlic extracts had a notable favorable impact, as it augmented the plants' disease resistance and increased their ability to absorb nutrients. Consequently, there was an increase in crop productivity. The results emphasize the significance of incorporating biocontrol agents into agricultural methods to support sustainable maize cultivation and conserve soil health and biodiversity.

Keywords: Maize, Biocontrol agents, *Fusarium verticillioides,* Neem, Garlic, Rosemary, Oleander

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INTRODUCTION

Maize (*Zea mays* L.) is an annual grass that belongs to the Poaceae family. Maize is a significant contributor to global grain production and ranks third after rice and wheat (Golob et al., 2004). The maize crop in Egypt is the second largest among the country's grain crops, according to Haggag (2013 & 2018). In 2019/20, the United States emerged as the leading global producer of corn, with an impressive volume of 345 million metric tons. This accounted for approximately one-third of the total global maize production. During that specific year, the United States emerged as the top producer of maize, followed by China and Brazil (Shahbandeh, 2021). Maize is a highly popular grain crop that is cultivated on a global scale. Numerous studies have highlighted the diverse uses and significance of maize as both a feed crop and a food crop. These studies have explored various aspects of maize versatility and importance (Ali et al., 2020 a, b, c&d; Adnan and Bilal, 2020; Adnan, 2020; Wasaya et al., 2019; Asif et al., 2020).

Inadequate crop management, a wide variety of diseases, and other biotic and abiotic variables all work together to reduce maize yields. Pathogens pose a threat to maize yield and quality (Oerke, 2006). Several diseases can infect maize crops, drastically reducing their yield and productivity (Costa et al., 2012). Fungal infection in maize can lower the maize yield by as much as 80% (Brito et al., 2011). There are a few fungal species that can attach themselves to corn seeds while they are in storage, making the seeds deteriorate or even infect young plants when they germinate. As many as 28 fungal infections, 12 of which are transmitted through seeds, impact crops during the seedling stage (Debnath et al., 2012). Researchers have found that some fungal taxa, including *Aspergillus*, *Fusarium*, *Curvularia*, *Bipolaris*, and *Penicillium*, can be found in maize seeds (Hussain et al., 2013). As a result of the systemic reduction in nutrient quality and quantity caused by these fungal infections, maize suffers from seed rot, seedling blight, germination failure, low seedling vigor, and poor crop performance (Hussain et al., 2013; Enyiukwu and Ononuju, 2016).

In agriculture, *Fusarium verticillioides* is still a major problem, especially regarding maize. This harmful fungus is ubiquitous in nature because it can withstand harsh environments, including high temperatures (Czembor et al., 2019, Soliman et al.,2022), and because it releases spores into the air.

As a member of the microbial community in the maize rhizosphere, this soil-borne pathogen can contaminate the seeds and roots of growing maize plants. The most encountered species of *Fusarium* on maize was *Fusarium verticillioides*, according to a study by Adejumo et al. (2007a, b). This species has a significant impact on corn yields, as it leads to plant diseases in infected ears and kernels, sometimes resulting in the production of harmful mycotoxins such as fumonisins. Research has demonstrated that fumonisins can cause equine leukoencephalomalacia (ELEM) in horses, pulmonary edema in pigs, and liver toxicity and cancer in rats. Studies have shown a correlation between the presence of *F. verticillioides* and the occurrence of esophageal cancer in both China and the Transkei region of southern Africa. The impact on plants and the environment, as well as the derivative it produces known as fumonisin, has been explored. Instead of relying on chemical pesticides, this research suggests a greater emphasis on biological control agents to address *F. verticillioides* during maize cultivation. By implementing this control approach in the field, one can achieve optimum yield and high-quality maize with minimal or no pesticides. In addition, it will reduce the presence of environmental spores in the air, resulting in a lower likelihood of contamination (Colmenarez et al., 2018, Soliman et al., 2022).

For a long time, synthetic fungicides have been used for controlling marigold fungal infections (Kumar, 2012). However, there are numerous severe side effects that have resulted from their widespread use and residual effects in soil, plants, and water. These include environmental disorders, risks to human health, harm to aquatic ecosystems, a decrease in beneficial soil microbes, the development of fungicide-resistant fungi, and ozone depletion (Seleiman et al., 2020, Abd-Ellatif et al., 2022).

Researchers have identified three ways in which plant extracts and essential oils can combat harmful fungi: by causing fungal death, by inhibiting fungal growth and development, and by stimulating the defense responses of infected plants to promote fungal growth (Draz et al., 2019). According to Lagrouh et al. (2017), plant extracts and essential oils possess various mechanisms that can effectively hinder the growth of harmful fungi. These mechanisms include disrupting electron transport in mitochondria, impeding cell division, interfering with nucleic acid and protein synthesis, and ultimately obstructing efflux pumps. The host plant activates various defense mechanisms, including the production of soluble sugars, phenols, flavonoids, hormones, and antioxidants, both enzymatic and nonenzymatic (Tarkowski et al., 2019; Al-Harbi et al., 2021). Plants depend on phenols and flavonoids to alter cellular processes and safeguard various cell components from damage (Walter et al., 2009). Another defense mechanism against pathogen invasion is the synthesis of enzyme-based antioxidants and the elimination of reactive oxygen species (ROS) (De Pinto and De Gara, 2004). Preventing the damage caused by the accumulation of ROS in cells can be achieved by activating antioxidants, including both enzymatic and nonenzymatic antioxidants (Barna et al., 2012; Soliman et al., 2022).

Thus, this study aimed to assess the effectiveness of various plant-based products in controlling *Fusarium verticillioides*, a fungal disease that affects maize seeds. An investigation will be conducted to assess the effectiveness of certain plants in reducing fungal infections in maize seeds, focusing on their potential fungicidal properties. This study aimed to assess the efficacy of four different plant extracts in managing seedborne fungal disease (*Fusarium verticillioides*) in maize seeds. The plant extracts evaluated were neem, rosemary, *Nerium oleander*, and garlic bulbs.

MATERIALS AND METHODS

Sample collection, identification, and preparation

Single-spore cultures of *F. verticillioides* were obtained from the Department of Plant Protection and Biomolecular Diagnosis, Arid Lands Cultivation Research Institute, Egypt, and monosporic cultures of *F. verticillioides* were maintained on potato dextrose agar with triple-cross 321 maize seeds.

Preparation of Plant Extracts

Fresh neem (*Azadirachta indica*) leaves, rosemary (*Rosmarinus officinalis*), *Nerium oleander*, and garlic (*Allium sativum*) bulbs were used. After that, we made aqueous extracts of all the plants. Following this, two different concentrations of each plant extract were made in water: 10% and 20%. The fresh leaves of the rosemary, neem, and oleander were rinsed with running water, dried in the shade for two days, and then processed into a powder using a blender. Next, one hundred millilitres of sterile distilled water was mixed with twenty grams of plant powder using an electric top-loading Mettler balance E1000. The mixture was then allowed to sit for twenty-four hours. To obtain the filtrate that served the 10% and 20% concentrations of each plant extract, each suspension was filtered through Whatman's filter paper No. 1. To

make garlic extract, fresh garlic bulbs were pulsed in a blender for 5 minutes with an appropriate amount of distilled water (1 milliliter of water for every 1 gram of garlic bulb weight) until the garlic juice was extracted. The obtained aqueous garlic extract was then filtered through one layer of sheath clothing, and the obtained aqueous extracts of all the plants were used immediately or stored at 4°C until use (Ismaiel, 2008).

Preparation of fungicide solution

The chemical fungicide Goldmil 72% WP (0.2% in water) was used as a positive control. The fungicide solution was prepared according to the instructions provided on the back of the fungicide bag (200 g/100 L water).

In Vitro Antifungal Activity of Plant Extracts

The antifungal effects of various plant extracts against *F. verticillioides* were examined using the agar diffusion method to determine their effectiveness. The fungal species that cause Maize Fusarium ear and stalk rot diseases were cultured and stored on modified potato dextrose agar (PDA) slants at 24°C. These slants are refreshed before they are used. The strains were regularly subcultured to maintain their viability throughout the study. To determine the antifungal properties of different plant extracts (*Allium sativum*, *Rosmarinus officinalis*, *Azadirachta indica*, and *Nerium oleander*), a mixture of 0.25 ml (10^6 cfu/ml) of *F. verticillioides* fungi was added to separate plates containing PDA media. This process was performed after the media had cooled and solidified. The chemical fungicides Goldmil 72% WP and Nystatin Stds were used as positive controls (50 ppm) and then poured into Petri dish wells. Three replicates (dishes) were utilized for each treatment and control. All plates were placed in an incubator set at a temperature of 28 ± 2°C for 7 days. After this time, the resulting inhibition zones were carefully measured. The plates were incubated for an additional 7 days to observe the long-term effects of the plant extract.

The MICs of the plant extracts were determined by conducting serial 2-fold dilutions of the test samples at various concentrations (10-150 µg/mL) in autoclaved distilled water. Ten microliters (10^6 cfu/ml) of *F. verticillioides* fungus were added to each tube at various concentrations. The tubes were then incubated at 28 ± 2°C for 7 days. The MIC is determined by the lowest concentration of the sample that prevents the visible growth of the microorganism during incubation, resulting in no turbidity.

Pot experiment

***Zea mays* L.:** *Zea mays* L. seeds were obtained from the Agriculture Research Center, Field Crop Research Institute, Giza, Egypt. Healthy seeds of the same stock were used in the experiment.

Soil inoculation: Soil-filled pots with a diameter of 15 cm were prepared using disinfested soil. The soil was added at a rate of 2.5 kg per pot, with a clay-to-sand ratio of 2:1 (v/v). The fungal inoculum (*F. verticillioides*) used in the experiment was prepared by cultivating the fungus in bottles filled with sterilized maize grain medium. The bottles were then incubated at a temperature of 25±2°C for 14 days. Soil infestation was accomplished by blending the fungal inoculum into the top 5 cm layer of the soil at a rate of 5% w/w for the pathogenic fungi under investigation. The soil was carefully prepared and regularly watered over a period of 7 days, with the temperature maintained at a consistent level. This was done to create optimal conditions for the fungi to thrive and spread throughout the soil. Control pots were prepared without the fungus. Each pot was filled with five robust maize seeds. Five pots were used per treatment to ensure accurate replication. The pots were carefully maintained under optimal conditions during the summer season at the Plant Pathology Department, Faculty of Agriculture, Mansoura University, Egypt. All plants were consistently watered with regular tap water to ensure that they received the optimal amount of hydration. The day temperature was maintained at approximately 22±3°C, while the night temperature was maintained at approximately 18±2°C. Additionally, the plants were exposed to a 16-hour photoperiod to mimic natural lighting conditions.

Planting and growth conditions: The seeds of Zea mays were surface sterilized using a 0.01% hypochlorite solution for a duration of 2 minutes. The seeds were then thoroughly rinsed with sterilized water to eliminate any remaining disinfectant residue. Seeds that had been sterilized were divided into 10 sets. The seeds of the first set were soaked in distilled water as a negative control, while those of Set 2 contained Goldmil 72% WP (0.2% in water) fungicide-soaked maize seeds grown in F. verticillioides inoculated soil (Fungicide+ Fusarium pathogen). The third set was soaked in a 10% neem extract (Pathogen (P)+10% neem), the fourth set in a 20% neem extract (P+20% neem), the fifth set in a 10% Nerium oleander extract (P+10% nerium), the sixth

set in a 20% Nerium oleander extract (P+20% nerium), the seventh set in a 10% garlic extract (P+10% garlic), the eighth set in a 20% garlic.

Disease severity: The disease severity (DS) of the fungi was assessed 15 days after infection using the scale described by (Filion et al., 2003).

$$\text{Disease severity (\%)} = \frac{\Sigma ab}{AK} \text{ x } 100$$

where (a) is the number of diseased plants with the same infection degree; (b) is the infection degree; (A) is the total number of assessed plants; and (K) is the greatest infection degree.

Morphological parameters: Sampling was conducted within a specific timeframe following treatment with various extracts. Measurements were taken on the morphological traits of both treated and untreated maize plants. Three plants from each experiment were harvested and brought to the laboratory (30 days old). The plants were gently uprooted, and their height and leaf number were measured. The plants were subsequently assessed for shoot and root fresh weight, as well as shoot and root dry weight, after being dried in an oven at 40°C for 48 hours.

Biochemical and phytochemical assessment: The malondialdehyde (MDA) content in fresh Zea leaves from plants treated with extracts and control plants was measured using the method described by Heath and Packer (1968). Briefly, a fresh Zea leaf weighing 0.5 g was mixed with 10 mL of ethanol and then spun at 12,000 × g for 15 minutes. Next, 1 mL of the supernatant was combined with a 2 mL mixture of thiobarbituric acid (0.65%) and trichloroacetic acid (20%). The mixture was heated for 30 minutes, cooled quickly, and then spun at a speed of 12,000 × g for 10 minutes. The MDA content in the supernatant was measured using a UV–VIS spectrometer (Jenway, Japan) at absorbance values of 532 and 600 nm. The total phenolic content (TPC) for all treatments was measured by dissolving 5 mg of air-dried leaf powder in 10 mL of methanol using the Folin–Ciocalteu reagent protocol (Slinkard and Singleton, 1977). The TFC for all treatments was measured using the aluminum chloride colorimetry method, as described by Chavan et al. (2013). The soluble protein content was determined for all treatments using the Bradford method (Bradford, 1976).

$$\text{Chla (µg mL−1)} = 16.82\ A665.2 - 9.28\ A652.4 \quad (3)$$

$$\text{Chlb (µg mL−1)} = 36.92\ A652.4 - 16.54\ A665.2 \quad (4)$$

Total anthocyanin analysis was conducted using the method described by Yang et al. (2015) with a few minor adjustments. The sample extracts were prepared in triplicate. In a short amount of time, a small amount of sample was subjected to sonication for an hour at 60°C. This was performed in a solution consisting of 2 mL of acidic MeOH with 1% HCl (v/v). The crude extract underwent centrifugation and filtration using the same method employed for the preprocessing of samples for total chlorophyll. Next, a portion of the filtered supernatant was mixed with MeOH containing 1% HCl to create a diluted solution with a volume of 1.5 mL. The absorbance of the solution was measured at 530 and 600 nm, and the resulting data were used to calculate the overall concentrations of anthocyanins (Yang, et al., 2015).

$$\text{y (µg g−1)} = (A530 - A600)\frac{V\ x\ n\ x\ Mw}{\varepsilon x\ m}$$

The absorbance at 530 nm is represented by A530, while the absorbance at 600 nm is denoted as A600. The total volume of the extracted solution is represented by V, and the dilution ratio is denoted as n. The molecular weight of cyanidin-3-glucoside is 449.4, Ɛ is the anthocyanin molar extinction coefficient (29.60 M−1.cm−1), and m is the sample mass.

Assays of Antioxidant Enzymes: Antioxidant enzymes were extracted by homogenizing 1 g of fresh maize leaf tissue in chilled 50 mM phosphate buffer (pH 7.0) supplemented with 1% polyvinyl pyrolidine and 1 mM EDTA. This was done using a prechilled mortar and pestle. After centrifuging at a high speed and low temperature, the resulting liquid was utilized for the enzyme assay. The superoxide dismutase (SOD) activity and NBT photochemical reductions were measured at 560 nm in a 1.5 mL assay mixture. The mixture consisted of sodium phosphate buffer (50 mM, pH 7.5), 100 µL of EDTA, L-methionine, 75 µM NBT, riboflavin, and 100 µL of enzyme extract. After 15 minutes of incubation, the light was turned off, and the activity was measured as EU per milligram of protein. The catalase activity assay (CAT, EC1.11.1.6) was conducted following the procedure outlined in (Luck, 1974). The absorbance at 240 nm was measured for 2 minutes, and an extinction coefficient of 39.4 mM−1 cm−1 was used for the subsequent calculations. The ascorbate peroxidase activity assay (APX, EC 1.11.1.11) was conducted by observing the change in absorption at 290 nm for 3 minutes. This was performed in a 1 mL reaction mixture consisting of potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, hydrogen peroxide, and enzyme

extract. A value of 2.8 mM−1 cm−1 was employed to calculate the extinction coefficient.

Scanning electron microscopy

SEM observations were conducted on seven-day-old fungal cultures of Fusarium verticillioides that had been treated with aqueous plant extracts at two different concentrations (10% and 20%), as well as the chemical fungicide Goldmil 72% WP. Fungal growth was examined using SEM to determine its response to different concentrations. The results were then compared to those of the untreated control. Segments measuring approximately 5× 10 mm were taken from cultures that were growing on plates. The segments were placed in Eppendorf tubes filled with 1.5 mL of modified Karnovisk's solution. After 24 hours, the segments were fully immersed in the solution. Next, the samples underwent a thorough washing process using aldehyde, with three 10-minute cycles in 0.05 M cacodylate buffer. They were then immersed in a 1% osmium tetroxide solution in 0.05 M cacodylate buffer with a pH of 7.2. This immersion took place at room temperature in a laminar flow chamber for a duration of 4 hours. Following this time frame, the samples were thoroughly rinsed in distilled water and subsequently dehydrated using a gradient of acetone concentrations (25%, 50%, 75%, 90%, and 100%). Each concentration was maintained for 10 minutes, and this process was repeated three times in 100% acetone solution. The specimens were dried using a critical point drying method (Autosamdri-815, USA). The dried specimens were carefully prepared for analysis, ensuring their stability and quality. They were mounted on SEM stubs using electrical silver paint and coated with gold-palladium membranes in a controlled environment. This process involved the precise application of the coating unit, using an atmosphere of argon for a specific duration and amount. The specimens were carefully examined and photographed using a state-of-the-art Jeol JSM-6510 L.V. SEM. The microscope was operated at accelerating voltages ranging between 20 and 30 kV at the electron microscopy unit of Mansoura University, Egypt.

SDS–PAGE Protein

The sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) procedure was conducted following the methods described by Laemmli (1970) and later modified by Studier (1973). Dry maize seeds were subjected to various plant extract treatments for 24 hours. A mixture of marker proteins was used to determine the molecular weight. A photograph of the banding of the profile in the gel was taken. Scoring was performed to determine the number of bands in each sample. The method for discontinuous SDS–PAGE was derived from the work of Laemmli (1970) and later modified by Studier (1973). The electrophoretic profile of the proteins in the seeds was quantified for each treatment, indicating the presence or absence of a band with a specific molecular weight. The protein profile was assessed using a Bio-Rad Gel Documentation System (Bio-Rad-Gel-Doc Model 2000). The experiment took place in the Botany Department of the Faculty of Science at Mansoura University.

Molecular analysis (inter simple sequence repeat (ISSR) analysis)

A gram of fresh leaves from *Zea mays* was used for DNA extraction, as described by Junghans and Metzlatt (1990) .For this investigation, nine primers from the ISSR marker were used. The sequences of the primers used are shown in Table 1. According to the studies conducted by Zietkiewicz et al. (1994) and Safhi et al. (2022), the DNA amplification process involved a PCR mixture of 20 µL. This mixture consisted of 10 µL of master (2X TOPsimpleTM DyeMIX-nTaq), 5 µL of each primer (0.1 µM), and 1 µL of genomic DNA (50 ng/µL). A final volume of 20 µL was achieved by adding sterile distilled water. The PCR conditions for the SimpliAmp™ Thermal Cycler involved an initial predenaturing step at 95°C for 5 minutes, followed by 45 cycles at 94°C for 30 seconds. The annealing step lasted for 40 seconds, and the extension step occurred at 72°C for 1 minute. Finally, the mixture was incubated for 5 minutes at 72°C. The products were successfully separated on a 1.5% agarose gel. Each treatment was evaluated and given a score of either 1 for presence or 0 for absence.

Table 1. List of primer names and nucleotide sequences used in the study for the ISSR procedure

Primer	Sequemces 5'-3'
Primer ISSR 1	TCCCGCCTAC
Primer ISSR 2	CCGTTGCCT
Primer ISSR 3	CTCTCCGCCA
Primer ISSR 4	CACCTTTCCC
Primer ISSR 5	TGCGAGAGTC
Primer ISSR 6	AGACGTCCAC
Primer ISSR 7	CCGCCCAAAC
Primer ISSR 8	GGGAAGGACA
Primer ISSR 9	TGCGCCCTTC

Estimation of genomic template stability (GTS)

Changes in the ISSR profiles revealed genotoxicity, as normal bands disappeared, and new bands appeared. Observing only clear and reproducible bands is crucial for assessing DNA order and disorder, as is demonstrating the percentage of genomic template stability (GTS%). The values for GTS were calculated for each sample using the formula provided by Sukumaran and Grant (2013) as follows:

$$GTS\ \% = (1- \frac{a}{n}) \times 100, \text{ where}$$

a: average number of polymorphic bands in the treated samples.
N: the number of total bands in the control.

The GTS% values were calculated for each sample as the loss/gain of bands, if the GTS% of the control was 100%.

Statistical analysis

The collected data were subjected to statistical analysis and are presented as the mean ± standard deviation (SD) according to SPSS version 16. One-way analysis of variance was used to assess the results and determine any significant differences at the alpha level of $p \leq 0.05$. A heatmap was created using TB tools to analyze and compare treatment responses across different genes (Chen et al. 2020).

RESULTS

In vitro antifungal activity

The antifungal activities of two different concentrations (10 and 20%) of the plant extracts were evaluated against the *F. verticillioides* pathogen in vitro. Figure 1 and Table 2 provide a visual representation of the average decrease in *F. verticillioides* radial growth resulting from the treatment. The inhibitory activity at all tested concentrations varied compared to that of the positive control fungicide nystatin (0.05%) and the untreated experimental control. The growth of mycelia was increasingly inhibited over time. After 7 days of incubation at 28 ±°C, the results revealed that the *Allium sativum* aqueous extract exhibited a lower MIC (1.5 µg/mL) than the standard fungicide nystatin. The ranking of inhibitory activity was as follows: *Allium sativum* (garlic) demonstrated the highest activity, followed by *Rosmarinus officinalis* (Rosemary), *Azadirachta indica* (Neem), and finally *Nerium oleander*.

***In Vivo* Trial**

The healthy and infected maize after treatment with the studied plant extracts are illustrated in Figure 2.

Disease assessments: After 18 days, the initial indications of *Fusarium* root rot disease became apparent (Figure 3a). Notably, the disease severity reached a peak of 94.01% when disease control measures were implemented. The data presented in Table 3 indicates that the extract from *Allium sativum*, at concentrations of both 10% and 20%, resulted in a significant decrease in disease severity compared to the other plant extracts. In addition, *Allium sativum* clearly had the greatest impact on reducing disease severity, with a rate of 27.12%. On the other hand, *Nerium* oleander had the lowest effect on disease severity, with a rate of 29.1%.

Morphological parameters: After 30 days of treatment with the studied plant extracts, there was a noticeable improvement in various morphological parameters. Compared with those of infected maize plants, the plant height, shoot and root fresh and dry weights, and leaf number significantly increased (Table 3 and Figure 3). An impressive increase in plant height was observed with the use of a 20% garlic plant extract, reaching a maximum height of 30.88± 0.02 cm. This height was compared to that of the control plant, which was 30.16± 0.02 cm (Figure 3b). Based on the leaf number, the application of 20% garlic resulted in a slight increase in this parameter (11.78) compared to that of the control plants (11.66) (Figure 3c). The application of 20% garlic resulted in a significant increase in shoot fresh weight (15.98 g), followed by 10% garlic (14.67 g), compared to that of the control plants (14.57 g). The use of garlic at a concentration of 20% resulted in a significant increase in shoot dry weight (14.28 g), closely followed by a 10% garlic concentration (13.97 g), compared to the control (13.34 g). The use of 20% garlic significantly increased the fresh weight of the roots (3.98 g) compared to that of the control (3.77 g) (Figure 3d).

Physiological and phytochemical characteristics: The highest value of total chlorophyll was recorded at 51.67 µg/g F W when 20% garlic was used (Figure 4 a). The maximum anthocyanin content reached 1.23 Ug-1 FW when garlic was used at a concentration of 20% (Figure 4 b). Garlic (20%) and garlic (10%) had the highest malondialdehyde (MDA) contents, which were 101.87 and 92.42 nmol/g F W, respectively (Figure 4c). The garlic samples had the highest total phenolic content (TPC), with values of 44.87 and 42.63 µmol g^{-1} FW at 20% and 10%, respectively

(Figure 4 d). The total flavonoid contents (TFC) for the control treatment and other treatments involving *F. verticillioides* were recorded, with the highest values of 122.76 and 117.07 µmol g^{-1} FW, respectively, observed when using 20% garlic and 10% garlic (Figure 4e). The protein content in maize plants increased when fungicide and plant extracts were applied. The highest recorded value was 33.98 nmol g^{-1} FW, which was observed when 20% garlic extract was used, as shown in Figure 4f.

Antioxidant and Enzyme Activities: The impact of water-based plant extracts on the activity of antioxidant enzymes (superoxide dismutase, catalase, and ascorbate peroxidase) in maize plants infected with *F. verticillioides* was evaluated, as depicted in Figure 5. According to the data, garlic treatment at 20% and 10% resulted in the highest values of this antioxidant enzyme activity, which were 68.8 and 67.13 U mg^{-1} protein, respectively. The treatments with garlic at 20% and 10% showed the highest recorded activity of CAT, with values of 89.76 and 88.28 U mg^{-1} protein, respectively. The treatments with 20% and 10% garlic exhibited the highest enzymatic activity of APX, with values of 30.98 and 28.41 U mg^{-1} protein, respectively.

Scanning electron microscopy

SEM of *Fusarium verticillioides* treated with water as a negative control revealed that the mycelia were well developed, inflated, and intact and had a smooth wall, and the conidia appeared normal in morphology (Figure 6 a,b). In the case of the treatment of *Fusarium verticillioides* with fungicide as a positive control, the mycelia were completely collapsed and lysed**.** Treatment of *F. verticillioides* with garlic extract (10%) caused the mycelia to undergo plasmolysis, deformation, distortion, squashing and collapse (Figure 6 c,d). Furthermore, treatment with garlic extract (20%) caused the mycelia to completely collapse, shrink, deform and lyse compared with treatment with 10% garlic (Figure 6 e-h)**.** In addition, treatment with neem leaf extract (10%) revealed that the mycelia were well developed and inflated and had a smooth wall and that the conidia appeared normal (Figure 6 i,j). Additionally, treatment with neem leaf extract (20%) resulted in plasmolysis, shrinkage and deformation of the mycelia (Figure 6 k,l). Treatment with rosemary leaf extract (10%) caused the mycelia to shrink, deform, and lyse (Figure 6 m,n), and treatment with rosemary leaf extract (20%) caused the mycelia to be completely plasmolysed, distorted, squashed and collapsed hyphae (Figure 6 o,p)**.** On the other hand, *Nerium oleander* leaf extract (10%) caused the disruption, squashing, and collapse of mycelia (Figure 6 q,r)**.** Furthermore, *Nerium oleander* leaf extract (20%) showed lysis, distortion, and mycelia (Figure 6 s,t).

SDS–PAGE protein profile

Figure 7 displays the protein profiles of maize seeds that were treated with four different plant extracts, as analyzed using SDS–PAGE. SDS–PAGE analysis revealed a total of 12 bands, each with a molecular weight ranging from 10 to 80 kDa. There was a total of 12 bands, with 9 being monomorphic and 3 being polymorphic. The molecular weight 62 kDa band was absent only in the 10% garlic sample, making it a suitable negative marker for 10% garlic. As indicated in Table 4, the garlic extract revealed 10 monomorphic bands and one polymorphic band. The absence of the 62 kDa molecular band in garlic (10%), as indicated in Table 4, suggested that this band could serve as a negative molecular marker for this concentration of garlic extract. Based on the Neem extract findings, there were 11 monomorphic bands and a single polymorphic band (Table 5). Based on the findings from the rosemary extract, there were a total of 11 monomorphic bands and 1 polymorphic band (Table 5). Based on the findings from the Nerium oleander extract, 11 monomorphic bands and no polymorphic bands were observed (Table 5). Table 5 shows that the Allium sativum extract had the highest percentage of polymorphism at 9.09%, while the Nerium oleander extract had the lowest value at 0%.

Molecular Analysis by using Inter Simple Sequence Repeats (ISSRs)

In this study, nine primers from Table 6 were utilized to investigate the impact of plant extracts on *Zea mays* L. Figure 8 shows that a significant number of well-defined and scorable ISSR markers were obtained from the fingerprinting of *Zea mays* seedlings treated with four plant extracts. The amplicon size ranged from 180 to 1600 bp. Out of the 75 amplified bands, 11 were found in both the control group and all the treatments with plant extracts (Allium sativum, Azadirachta indica, Rosmarinus officinalis, and Nerium oleander), while 64 bands showed polymorphism. There was a range of polymorphic bands observed across different primers, with varying numbers. Primer ISSR 1 had eight bands, primer ISSR 2 had four bands, primer ISSR 3 had six bands, primer ISSR 4 had seven bands, primer ISSR 5 had eight bands, primer ISSR 6 had five bands, primer ISSR 7 had nine bands, primer ISSR 8 had seven bands, and primer ISSR 9 had ten bands.

Table 2. Antifungal activity of plant extracts against plant pathogenic fungus (*Fusarium verticillioides*)

Conc.	*Zone of inhibition* (mm)					
	***Allium sativum* extract**	***Azadirachta indica* extract**	***Rosmarinus officinalis* extract**	***Nerium oleander* extract**	**Nystatin 50 ppm**	**Goldmil 72% WP 50 ppm**
10%	24	16	18	14		
	22	18	19	15		
	27	18	21	14		
20%	28	22	24	22		
	31	19	28	20	2.25-2.8	2.0-2.25
	27	19	22	18		
MIC	1.5 µg/mL	2.2 µg/mL	1.7 µg/mL	2.4 µg/mL	0.6 µg/mL	0.9 µg/mL

Table 3. Effect of aqueous plant extracts on disease severity, plant height, shoot (fresh and dry) weight, root (fresh and dry) weight and leaf number of maize plants infected with *Fusarium verticillioides* (M+P) = maize + pathogen.

Treatment	Disease severity (%)	Plant height (cm)	Shoot Fresh wt (g)	Shoot dry wt (g)	Root fresh wt (g)	Root dry wt (g)	Mean Leaf No.
Control	0	30.16	14.57	13.34	3.77	1.45	11.66
M+P	94.01	19.15	8.18	7.23	0.99	0.24	7.66
Fungicide	29.16	25.26	9.56	8.09	1.89	0.87	9.66
Allium sativum (10%)	28.65	29.12	14.67	13.97	3.18	1.03	10.33
Allium sativum (20%)	27.12	30.88	15.98	14.28	3.98	1.43	11.78
Azadirachta indica (10%)	38.98	20.65	11.34	10.99	2.13	0.77	9.12
Azadirachta indica (20%)	35.34	20.88	11.56	11.06	2.67	0.87	9.88
Rosmarinus officinalis (10%)	34.12	21.13	12.87	11.77	2.76	0.88	9.88
Rosmarinus officinalis (20%)	33.14	22.45	12.98	12.08	2.89	0.98	10.12
Nerium oleander (10%)	29.89	21.76	13.76	12.54	2.98	1.02	10.34
Nerium oleander (20%)	29.1	22.98	14.1	13.87	3.11	1.23	10.76

Table 4. SDS–PAGE of the protein banding pattern of *Zea mays* L. seeds treated with plant extracts (*Allium sativum* (garlic)*, Azadirachta indica* (Neem)*, Rosmarinus officinalis* (rosemary)*, Nerium oleander)* for 24 hr.

Band No.	Molecular weight (kDa)	1	2	3	4	5	6	7	8	9	10
1	80	1	1	1	1	1	1	1	1	1	1
2	70	1	1	1	1	1	1	1	1	1	1
3	65	1	1	1	1	1	1	1	1	1	1
4	62	1	1	0	1	1	1	1	1	1	1
5	50	1	1	1	1	1	1	1	1	1	1
6	45	0	0	0	0	0	1	1	1	1	1
7	33	1	1	1	1	1	1	1	1	1	1
8	31	1	1	1	1	1	1	1	1	1	1
9	27	1	1	1	1	1	1	1	1	1	1
10	25	1	1	1	1	1	1	1	0	0	0
11	15	1	1	1	1	1	1	1	1	1	1
12	10	1	1	1	1	1	1	1	1	1	1
Total bands		11	11	10	11	11	12	12	11	11	11
Polymorphic bands		2	2	1	2	2	3	3	2	2	2
% of polymorphism		18.18	18.18	9.09		8.33		8.33		0	
% of polymorphism for all total treatments		6.52%									

Monomorphic band, 1= control with water, 2= control with fungicide (Goldmil 72% WP (0.2% in water), 3= Garlic 10%, 4= Garlic 20%, 5= Neem 10%, 6= Neem 20%, 7= Rosemary 10%, 8= Rosemary 20%, 9= *Nerium oleander* 10%, and 10= *Nerium oleander* 20%.

Percentages of polymorphisms

The percentage of polymorphic bands of the plant extracts under study can be found in Table 6. In terms of the percentage of polymorphisms observed in all treatments of plant extracts, the highest value of 100% was recorded for primer ISSR 1 with the sequence TCCCGCCTAC, primer ISSR 2 with the sequence CCGTTGCCT, primer ISSR 3 with the sequence CTCTCCGCCA, primer ISSR 5 with the sequence TGCGAGAGTC, primer ISSR 7 with the sequence CCGCCCAAAC, and primer ISSR 8 with the sequence GGGAAGGACA. On the other hand, the lowest value of 50% was observed for primer ISSR 6 with the sequence AGACGTCCAC.

Genomic template stability (GTS%)

Based on the molecular findings presented in Table 7, the percentage of genetic template stability (GTS) was highest in the groups treated with 10% and 20% aqueous garlic extract, with values of 65.85% and 63.41%, respectively, compared to the control group,

Table 5. Polymorphic bands of *Zea mays* L. seeds treated with four plant extracts (*Allium sativum* (garlic)*, Azadirachta indica* (Neem)*, Rosmarinus officinalis* (rosemary)*, and Nerium oleander)*.

Primer code	Amplified fragments					
	Molecular size range ((pb)	Monomorphic bands	Polymorphic Bands		Total bands	Polymorphism (%)
			Unique bands	Nonunique bands		
Primer ISSR 1	420-1600	0	0	8	8	100
Primer ISSR 2	200-450	0	0	4	4	100
Primer ISSR 3	200-1500	0	0	6	6	100
Primer ISSR 4	180-900	3	2	5	10	70
Primer ISSR 5	400-1500	0	1	7	8	100
Primer ISSR 6	360-1600	5	1	4	10	50
Primer ISSR 7	250-1000	0	1	8	9	100
Primer ISSR 8	400-1000	0	1	6	7	100
Primer ISSR 9	240-1600	3	1	9	13	76.92
Total		11	7	57	75	85.33

Table 6. Polymorphic bands and percentage of polymorphisms in *Zea mays* L. seedlings treated with four plant extracts (*Allium sativum, Azadirachta indica, Rosmarinu officinalis and Nerium oleander)*.

Bands / Plant extracts	Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
		Unique bands	Nonunique bands		
Allium sativum	10	0	1	11	9.09
Azadirachta indica	11	0	1	12	8.33
Rosmarinus officinalis	11	0	1	12	8.33
Nerium oleander	11	0	0	11	0

Table 7. Band sharing index (BSI), number of new bands that appeared (a), and number of bands that disappeared (b), as related to the control and genome template stability (GTS) percentage in *Zea mays* L. seedlings treated with four plant extracts using ISSR markers.

Primers	No. of bands in control	Treatments																	
		Fungicide		Garlic 10%		Garlic 20%		Neem 10%		Neem 20%		Rosemary 10%		Rosemary 20%		*Nerium oleander* 10%		*Nerium oleander* 20%	
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
ISSR1	6	2	1	0	0	0	1	1	0	1	0	0	0	2	1	2	1	2	1
ISSR2	1	0	3	2	0	1	0	2	2	1	2	0	2	1	0	0	2	0	2
ISSR3	2	1	2	1	1	2	0	1	1	1	1	0	2	1	2	1	1	1	0
ISSR4	7	0	1	1	0	0	0	1	0	1	1	1	2	0	0	0	1	1	1
ISSR5	1	0	3	3	0	2	0	2	3	1	4	0	2	0	2	0	3	0	1
ISSR6	7	0	2	2	0	1	0	3	1	2	2	0	2	0	1	0	2	0	2
ISSR7	7	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	4	0
ISSR8	4	1	1	0	3	1	1	0	0	0	0	2	0	0	1	1	1	1	0
ISSR9	6	0	5	3	0	3	2	1	2	0	3	0	3	1	2	0	4	1	0
Total	41	4	18	12	4	10	4	11	10	7	13	3	13	5	10	4	15	10	7
a+b		22		16		14		21		20		16		15		19		17	
GTS%		46.34		65.85		63.41		48.78		51.21		60.97		60.97		53.65		58.53	

which had 100% stability. The percentage of GTS decreased in comparison to that in the control group, reaching 46.34% for fungicide (Figure 8). Overall, the percentage of stable genetic template application was greater in all treatments of *Zea mays* with aqueous plant extracts than in those with fungicide extracts. This result was most significant because the plant extracts provided stable genomic DNA, whereas chemical fungicides decreased DNA stability.

Statistical analysis

The hierarchical cluster analysis of the heatmap yielded two distinct types of clusters dendrograms: one representing the control and different treatments with two concentrations of 10% and 20% in a vertical orientation and the other representing various parameters (morphological, physiological and antioxidant enzymes) in a horizontal orientation. There are two primary groups of vertical orientation according to their classification: the first group consists of the control group, while the second group is further divided into two subgroups. The initial subgroup yielded garlic extracts with percentages of 10% and 20%. The second subgroup consisted of other aqueous plant extracts. The red hue signifies the maximum level of resemblance between the isolated microorganisms, while the blue color

represents a lower level of similarity, as shown in Figure 9. The Pearson correlations among the different parameters (physiological and biochemical parameters) showed the relationships between them. The highest positive correlation between morphological parameters was 0.98 between shoot dry weight and shoot fresh weight, and the lowest positive correlation was 0.58 between shoot dry weight and plant height. For the phytophysiological parameters, the most significant positive correlation was 0.99 between total phenol content and MDA, followed by 0.95 between total phenol content and total flavonoids. Finally, for antioxidant enzymes, the highest positive correlation was 0.99 between APX and SOD, followed by 0.97 between CAT and APX and between SOD and CAT (Figure 10).

DISCUSSION

Although synthetic fungicides were initially used to combat plant infections, their use is heavily regulated on a global scale because of concerns about their impact on the environment and human health, as well as consumer desire for safer, more natural alternatives (Hewedy et al., 2020). The EU has pushed for new regulations outlining a number of these fungicides (Hillocks, 2012). As a result, researchers must immediately begin looking for new forms of control. In this study, the antifungal effects of four different plant extracts on Fusarium root rot in maize were studied. Multiple studies have shown that medicinal and aromatic herbs have biofungicide potential in agriculture (Ribeiro et al., 2010; Mohsen et al., 2023). Plants can produce a wide variety of secondary metabolites that defend them from predators, including insects, rodents, and fungi, which is why these compounds have been the target of fungicide research (Ahmad and Beg, 2001). Compared to fungicides, it is also biodegradable, has low toxicity to humans, and is generally safe (MartÍnez, 2012). The ability of the extracts to extract components with antifungal action could be blamed. Biologically active components found in the studied extracts may be responsible for their inhibitory impact (Abdel-Monaim et al., 2011; Atta et al., 2013). Tannins, sterols, saponins, trepenoids, phenolics, alkaloids, flavanoids, glycoside compounds, and quercetin and kaemfericitin are among the phytochemicals found in the plant extracts of the leaves, according to the literature. According to Goss et al. (2017), the antifungal characteristics of leaf and bulb extracts were found to suppress the growth of R. solani and F. solani. The effectiveness of moringa extracts as antifungals was affected by their concentration (Soliman et al., 2021; Abd-Ellatif et al., 2022).

Allium sativum aqueous extract had the lowest MIC (1.5 µg/mL) and the greatest inhibition zone diameter, followed by *Rosmarinus officinale* rosemary. Plants may produce a variety of chemicals with biological characteristics that help protect them from diseases (Hancock et al., 2015). An important source of bioactive compounds—antibacterial, insecticidal, fungicidal, nematicidal, and herbicidal—among these molecules, more than three thousand essential oils have been found (Shaaban et al., 2012). Some examples of these chemicals include phenols, aldehydes, alcohols, ketones, esters, amines, ethers, and acids, as well as terpenes (mono-, sesqui-, and diterpenes) (Zorzi-Tamazoni et al., 2018).

Fungicidal action hinders fungal growth through various mechanisms. These include interfering with cell wall biosynthesis and ion channel formation on the cell membrane (Hu et al., 2010), inhibiting the normal functions of topoisomerase enzymes (Kawakami et al., 2015), increasing cell wall permeability (Nene and Thapliyal, 1993), targeting the plasma membrane (Ahmed et al., 2021), interfering with sterol biosynthesis (Adil et al., 2017), and inhibiting ergosterol biosynthesis (Goodman et al., 1986), resulting in irreversible cell wall damage. The results showed that maize morphology was greatly enhanced at both concentrations after treatment with the plant extracts. The shoot dry and fresh weights, root dry and fresh weights, plant height and number of leaves increased in both the 10% and 20% Allium extracts. In addition, the phytochemical characteristics were enhanced by these botanicals. Previous studies have revealed comparable outcomes (Ahmed et al., 2021).

Indirectly by increasing plant systemic resistance or directly by influencing pathogen physiology, morphology, and ultrastructure, phenols protect plants from harmful fungi (Mohamed et al., 2016). Like phytoalexins, phenols are toxic and can modify pathogen toxicity and activate resistance genes in pathogens (Nicholson and Hammerschmidt, 1992). An additional structural barrier that prevents the infection from spreading into plant tissues is increased by phenol's involvement in lignification of the cell wall. Additionally, lignin may lessen the host cell's ability to supply nutrients to the virus (Zaynab et al., 2018). The antioxidant, antifungal, antiviral, and antibacterial properties of flavonoids make them one of the most abundant types of polyphenols found in

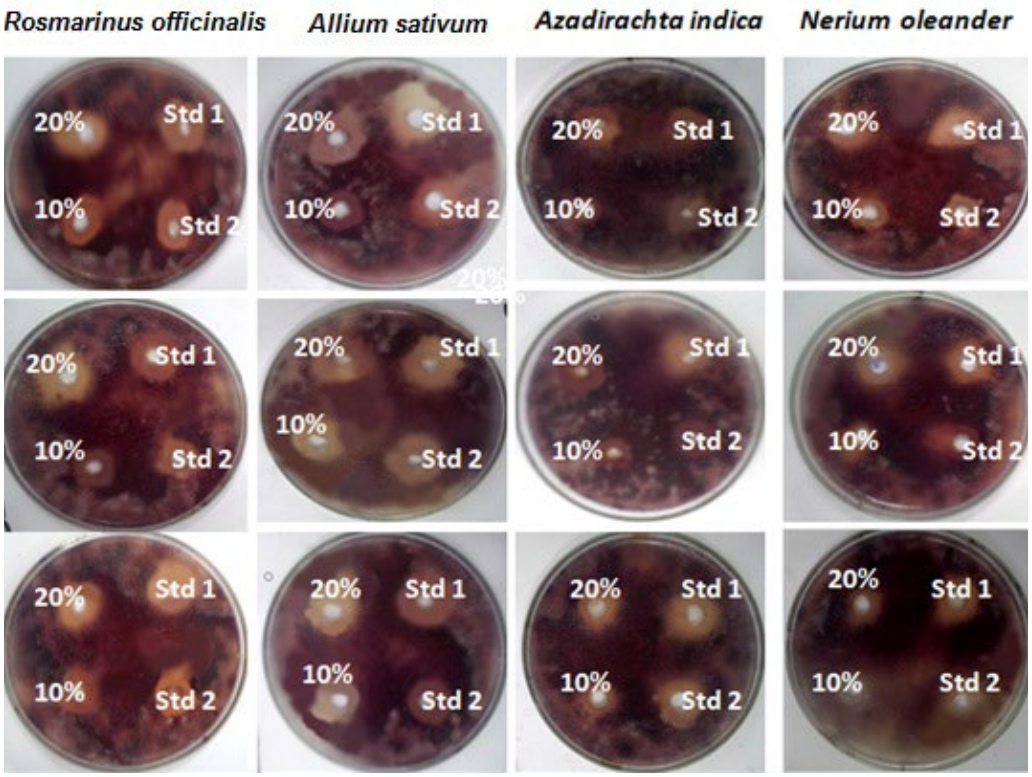

Figure 1. The antifungal activity of the studied plant extracts at different concentrations against *F. verticillioides*. where Std1: Nystatin (0.1µg/mL), Std2: Goldmil 72% WP (2.0µg/mL)..

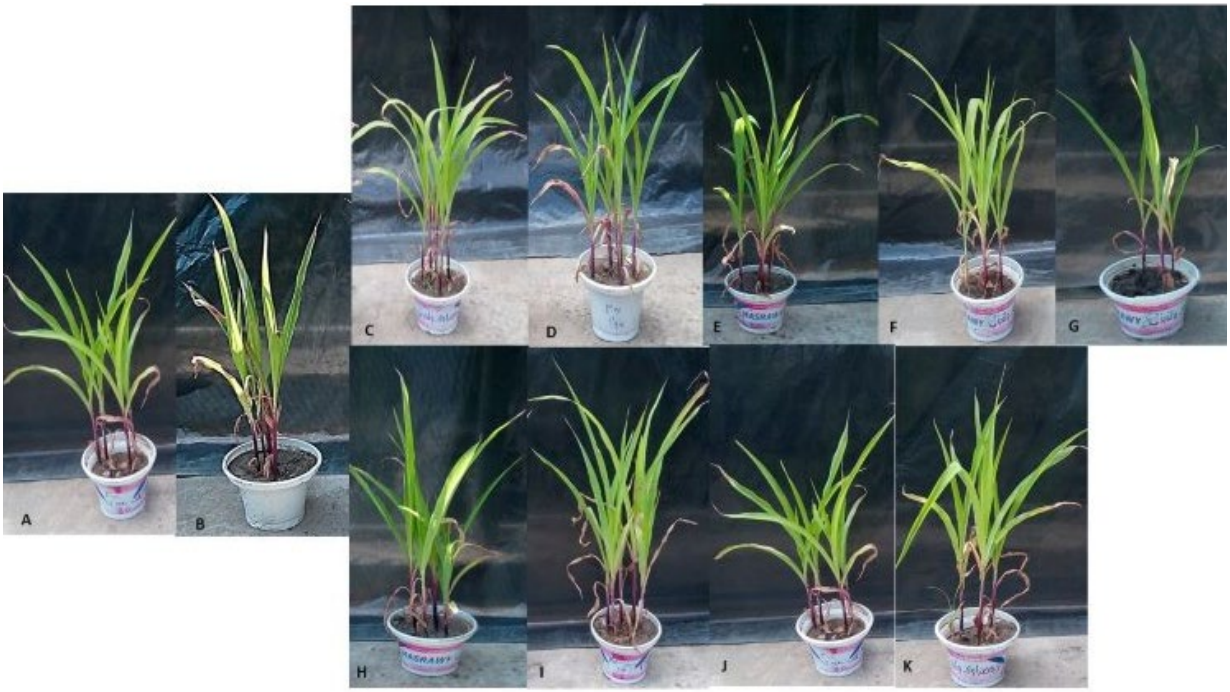

Figure 2. Effects of aqueous plant extracts (10% and 20%) on *Zea mays* L. seedling (30 days old) growth potential under *Fusarium* infection. A = control (healthy maize), B = infected maize, C = fungicide (0.2% in water), D = 10% garlic extract, E = 20% garlic extract, F=10% neem extract, G= 20% neem extract, H=10% rosemary extract, I=20% rosemary extract, J= 10% *nerium oleander* extract, and K=20% *nerium oleander* extract.

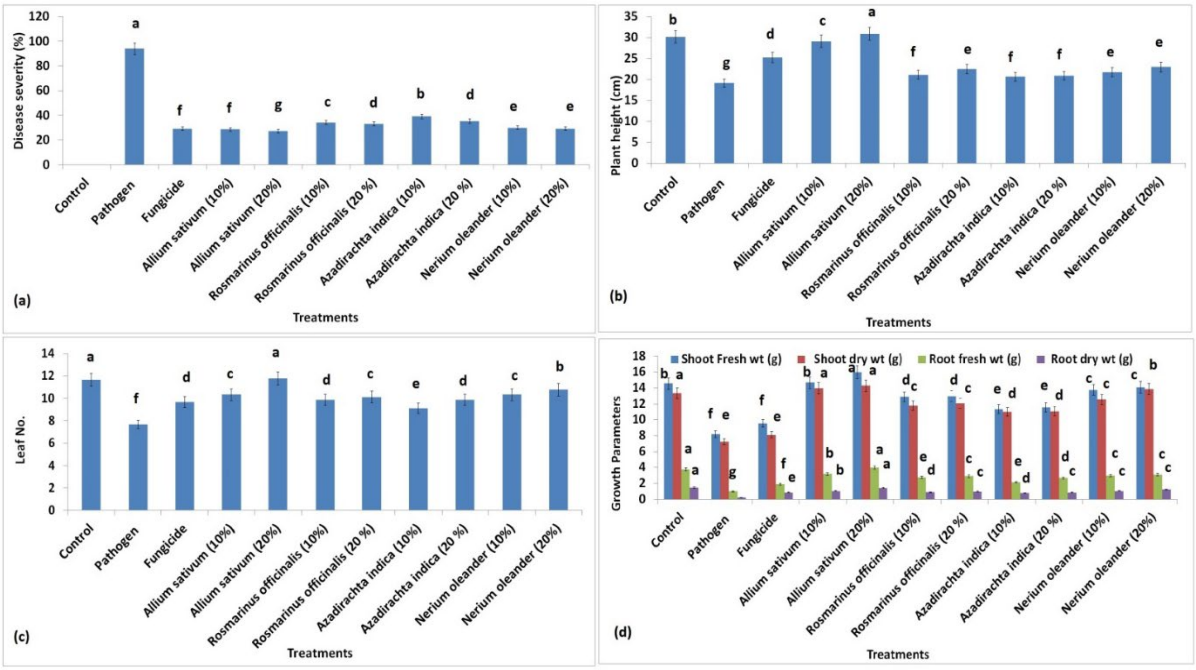

Figure 3. Effects of the studied plant extract on disease severity and morphological parameters of *Zea mays* seedlings. Bars with different letters indicate significant differences between treatments, expressed as the mean of three replicates ± SDs.

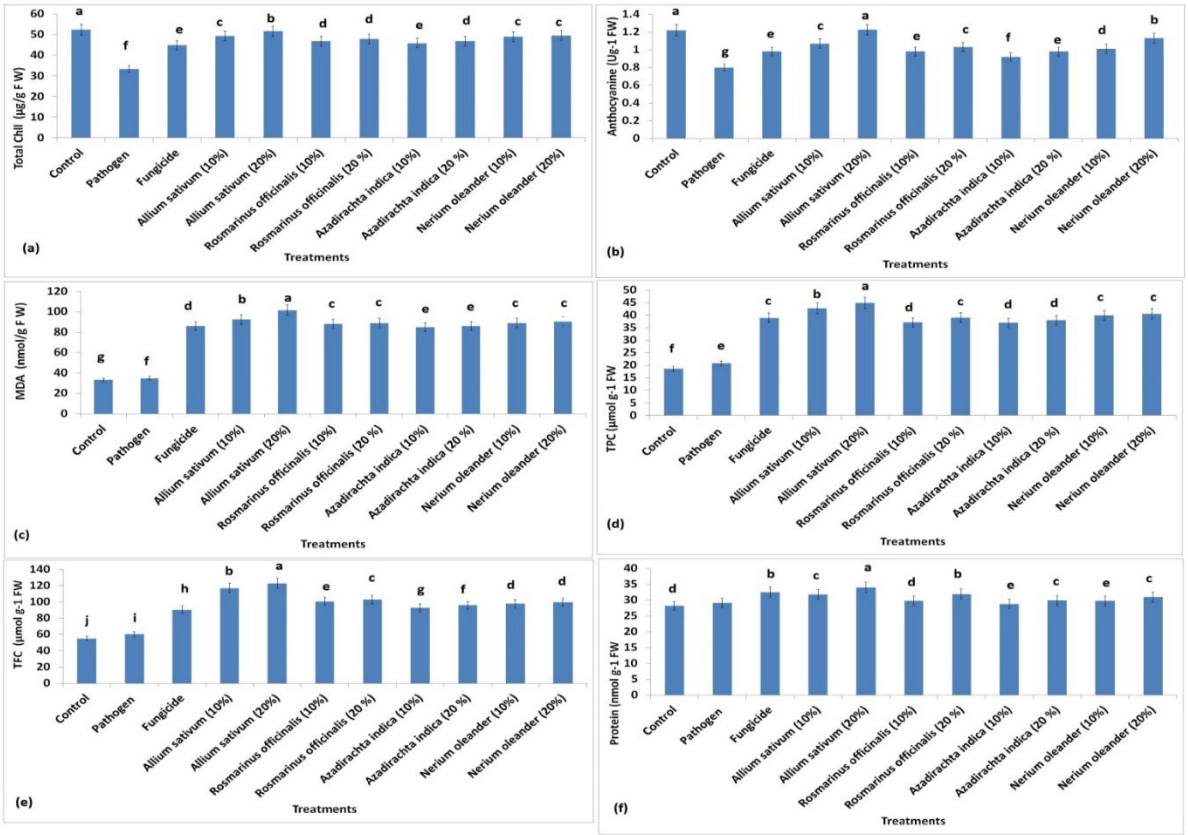

Figure 4. Effects of the studied plant extracts on total chlorophyll, anthocyanin, MDA, TPC, TFC, and PC levels in *Zea mays* plants under conditions of *Fusarium* root rot disease infection. Bars with different letters indicate significant differences between treatments, expressed as the mean of three replicates ± SDs.

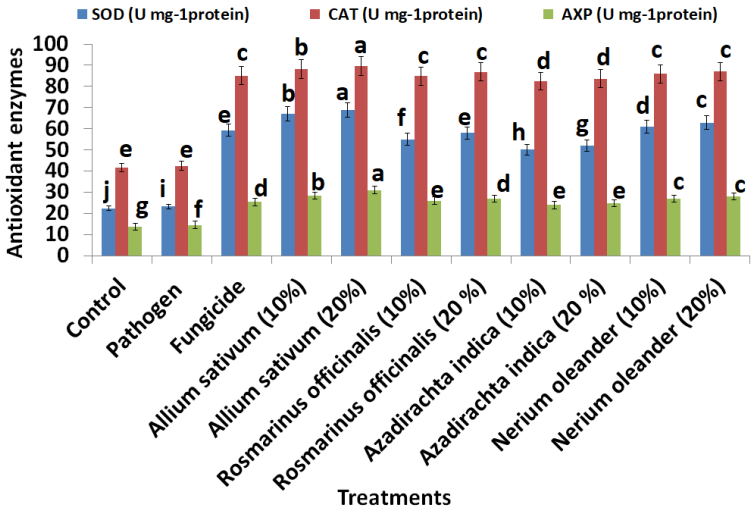

Figure 5. Effects of the studied plant extracts on SOD, CAT, and APX enzyme activity in *Zea mays* seedlings with *Fusarium* root rot disease infection. Bars with different letters indicate significant differences between treatments, expressed as the mean of three replicates ± SDs.

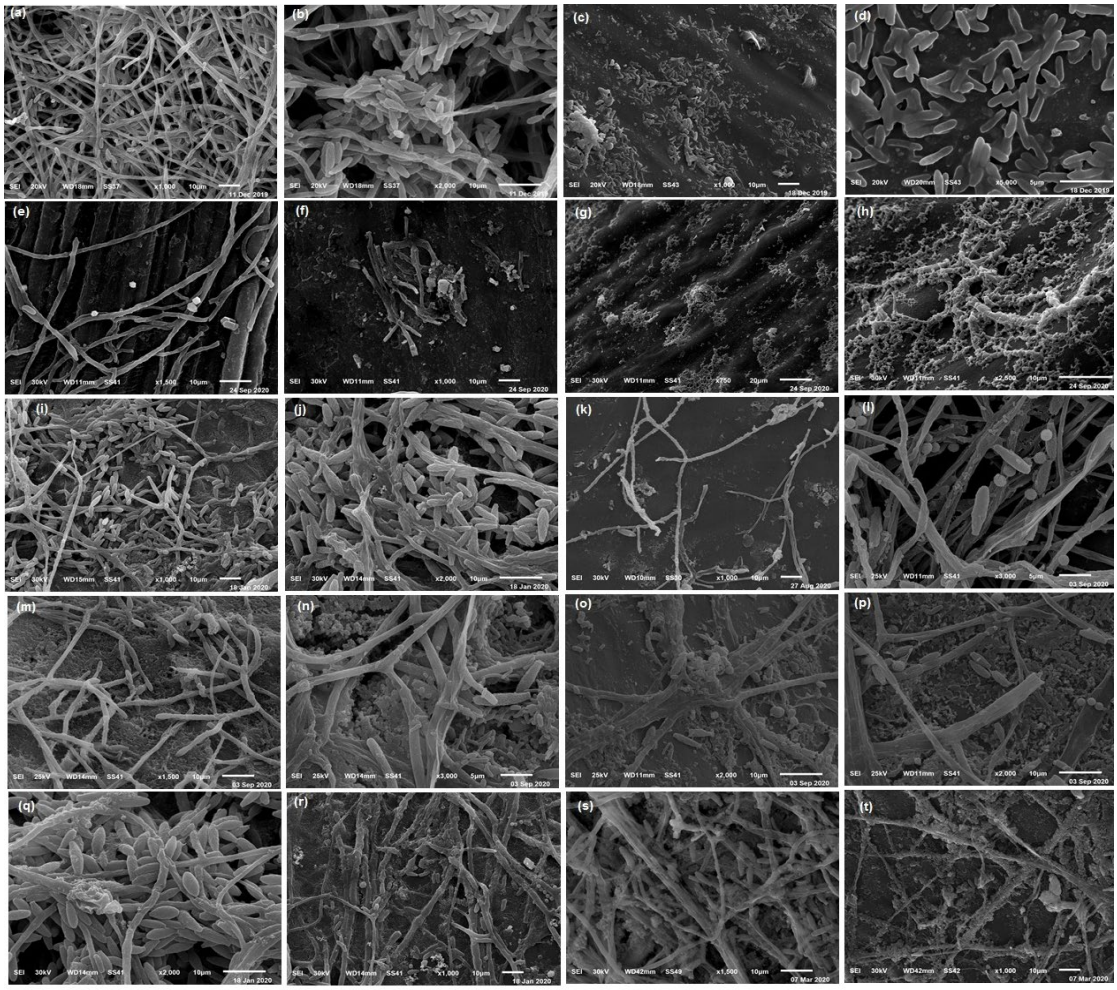

Figure 6. Scanning electron micrographs of *Fusarium verticillioides*-infected *Zea mays* grains treated with aqueous plant extracts. a, b: Treatment with water as a negative control; c, d: treatment with fungicide as a positive control; e, f: treatment with 10% garlic; g, h: treatment with 20% garlic; i, j: treatment with 10% neem; k, l: treatment with 20% neem; m, n: treatment with 10% rosemary; o, p: treatment with 20% rosemary; q, r: treatment with 10% *Nerium oleander;* and s, t: treatment with 20% *Nerium oleander*.

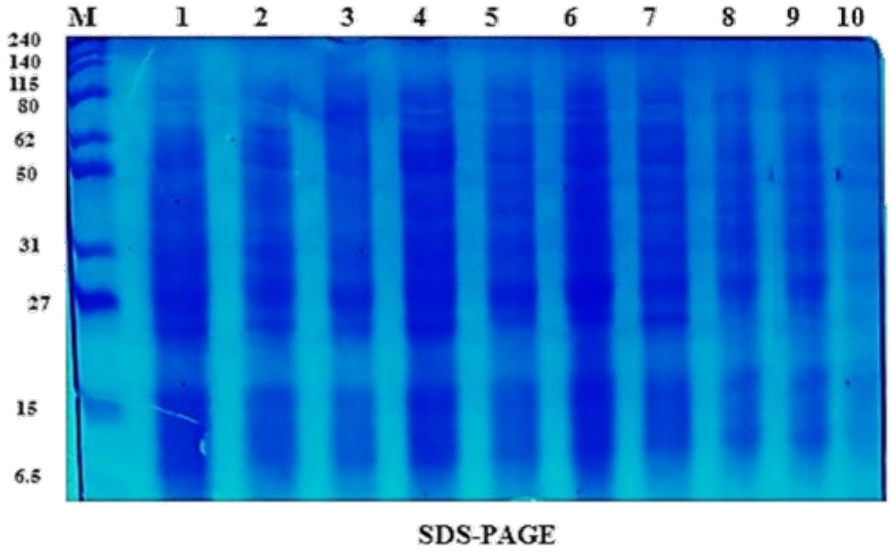

Figure 7. SDS–PAGE of protein banding pattern gels of *Zea mays* L. seeds treated with plant extracts for 24 hr. M= Marker, 1= Control with water, 2= Control with fungicide (Goldmil 72% WP)(0.2 in water), 3= Garlic 10%, 4= Garlic 20%, 5= Neem 10%, 6= Neem 20%, 7= Rosemary 10%, 8= Rosemary 20%, 9= *Nerium oleander* 10%, and 10= *Nerium oleander* 20%.

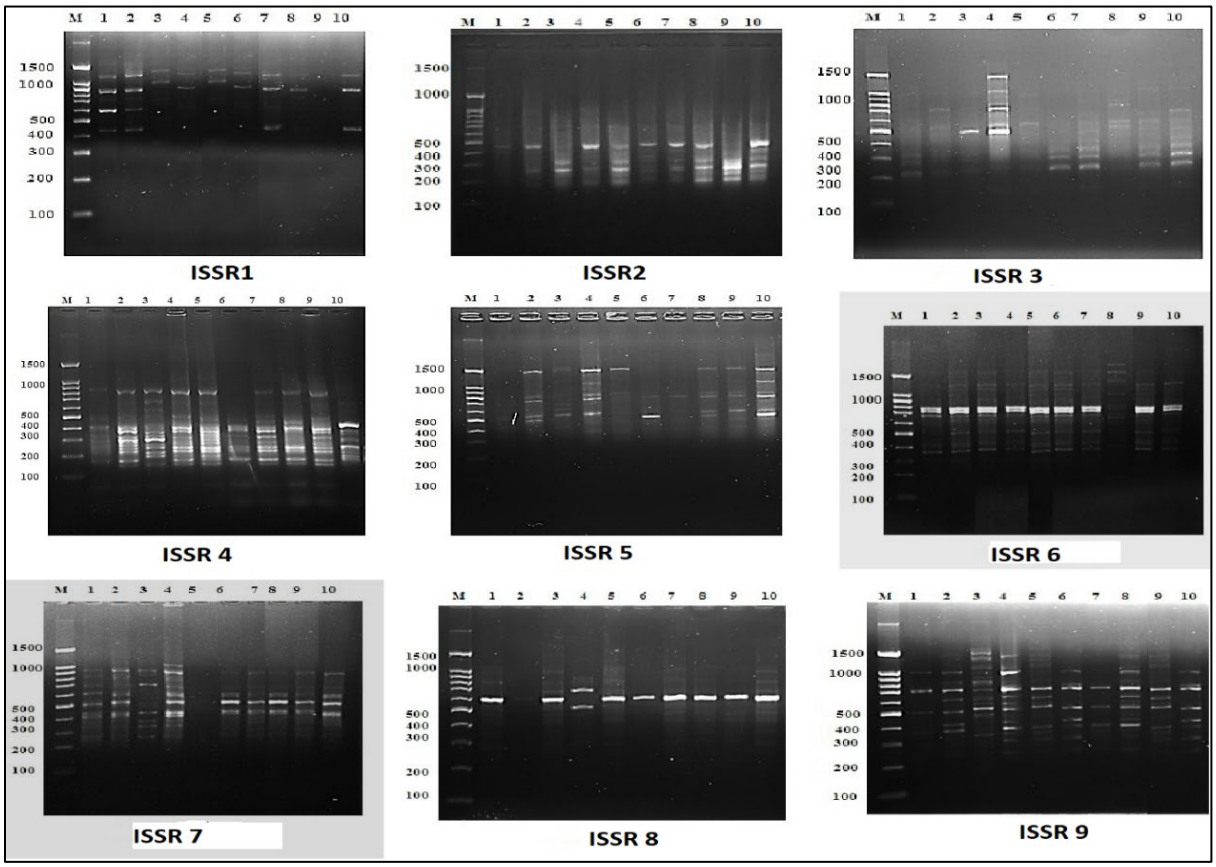

Figure 8. The amplification profiles of *Zea mays* L. seedlings after ten treatments with four plant extracts; M= Marker, 1= control with water, 2= control with fungicide (Goldmil 72% WP (0.2 in water)), 3= Garlic 10%, 4= Garlic 20%, 5= Neem 10%, 6= Neem 20%, 7= Rosemary 10%, 8= Rosemary 20%, 9= *Nerium oleander* 10%, and 10= *Nerium oleander* 20%.

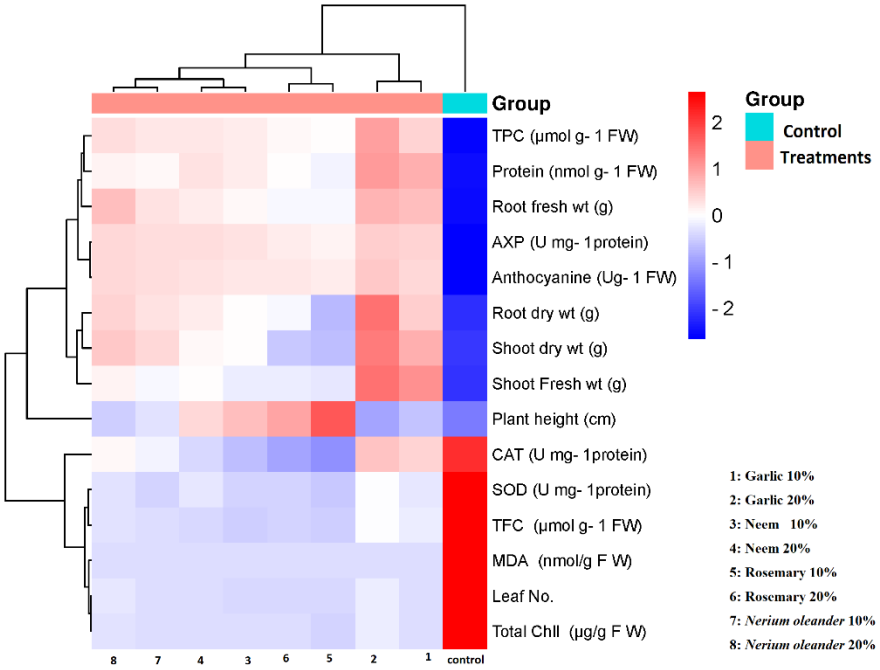

Figure 9. Hierarchical cluster heatmap between the different aqueous plant extracts and the different physiological, morphological and biochemical parameters of *Zea mays* after treatment.

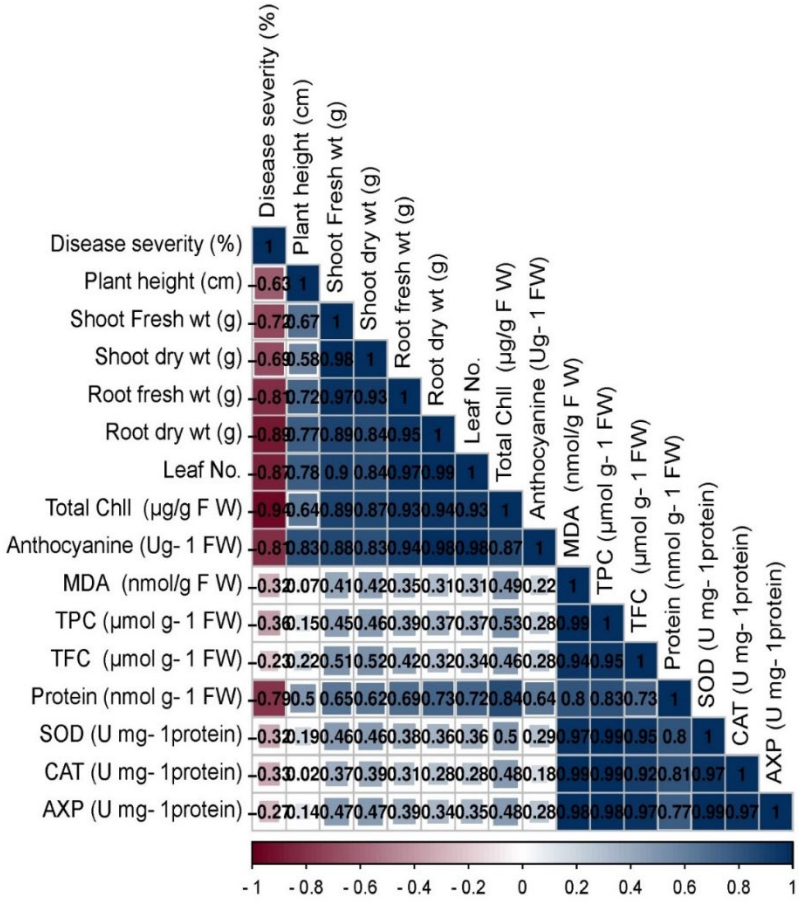

Figure 10. Pearson correlation among the different aqueous plant extracts and the different physiological, morphological and biochemical parameters of *Zea mays* after treatment.

nature (Özçelik et al., 2008). The presence of flavonoids is primarily responsible for inhibiting fungal development, according to Mohamed et al. (2016). This research demonstrated that catalase, ascorbate peroxidase, and superoxide dismutase enzyme activities were significantly elevated. These outcomes are consistent with what was previously reported (Pastuszak et al., 2021). To identify and withstand fungal invasion, plants can develop a variety of defense mechanisms that activate intricate defense responses (Dangl and Jones, 2001).

Multiple studies have utilized SDS–PAGE to assess how environmental stress manifests in protein patterns (Shehab et al., 2004, Soliman et al., 2023; Alqahtani et al., 2024; Abd El-Moneim et al., 2021). Comprehensive investigations attempting to explain the molecular mechanisms underlying the effect of plant extracts on plants should consider protein profiles since Vannini et al. (2014) demonstrated that certain proteins are altered by AgNP exposure. Compared with those of the untreated control, the protein profiles of the treated seeds of the maize plants varied greatly, with 12 bands, 9 monomorphic bands and 3 polymorphic bands. The percentage of polymorphisms for all total treatments was 6.52%. Mutations in the regulatory genes that halt or postpone transcription could explain, in general, any change in protein bands compared to the control sample, including the loss of some bands altogether (Muller and Gottschelk, 1973; Mesfer ALshamrani et al., 2022;).

One of the most widely used markers in molecular procedures utilizing polymerase chain reaction (PCR) is the intersimple sequence repeats (ISSRs) method. It has been studied and referenced by various researchers (Atienzar et al., 2002, Essa et al., 2024; Salem et al., 2024). The discovery of variations in intermicrosatellite loci forms the foundation for ISSRs, which serve as DNA-based markers (Cenkci et al., 2009; Alqahtani et al., 2024). The findings of the study revealed that the percentage of polymorphisms was 50% when utilizing the primer ISSR6, which increased to 70% when using the primer ISSR4.

Overall, the total polymorphism percentage for all treatments was 85.33%. Disruptions at the DNA level, such as DNA damage and instability, can have a significant impact on the stability of the genomic template. These genotoxic effects can be assessed to determine the qualitative changes in genomic template stability (GTS) (Erturk et al., 2013). The genomic template stability assay is a qualitative method that detects changes in ISSR profiles caused by plant extracts compared to the profiles obtained from the control sample. Genomic template stability (GTS) can be influenced by DNA damage, as observed in previous studies (Sukumaran and Grant, 2013; Soliman et al., 2023). This study revealed that the genomic template stability varied among the different treatments. The lowest value, 48.78%, was observed in the neem 10% treatment, while the highest value, 65.85%, was found in the garlic 20% treatment.

To summarize, this work highlights the possibility of using biocontrol agents, particularly plant extracts such as garlic, rosemary, neem, and Nerium oleander, to improve the physiological and genetic characteristics of maize and effectively control mycoflora infestations. The use of these natural extracts as substitutes for commercial fungicides demonstrated both a high level of efficacy in managing Fusarium verticillioides and substantial enhancements in the growth and development of afflicted maize plants. The significant increase in plant height and root and shoot weights, together with improved disease resistance and nutrient absorption, indicate the possible contribution of biocontrol agents to advancing sustainable agricultural practices and enhancing crop yield. Integrating biocontrol agents into agricultural practices can be an effective technique for supporting maize development, reducing environmental concerns linked to chemical use, and promoting soil health and biodiversity conservation.

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