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Research Article

Evaluation of the Efficiency of Active Ingredients of Some Plant Extracts as Sustainable Alternatives for Controlling *Fusarium oxysporum* on Eggplant

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ABSTRACT

Fusarium oxysporum is a destructive soil-borne pathogen responsible for Fusarium wilt in eggplant, causing substantial reductions in plant growth and productivity. The present study aimed to isolate, identify, and molecularly characterize *F. oxysporum* and evaluate the antifungal potential of hot aqueous and alcoholic extracts of *Syzygium aromaticum* (clove), *Cinnamomum verum* (cinnamon), and *Mentha piperita* (peppermint), individually and in combination, alongside Chinosol for disease management. Six *F. oxysporum* isolates were recovered and identified morphologically. PCR amplification of the ITS-rDNA region generated a 550-bp fragment, confirming the identity of the most virulent isolate (FOL5), which exhibited 97% sequence similarity with *F. oxysporum* in GenBank (accession no. PX251629). Pathogenicity assays revealed significant variability among isolates, with FOL5 causing the highest disease incidence (86.7%) and severe reductions in seed germination. In vitro, all plant extracts exhibited concentration-dependent antifungal activity. Clove extract completely inhibited mycelial growth at 5-7%, whereas cinnamon and peppermint achieved complete inhibition at higher concentrations. Chinosol also showed strong fungicidal activity, completely suppressing fungal growth at ≥1000 mg L^{-1}. Under pot conditions, all botanical treatments significantly reduced Fusarium wilt incidence and severity compared with the pathogen-inoculated control. Combined extracts of clove, cinnamon, and peppermint provided complete disease suppression (0% incidence and severity) and significantly enhanced plant biomass. These findings demonstrate that combined botanical extracts, particularly clove-based formulations, represent effective, environmentally safe alternatives to synthetic fungicides and have considerable potential for sustainable integrated management of Fusarium wilt in eggplant.

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Introduction

Eggplant (*Solanum melongena* L.) also known as brinjal and aubergine and being a member of the Solanaceae family, is one of the most economically significant vegetables widely grown in the tropical and subtropical countries of the world. It is widely cultivated for its medicinal applications, nutritional, culinary, and economic importance, especially in Asia, where it represents the highest proportion of the total production (Zokian and Al-Musawi, 2015). Eggplant fruits are an important food component in human nutrition and food security because of their high nutritional value, including high contents of carbohydrates, dietary fibers, vitamins, minerals, phenolic compounds and antioxidants, but are low calorie. The

Food and Agriculture Organization estimates that eggplant is grown on about two million hectares worldwide, with almost 1,700,000 ha occupied in Asia. Eggplant is one of the main vegetable crops in Iraq with an estimated area of 8,000-9,000 ha and a total production of 183,000 tons per year in 2025 (Al-Sudani et al., 2025).

Eggplant, due to its economic significance, is highly vulnerable to many biotic stresses including soil-borne fungal diseases that are especially damaging. These disease inciting agents can cause seed rotting, pre and post emergence damping off, root rots, crown rots, vascular wilts and finally the death of plants throughout their growing season, which can significantly reduce yields and fruit quality. *Fusarium*, *Rhizoctonia*, *Pythium* and *Phytophthora* are the most common soil-borne pathogens associated with eggplant (Al-Sudani et al., 2025). *Fusarium* species, among these, are considered one of the most lethal fungal pathogens due to their capacity to survive in the soil for extended periods of time in the form of resistant survival structures, their ability to infect a broad range of hosts and their persistence in the soil. Typical symptoms of infection include seedling death, root and crown rot, vascular discoloration, wilting, retarded growth and premature death of plants (Sarnaik et al., 2025). Thus, *Fusarium* spp. have emerged as significant challenges to lucrative production of eggplant in open fields and protected agriculture systems, leading to significant losses in yields and economic return globally (Chehri and Godini, 2017).

Control of *Fusarium* diseases is difficult since the fungus can persist in soils and in plant debris for several years and is hard to control once the disease is established. Disease management has therefore been highly reliant on multiple applications of synthetic fungicides. Indiscriminate and prolonged applications of fungicides, although provide short term control of the pathogen, have resulted in the emergence of populations of pathogens that are resistant to the fungicides, harmful impacts on soil microbial communities, build-up of fungicide residues in the environment, contamination of the environment and growing concerns about food safety and human health (Lamichhane et al., 2017). This has dramatically increased the global challenge of creating sustainable and environmentally friendly disease management solutions, which aim to decrease the use of synthetic pesticides without compromising crop productivity.

Increasing cognizance of the environmental and health hazards of overuse of pesticides has led to a shift towards the use of integrated disease management strategies. The global organizations such as World Health Organization (WHO), United States Environmental Protection Agency (US EPA), and Food and Agriculture Organization (FAO) are still calling for the decrease of hazardous pesticide use and the introduction of biologically-based and environmentally friendly alternatives in sustainable agriculture (Ahmad et al., 2024). Among these options, the use of plant-based extracts has garnered significant interest due to their biodegradability, relative toxicity to non-target organisms, environmental compatibility, and abundance of bioactive secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, terpenoids, and essential oils. They also possess a broad spectrum of antifungal activities and have demonstrated significant potential against various soil-borne pathogens of economic importance (Adhab et al., 2025).

The antifungal effects of plant-derived extracts are due to several mechanisms. Bioactive phytochemicals inhibit spore germination and growth of hyphae, disrupt cell membrane integrity and permeability, interfere with essential metabolic pathways, inhibit enzyme activity, and finally inhibit pathogen growth and colonization. Furthermore, certain plant extracts have been found to trigger host defense mechanisms and boost resistance against the attack of pathogen infections, thus lowering the incidence and severity of diseases (Al-Ani et al., 2011; Ali et al., 2025). The various modes of action indicate that botanical products have a potential role as valuable tools in integrated disease management programs for sustainable vegetable production.

In view of the growing significance of disease management in respect to environmental concerns and the considerable losses attributed to *Fusarium* root and crown rot of eggplant, the present study was designed to assess the antifungal activity of certain plant extracts in laboratory and greenhouse settings against the causal agent. The results will help develop alternative sustainable, eco-friendly and economically viable management strategies to soil borne diseases for eggplant production systems.

Materials and Methods

Isolation and identification of the pathogen

Fusarium oxysporum was isolated from the roots of eggplant plants exhibiting typical symptoms of vascular wilt, including leaf yellowing and wilting. Diseased plants were collected from commercial greenhouses in Baghdad

Governorate, Iraq. Root tissues were cut into approximately 1-cm-long segments and surface-sterilized in 1% sodium hypochlorite solution for 2 min, followed by three rinses with sterile distilled water. The sterilized tissues were dried on sterile filter paper and transferred onto Potato Dextrose Agar (PDA) supplemented with tetracycline (100 mg L^{-1}) to suppress bacterial contamination. Four tissue segments were placed in each 9-cm Petri plate and incubated at 25 ± 2°C for 3 days.

Hyphal-tip technique was used to purify the emerging fungal colonies showing morphological characteristics typical of *F. oxysporum* and subsequently transferred to fresh PDA plates to get pure cultures. After an additional 4 days of incubation, the isolates were identified according to the standard taxonomic descriptions, which include the microscopic features, morphology, size and the presence or absence of septum in macroconidia, microconidia and chlamydospores (Booth, 1977; Rabiei-Motlagh et al., 2017; Rezaee et al., 2018).

Pathogenicity assay

The pathogenicity of six isolates of *F. oxysporum* was tested in the greenhouse in the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, using completely randomized design (CRD). The fungal isolates were inoculated on millet grains which were used as inoculum source.

A loam soil mixture was autoclaved (121°C for 20 min) three times at 24 h intervals. Sterilized soil was well mixed with fungal inoculum at the rate of 1% (w/w) and potted in plastic pots (3 kg of soil per pot). The inoculated pots were then watered, covered with perforated polyethylene sheets and incubated for 3 days to enable the establishment of the fungi.

The seeds of eggplant were surface sterilized using 1% sodium hypochlorite, washed with sterile distilled water and planted at 10 seeds per pot. Sterilized millet grains without fungal inoculum were added to control pots. Seven days after sowing, the germination was recorded and was continued until all the seeds in the control treatment germinated. The percentage germination of seeds was determined as follows:

$$\text{Germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total number of seeds}} \times 100$$

Isolate FOL5 was chosen for all further tests because of its high pathogenicity.

Molecular characterization of *F. oxysporum*

The purified fungal cultures were used for genomic DNA extraction using a commercial DNA extraction kit (Zymo Research, USA) as per manufacturer's instructions and the protocol outlined by Sidharthan et al. (2018). Molecular identification was carried out using the internal transcribed spacer (ITS) region of fungus by polymerase chain reaction (PCR) according to the method developed by Homa et al. (2018). For the PCR reaction, 2 µL each of the forward and reverse primers was used (Table 1). Amplified PCR products were commercially sequenced by Macrogen (Seoul, South Korea). The nucleotide sequences obtained were compared with those in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to verify the identity of the species.

Preparation of plant extracts

Three species of medicinal plants (Table 2) were chosen to test their fungicidal activities against a highly virulent isolate of *F. oxysporum* (FOL5). Both hot aqueous and ethanolic extracts were prepared as described below.

Table 1. Nucleotide sequence of the ITS rDNA region of the Fusarium oxysporum isolate.

ITS r DNA Primer gene nitrogenous base sequence	Primer
3′ –TCCGTAGGTGAACCTGCGG – 5′	ITS_1
3′ –TCCTCCGCTTATTGATATGC– 5′	ITS_4

Preparation of hot aqueous extracts

Hot aqueous extracts were prepared according to the protocol of Seema et al. (2011) with minor changes. In brief, 150 g each of finely powdered plant materials from clove, cinnamon and peppermint was individually added to 1-L Erlenmeyer flasks. To each flask, 850 mL of boiling distilled water was added, which was then maintained in a water bath for 12 h at 30°C.

Table 2. Medicinal plants evaluated and their relevant characteristics.

Local Name	Scientific Name	Family	Used part
Cloves	*Syzygium aromaticum*	Myrtaceae	Flower buds
Cinnamon	*Cinnamomum verum*	Lauraceae	Bark
Peppermint	*Mentha arvensis*	Lamiaceae	Leaves

Using a Büchner funnel, the extracts were filtered under vacuum using Whatman No. 1 filter paper. This extraction was repeated two times and the filtrates were mixed together. The combined filtrates were concentrated at 45°C in a drying oven to get a semi-solid paste. The crude extracts were weighed, put into sterile, airtight, amber colored glass bottles and kept at 4°C until their further use.

Preparation of ethanolic extracts

Ethanolic extracts were made following the method of Seema et al. (2011). In brief, 150 g of the pulverized plant material was added to 850 mL of 99% ethanol in 1-L Erlenmeyer flasks. These mixtures were sealed and incubated for 12 h in a water bath at 30°C.

Using Büchner funnel, the extracts were filtered twice under vacuum with Whatman No. 1 filter paper. The combined filtrates were concentrated by drying at 45°C in a drying oven until the solvent was completely evaporated. The crude dried extracts were weighed and placed in sterile amber glass bottles and kept at 4°C until their further use.

In vitro* evaluation of hot aqueous and ethanolic plant extracts against *F. oxysporum

The antifungal activity of hot aqueous and ethanolic plant extracts against *F. oxysporum* isolate FOL5 was evaluated using the poisoned food technique. Five extract concentrations (0, 3, 5, 7, and 9%, w/v) were tested.

Prior to incorporation into the medium, the extracts were sterilized by membrane filtration through 0.22-µm Millipore filters. Sterile extracts were incorporated separately into molten PDA medium, mixed thoroughly to ensure uniform distribution, and poured into sterile 9-cm Petri dishes.

After solidification, a 5-mm-diameter mycelial plug excised from the actively growing margin of a 5-day-old *F. oxysporum* culture was placed at the center of each plate. Three replicate plates were prepared for each treatment. Control plates contained PDA without plant extracts. All plates were incubated at 25 ± 2°C until the control colonies reached the edge of the plates.

The percentage inhibition of mycelial growth was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Rc} - \text{Rt}}{\text{Rc}} \times 100$$

Where:

Rc = radial mycelial growth in the untreated control (mm)

Rt = radial mycelial growth in the treated plates (mm)

***In vitro* evaluation of chinosol fungicide**

The antifungal activity of the commercial fungicide Beltanol® (Probelte S.A., Spain), containing 50% Chinosol as the active ingredient, was evaluated against *F. oxysporum* isolate FOL5 using the poisoned food technique.

Four concentrations (500, 750, 1000, and 1500 mg L^{-1} active ingredient) were incorporated individually into molten PDA medium and mixed thoroughly before pouring into sterile 9-cm Petri dishes.

Following solidification, a 5-mm agar disc taken from the actively growing margin of a 5-day-old *F. oxysporum* culture was placed at the center of each plate. Three replicate plates were prepared for each concentration. Control plates containing PDA without fungicide served as the untreated control. The plates were incubated at 25 ± 2°C for 5 days. Radial colony growth was measured as the average of two perpendicular colony diameters, and the percentage inhibition of mycelial growth was calculated using the equation described above.

Greenhouse evaluation of hot aqueous and ethanolic plant extracts against *Fusarium* wilt of eggplant

Based on the results of the *in vitro* assays, the efficacy of selected hot aqueous and ethanolic plant extracts was further evaluated under greenhouse conditions for the management of *Fusarium* wilt caused by *F. oxysporum*. The experiment was conducted in the greenhouse of the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad. A sterilized soil mixture consisting of loam soil and peat moss was prepared by autoclaving at 121°C for 20 min on three successive occasions at 24-h intervals. Sterile soil was transferred into plastic pots (25 cm diameter) at a rate of 3 kg per pot. The experiment consisted of 31 treatments given in Table 3.

Greenhouse evaluation of plant extracts and beltanol against *F. oxysporum* on eggplant

Alcoholic plant extracts were applied as a soil drench at a concentration of 5% (100 mL pot^{-1}), whereas hot aqueous extracts were applied at a concentration of 7% (100 mL pot^{-1}). The inoculum of *F. oxysporum*, prepared on locally produced millet seeds, was incorporated into the soil at 1% (w/w) three days before sowing in all treatments requiring pathogen inoculation. The commercial fungicide Beltanol was applied at the manufacturer's recommended rate of 1 mL L^{-1} (100 mL pot^{-1}) at the time of sowing.

Table 3. Detail of treatments.

Sr. No.	Description of treatments
1	Sterilized soil only
2	Sterilized soil + *F. oxysporum* (isolate FOL5)
3	Sterilized soil + clove alcohol extract
4	Sterilized soil + clove hot aqueous extract
5	Sterilized soil + cinnamon alcohol extract
6	Sterilized soil + cinnamon hot water extract
7	Sterilized soil + peppermint alcohol extract
8	Sterilized soil + peppermint hot water extract
9	Sterilized soil + clove + cinnamon alcohol extract
10	Sterilized soil + clove +cinnamon hot aqueous extract
11	Sterilized soil + clove + peppermint alcohol extract
12	Sterilized soil + clove + peppermint hot aqueous extract
13	Sterilized soil + cinnamon + peppermint alcohol extract
14	Sterilized soil + cinnamon + peppermint hot aqueous extract
15	Sterilized soil + clove + cinnamon + peppermint alcohol extract
16	Sterilized soil + clove + cinnamon + peppermint hot aqueous extract
17	Sterilized soil + clove alcohol extract + FOL5
18	Sterilized soil + clove hot aqueous extract + FOL5
19	Sterilized soil + cinnamon alcohol extract + FOL5
20	Sterilized soil + cinnamon hot water extract + FOL5
21	Sterilized soil + peppermint alcohol extract + FOL5
22	Sterilized soil + peppermint hot water extract + FOL5
23	Sterilized soil + clove+ cinnamon alcohol extract + FOL5
24	Sterilized soil + clove+ cinnamon hot aqueous extract + FOL5
25	Sterilized soil + clove+ peppermint alcohol extract + FOL5
26	Sterilized soil + clove+ peppermint hot aqueous extract + FOL5
27	Sterilized soil + cinnamon + peppermint alcohol extract + FOL5
28	Sterilized soil + cinnamon + peppermint hot aqueous extract + FOL5
29	Sterilized soil + clove+ cinnamon + peppermint alcohol extract + FOL5
30	Sterilized soil + clove + cinnamon + peppermint hot aqueous extract
31	Sterilized soil + Chinosol + FOL5

The eggplant cultivar “Wissam” was used throughout the experiment. Each pot was sown with five seeds. For comparison treatments receiving hot aqueous or alcoholic extracts of clove, cinnamon, or peppermint without pathogen challenge, identical procedures were followed except that the soil was not inoculated with *F. oxysporum*. In treatments involving the combined application of two factors, each component was applied at 50% of its individual concentration. The negative control consisted of sterilized soil amended with sterile millet seeds without fungal inoculation.

All pots were maintained under greenhouse conditions and irrigated as required throughout the experiment. Disease symptoms, including leaf yellowing and wilting, were first observed 30 days after sowing (DAS). Disease incidence was calculated using the following equation:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings}} \times 100$$

Two months after sowing, leaf chlorophyll content was determined using a SPAD-502 chlorophyll meter (Minolta, Japan). Three fully expanded leaves were randomly selected from each replicate, and SPAD values were

recorded at different canopy positions. The average of the three readings was used for statistical analysis (Al-Mayahi and Hassan, 2021). Following chlorophyll measurements, plants were harvested and disease severity was assessed using a five-point disease rating scale:

0 = Healthy plant with no visible symptoms,

1 = Yellowing of 1-25% of the leaves near the stem base,

2 = Yellowing and wilting of 26-50% of the leaves accompanied by slight brown discoloration of the vascular tissue,

3 = Yellowing and wilting of 51-75% of the leaves with pronounced dark-brown vascular discoloration,

4 = Severe wilting, collapse, and death of 76-100% of the foliage.

Disease severity (%) was calculated according to the formula of McKinney (1923):

$$\text{Disease severity (\%)} = \frac{\Sigma\ (\text{Disease rating} \times \text{Number of plants in each rating class})}{\text{Total number of plants assessed} \times \text{Maximum disease rating}} \times 100$$

To fulfill Koch's postulates and confirm pathogen infection, root samples were collected from symptomatic plants, surface-sterilized, plated on PDA, and the recovered fungus was identified microscopically.

Plant growth was evaluated by determining fresh biomass immediately after harvest, followed by dry biomass after oven-drying the samples at 50°C for 72 h, or until a constant weight was achieved.

For biochemical analyses, leaf samples were collected from pathogen-inoculated plants receiving the different treatments. Samples were harvested 40 days after sowing to determine total soluble protein according to Kalra (1971). In addition, polyphenol oxidase (PPO) activity, an important biochemical marker associated with induced resistance against *F. oxysporum*, was quantified using the method described by Ojha and Chatterjee (2012). Leaf samples for PPO analysis were collected 15 days after fungal inoculation.

Statistical analysis

The greenhouse experiment was conducted using a completely randomized design (CRD) consisting of 31 treatments, each replicated three times. Experimental data were subjected to analysis of variance (ANOVA), and treatment means were compared using the least significant difference (LSD) test at the 5% probability level ($P \leq 0.05$).

Results and Discussion

Isolation and identification of the pathogen

The isolation and identification procedures revealed six distinct isolates of *F. oxysporum* (Figure 1). These isolates demonstrated considerable variations in growth rates and mycelial densities on PDA medium. Moreover, they showed a range of pigmentation, starting from a cottony white appearance to pink or deep purple color. After microscopic examination, it was found to contain three asexual structures.

Microconidia

The microconidia were usually unsegmented and observed on conidiophores. They were cylindrical to oval or kidney shaped. They also formed pseudo heads on a single conidiophore.

Macroconidia

The second type of asexual structure was the macroconidia with 3-6 septa. They were spindle-shaped with tapered ends and a sharp tip that might sometimes be curved. They had uneven dimensions, as these spores were formed on small lateral branches of conidia that were clustered or united together, creating what is known as the fungal cushion (sporodochia).

Chlamydospores

The third type of spores was the chlamydospores. They were usually formed in singles or in pairs (doublets). They were produced either terminally on short lateral branches or intercalarily along the hyphae. In addition, they were often observed in chains, a strategy employed to ensure the survival of the fungus during unfavorable conditions. The walls of these structures might be characterized as either rough or smooth, depending on the specific characteristics of the fungus in question (Booth, 1977; Leslie and Summerell, 2006).

Effect of *F. oxysporum* on the germination of eggplant seeds in pots

All fungal isolates were shown to be pathogenic based on their pathogenicity results (Table 4) which resulted in a notable decrease in eggplant seed germination and wilting. Moreover, there was a notable difference from the control treatment (fungus-free), which had 0.00% infection rate. The infection rate significantly increased to 86.7% with isolate FOL5. The pathogenicity of the isolates varied significantly, with FOL3 and FOL4 isolates showing the maximum virulence and both caused individual infection rates of 73.3%. The pathogenicity of the fungus might be explained by its

capacity to attack seeds, causing them to rot and decompose, or by variations in the isolates' genetic makeup, the type of enzymes they secrete, and the quantity of toxins and metabolic compounds they release. In the early stages of infection, the fungus secretes pectin and cellulose-degrading enzymes, which facilitates penetration of the host (Manikandan et al., 2024).

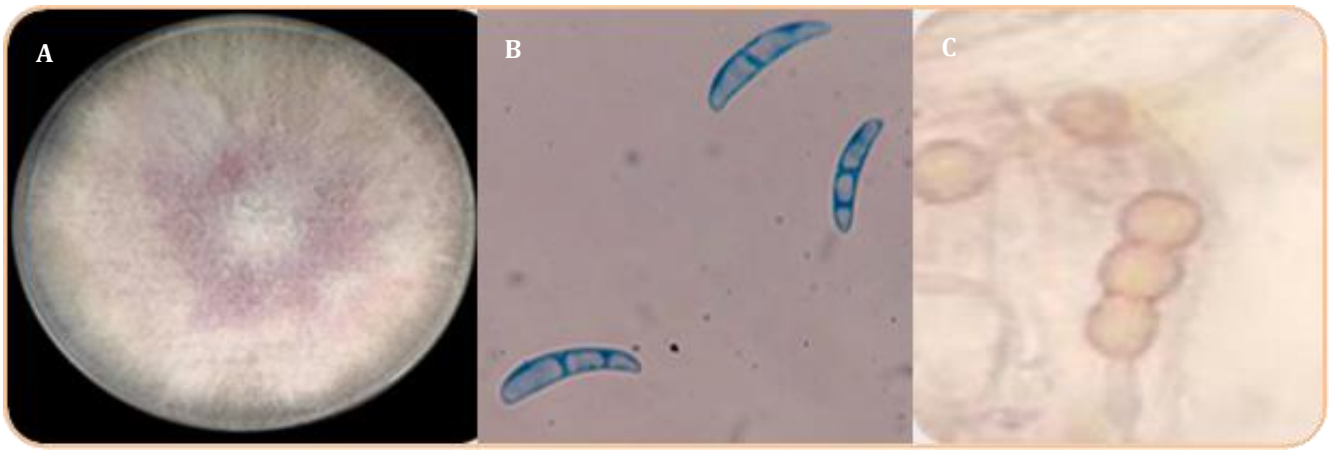


Figure 1. Morphological characteristics of the pathogenic fungus. A: *F. oxysporum* fungal culture on PDA culture medium after 5 days, B: Macroconidia under 40× magnification, C: Sporochlamydia under 40× magnification.

Table 4. Pathogenicity of *F. oxysporum* isolates on eggplant seed germination.

Sr. No.	Isolation symbol	% infection
1	FOL1	46.7
2	FOL2	56.7
3	FOL3	73.3
4	FOL4	73.3
5	FOL5	86.7
6	FOL6	66.7
7	Control	00.0
8	L.S.D $_{0.05}$	9.36

Each value represents the average of 3 replicates.

Molecular confirmation of *F. oxysporum* by PCR

The molecular confirmation of *F. oxysporum* isolate was done by amplifying the ribosomal DNA internal transcribed spacer (rDNA-ITS) region by employing polymerase chain reaction (PCR). From purified cultures of *F. oxysporum*, genomic DNA was extracted, its quality was measured using spectrophotometer by determining the absorbance ratio at 260/280 nm. An A260/A280 ratio of about 2 shown by the extracted DNA indicated its high purity and suitability for PCR amplification. By using species-specific primers, amplification of the rDNA-ITS region produced a single, distinct amplicon of about 550 bp, which confirmed the successful amplification of the target region (Figure 2). The molecular identity of the isolate as *F. oxysporum* was further verified by the presence of the expected PCR product.

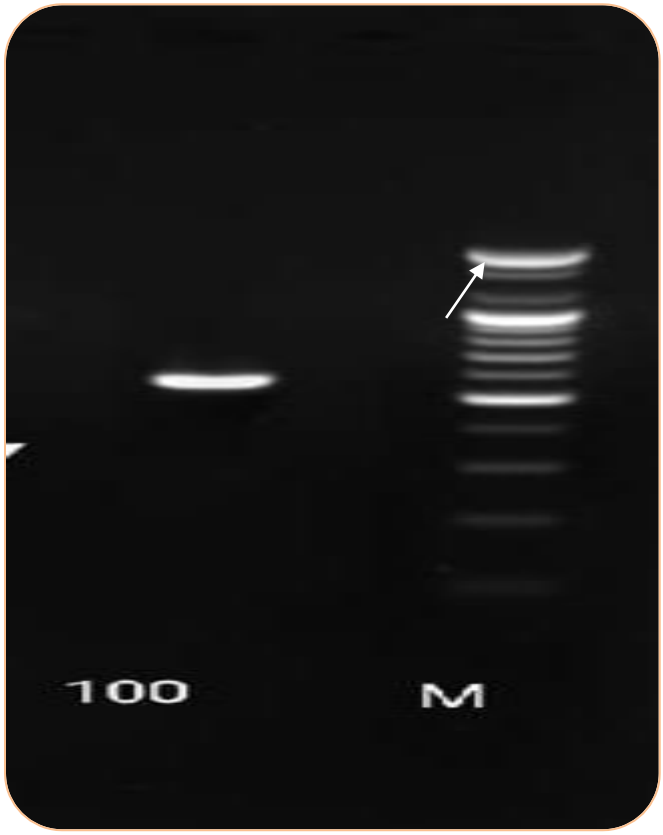


Figure 2. Gel electrophoresis profile of *F. oxysporum* isolate FOL5, illustrating amplified DNA fragments

produced by the ITS primer set, with an approximate molecular size of 550 base pairs (lanes 1 and 2 from the left). Lane M denotes the molecular weight marker derived from a 100 bp DNA ladder.

Sequence analysis of the PCR amplicon

The PCR amplicon was submitted to Macrogen Inc. (South Korea) for sequencing. The resulting nucleotide sequence of the amplified ITS rDNA region was analyzed using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). BLAST analysis revealed a 97% similarity between this isolate and the corresponding nucleotide sequences of the ITS region from *F. oxysporum* isolates deposited in the NCBI database.

This molecular result corroborated the morphological identification of the isolate. The nucleotide sequence of the amplified fragment was submitted to GenBank under the accession number PX251629.

Evaluation of the antifungal efficacy of hot aqueous and alcoholic plant extracts against *F. oxysporum* (FOL5) using the poisoned food technique

Table 5 shows the fungicidal activities of hot aqueous and alcoholic extracts of *S. aromaticum*, *C. verum* and *M. piperita* against FOL5 isolate of *F. oxysporum* on PDA amended with extract concentrations of 3, 5, 7 and 9% using the poisoned food technique.

Clove extract, among all the tested botanicals, showed the highest fungicidal activity regardless of the extraction technique. The alcoholic and hot aqueous extracts completely inhibited the mycelial growth of the pathogen at concentrations of 5 and 7% resulting in 100% growth inhibition. The untreated control (0% extract) had a colony diameter of 9.0 cm and no inhibition was detected. As a whole, the effect of the clove extract on the fungus was found to be dose-dependent: the more concentrated the extract, the stronger the antifungal effect.

Cinnamon extracts were also found to be highly antifungal. The alcoholic and hot aqueous extracts showed significant inhibitory effect at 5% and 7% with inhibition of 75.77% and 89.88% respectively, with colony diameters of 2.18 and 0.91 cm respectively. At the highest effective concentration evaluated, complete inhibition of mycelial growth (0.0 cm colony diameter; 100% inhibition) was achieved.

Peppermint extract had relatively weak fungicidal activity compared to cinnamon and clove extracts. Nevertheless, the highest concentration of 9% at both extraction methods completely suppressed the fungal growth suggesting that the effect of peppermint extract on fungal growth depended on concentration.

The greater fungicidal activity of clove extract is thought to be due to the high amounts of phenolic compounds, especially eugenol, which makes up about 72-90% of the essential oil. Clove also contains several other biologically active ingredients that synergize its antifungal activity. These biologically active constituents include the flavonoids (eugenin, eugenitin, and kaempferol), phytosterols (stigmasterol), triterpenoids (oleanolic acid), and hydrolysable tannins (bicornin). All of these compounds act by disrupting the fungal cell membranes, inhibiting the activity of enzymes, and causing damage to the fungal cells (Elshahawy et al., 2017; Hassan et al., 2021).

The fungicidal action of cinnamon extract is attributed to its wide composition of phytochemicals such as cinnamaldehyde, flavonoids, eugenol, phenolic acids, and other polyphenolic compounds which disrupt the integrity of the fungal cell wall, permeability of the cell membrane, and essential metabolic pathways. Similarly, the antifungal properties of peppermint extract might be ascribed to a variety of bioactive metabolites such as alkaloids, ascorbic acid, amino acids, carbohydrates, flavonoids, menthol, menthone, phenolic acids (including caffeic and salicylic acids), and proteins which affect fungal growth by different biochemical mechanisms (Kamal, 2015; Martinko and Mioč, 2024). The results reported in this study indicate that there is a significant potential for using plant-based extracts, especially clove and cinnamon, as environmentally benign substitutes to deleterious synthetic fungicides for the control of *F. oxysporum*.

Effect of Chinosol in inhibiting the mycelial growth of *F. oxysporum* isolate FOL5 on PDA

In vitro evaluation of Chinosol (commercial formulation: Beltanol) for its antifungal activity against *F. oxysporum* isolate FOL5 was carried out at the concentrations of 500, 750, 1000 and 1500 mg L^{-1} (Table 6). The results showed that the effect of inhibition of mycelial growth was clearly concentration dependent. The concentrations of 1000 mg L^{-1} and 1500 mg L^{-1} showed complete inhibition of the fungal growth (100%) with 0.00 cm colony diameter compared with the untreated control which had 9.00 cm colony diameter with no inhibition of growth. Chinosol inhibited the growth of

the colony by 93.05% at the rate of 750 mg L^{-1}, giving an average colony diameter of 0.62 cm. The lowest concentration tested (500 mg L^{-1}) also showed significant antifungal activity with the colony diameter reduced to 3.31 cm and 63.19% growth inhibition.

In general, the antifungal activity of Chinosol was found to be high against *F. oxysporum* isolate FOL5 at all the concentrations tested with complete inhibition of mycelial growth at ≥ 1000 mg L^{-1}. The high fungicidal activity of Beltanol might be due to the presence of an active ingredient, Chinosol (8-hydroxyquinoline sulfate), that acts as a metal-ion chelator to produce its antimicrobial effect. Chinosol forms stable complexes with essential metal ions needed for fungal metabolism which results in impaired enzyme function, disruption of membrane integrity and disturbance of key cellular processes that inhibit fungal growth and finally lead to cell death. In addition, its chelating activity might improve binding with metal ions like copper in host tissues resulting in improved antimicrobial activity and pathogen growth inhibition. These results are in line with previous work showing the remarkable fungicidal activity of Chinosol against various plant pathogenic fungi (Al-Mayahi and Hassan, 2021; Mousa and Hassan, 2023).

Effect of hot aqueous and alcoholic plant extracts on incidence and severity of *F. oxysporum* infection in eggplant

All the tested plant extracts individually and in various combinations significantly decreased the incidence and severity of *F. oxysporum* infection over the pathogen-inoculated control plants (Table 7). The combined alcoholic extract of clove, cinnamon and peppermint was observed as the most effective treatment with 0.00% disease incidence and 0.00% disease severity, while the pathogen inoculated control had 76.66% disease incidence and 71.67% disease severity. This treatment, however, was not significantly different than the combined hot aqueous clove, cinnamon and peppermint extracts and the alcoholic combinations of cinnamon + peppermint, clove + peppermint, and clove + cinnamon, which also achieved complete disease suppression (0.00% incidence and 0.00% severity). The rest of the treatments showed moderate disease control.

Effect of hot aqueous and alcoholic plant extracts on fresh and dry biomass per plant

The combination of clove, cinnamon and peppermint extracts effectively improved plant growth besides controlling the disease. This treatment resulted in the highest fresh and dry biomass of 24.5 and 12.3 g $plant^{-1}$, respectively, which is significantly higher than the pathogen-inoculated control and all other treatments (Table 7; Figures 3, 4, 5). The results showed that the combinations of plant extracts resulted in not only effective protection against *F. oxysporum*, but also plant vigor under pathogen stress.

The exceptional fungicidal activity reported in the present study is in agreement with numerous previous reports that have shown the multiplicate antimicrobial properties of clove, cinnamon and peppermint extracts against a wide range of phytopathogenic fungi (Adhab et al., 2025). The effectiveness of their use is largely ascribed to the rich presence of bioactive secondary metabolites such as flavonoids, essential oils, phenolics, terpenoids, and others, which are effective in disrupting cell membrane of fungi, inhibiting spore germination, suppressing mycelial growth and interfering with the metabolism of fungi.

Eugenol, a phenolic compound, is a major compound of clove extract that possesses strong fungicidal and antioxidant properties. Eugenol helps to reduce oxidative damage caused by pathogen infection, thus ensuring the maintenance of cellular metabolism and physiological homeostasis (Muñoz-Castellanos et al., 2020; Martinko and Mioč, 2024). In addition, it has been reported that clove extract can stimulate antioxidant defense systems by increasing the activity of antioxidant enzymes like peroxidase, which in turn inhibits the production of ROS caused by attack by pathogens (Agbo et al., 2022). Clove-mediated disease suppression has also been found to be associated with systemic resistance via activation of defense-related enzymes, such as chitinase and β-1,3-glucanase, which break down the cell walls of fungi and boost host immunity. As a result the treated plants grow better, absorb higher levels of nutrients and are more resistant to diseases and environmental stress.

The enhanced activity of the mixture of extracts prepared from clove, cinnamon and peppermint might also be attributed to the synergic action of the extracts on plant nutrition and defense. These extracts have been documented to have the ability to boost the absorption and accumulation of mineral nutrients such as Fe, Zn, Mn, Cu, N, P, and K, and to stimulate the activity of defence-related enzymes and promote beneficial rhizosphere microorganisms such as yeasts and algae.

These synergistic effects promote soil health, plant vigor and tolerance to pathogen infections (Muñoz-Castellanos et al., 2020). Furthermore, the clove extract improves the root growth, uptake of nutrients and the photosynthetic efficiency thus facilitating biomass accumulation (Rubinskienė et al., 2015).

The positive effects of cinnamon extract are due to its capability of improving physiological performances of plants through promoting nitrogen assimilation, as well as increasing chlorophyll biosynthesis and stability that prevents the photosynthetic pigments from oxidative degradation. Cinnamon also stimulates shoot and root growth in a similar way as plant growth regulators do, thus, enhancing the biomass formation and development of the plant (Jukanti and Bhatt, 2015). Furthermore, cinnamon plays an important role in improving tolerance to stress by regulating electron transport processes, cell division, and respiration, and by protecting the chloroplasts from oxidative damage, thereby increasing the efficiency of the photosynthetic processes and increasing plant resistance to pathogen stress (Muñoz-Castellanos et al., 2020).

In general, the combination of clove, cinnamon and peppermint extracts proved to be very effective in controlling *F. oxysporum* infection, as well as improving plant growth and physiological performance, thus providing great potential as environmentally friendly botanical alternatives for integrated management of *Fusarium* wilt in eggplant.

Table 6. Antifungal activity of Chinosol against *F. oxysporum*.

Concentrations mg/L	Fungal radial growth rate	Inhibition %
0	9.00	0.00
500	3.31	63.19
750	0.62	93.05
1000	0.00	100.00
1500	0.00	100.00
L.S.D $_{0.05}$	0.53	6.94

Each value represents the average of 3 replicates.

Table 5. Effect of hot aqueous and alcoholic extracts on colony diameter, growth rate, and inhibition percentage of F. oxysporum under in vitro conditions.

Treatments	Concentration	Hot aqueous extracts		Alcoholic extracts	
		Average colony diameter (cm)	% Inhibition	Average colony diameter (cm)	% Inhibition
Cloves	3	7.40	17.77	4.34	51.77
	5	4.17	53.67	0.00	100.00
	7	0.00	100.00	0.00	100.00
	9	0.00	100.00	0.00	100.00
	0	9.00	0.00	9.00	0.00
Cinnamon	3	7.80	13.33	6.58	26.88
	5	4.50	50.00	2.18	75.77
	7	0.91	89.88	0.00	100.00
	9	0.00	100.00	0.00	100.00
	0	9.00	0.00	9.00	0.00
Peppermint	3	7.80	13.33	7.12	20.88
	5	5.37	40.33	5.78	35.77
	7	1.8	80	2.32	74.22
	9	0.00	100.00	0.00	100.00
	0	9.00	0.00	9.00	0.00
L.S.D $_{0.05}$		Treatment = 0.17 Conc. = 0.23 Interactions = 0.40	Treatment = 1.98 Conc. = 2.56 Interactions = 4.44	Treatment = 0.17 Conc. = 0.38 Interactions = 0.66	Treatment = 0.27 Conc. = 0.60 Interactions = 1.04

Each value represents the average of 3 replicates.

Table 7. Effect of hot aqueous and alcoholic extracts against per cent disease incidence and severity and fresh and dry weights of *F. oxysporum* under *in vitro* conditions.

Treatments	% Incidence	% Severity	Fresh Weight (g/plant)	Dry Weight (g/plant)
Control(Sterilized soil)	0.00	0.00	24.0	11.2
F.oxysporum(FOL5)	76.66	71.67	12.4	3.6
Sterilized soil + clove alcohol extract	0.00	0.00	26.2	11.8
Sterilized soil + clove hot aqueous extract	0.00	0.00	26.1	11.2
Sterilized soil + cinnamon alcohol extract	0.00	0.00	26.0	10.9
Sterilized soil + cinnamon hot aqueous extract	0.00	0.00	25.7	10.7
Sterilized soil + peppermint alcohol extract	0.00	0.00	25.9	10.5
Sterilized soil + peppermint hot aqueous extract	0.00	0.00	25.4	10.3
Sterilized soil + clove+ cinnamon alcohol extract	0.00	0.00	26.8	11.9
Sterilized soil + clove+ cinnamon hot aqueous extract	0.00	0.00	26.0	11.3
Sterilized soil + clove+ peppermint alcohol extract	0.00	0.00	26.7	11.6
Sterilized soil + clove+ peppermint hot aqueous extract	0.00	0.00	26.7	11.5
Sterilized soil + cinnamon + peppermint alcohol extract	0.00	0.00	25.6	10.5
Sterilized soil + cinnamon + peppermint hot aqueous extract	0.00	0.00	25.3	10.1
Sterilized soil +Cloves+ cinnamon + peppermint hot aqueous extract	0.00	0.00	27.3	12.3
Sterilized soil +Cloves+ cinnamon + peppermint alcohol extract	0.00	0.00	26.9	11.8
Sterilized soil + clove alcohol extract+FOL5	6.66	3.33	21.7	7.3
Sterilized soil + clove hot aqueous extract+FOL5	13.33	10.50	20.8	6.3
Sterilized soil + cinnamon alcohol extract+FOL5	13.33	10.50	20.2	6.1
Sterilized soil + cinnamon hot aqueous extract+FOL5	20.00	15.67	20.0	6.0
Sterilized soil + peppermint alcohol extract +FOL5	26.66	21.55	20.2	6.3
Sterilized soil + peppermint hot aqueous extract +FOL5	33.33	25.50	20.1	6.2
Sterilized soil + clove+ cinnamon alcohol extract+FOL5	0.00	0.00	22.5	11.5
Sterilized soil + clove+ cinnamon hot aqueous extract+FOL5	6.66	3.33	21.6	11.2
Sterilized soil + clove+ peppermint alcohol extract+FOL5	0.00	0.00	22.7	11.3
Sterilized soil + clove+ peppermint hot aqueous extract+FOL5	6.66	3.33	21.6	10.9
Sterilized soil + cinnamon + peppermint alcohol extract+FOL5	0.00	0.00	21.8	11.2
Sterilized soil + cinnamon + peppermint hot aqueous extract+FOL5	13.33	10.50	21.7	11.1
Sterilized soil +Cloves+ cinnamon + peppermint alcohol extract+FOL5	0.00	0.00	24.5	12.3
Sterilized soil +Cloves+ cinnamon + peppermint hot aqueous	0.00	0.00	24.0	11.9
Sterilized soil + Chinosol+FOL5	46.66	36.78	17.3	4.8
L.S.D $_{0.05}$	12.57	10.90	1.08	0.46

Each value represents the average of 3 replicates.

Conclusions

In the present study, six pathogenic isolates of *F. oxysporum* were successfully isolated and identified, of which the isolate FOL5 was found to be the most virulent towards eggplant. The pathogen was further confirmed by morphological characterization, PCR amplification and sequencing of the rDNA-ITS region. *S. aromaticum* (clove) had the greatest *in vitro* antifungal activity, followed by *C. verum* (cinnamon), while *M. piperita* (peppermint) was relatively weak when used alone. Chinosol (Beltanol) also exhibited very good antifungal activity, completely inhibiting fungal growth at 1000 mg L^{-1} and higher concentrations. Clove, cinnamon, and peppermint extracts when applied in combination, especially the alcoholic extracts, resulted in complete suppression of *Fusarium* wilt, caused significant reduction in disease incidence and severity and a significantly increased plant biomass under pot conditions. The results showed that using botanical extracts, particularly in combination, is effective, environmentally friendly and sustainable alternatives to detrimental synthetic fungicides in controlling *Fusarium* wilt of eggplant disease.

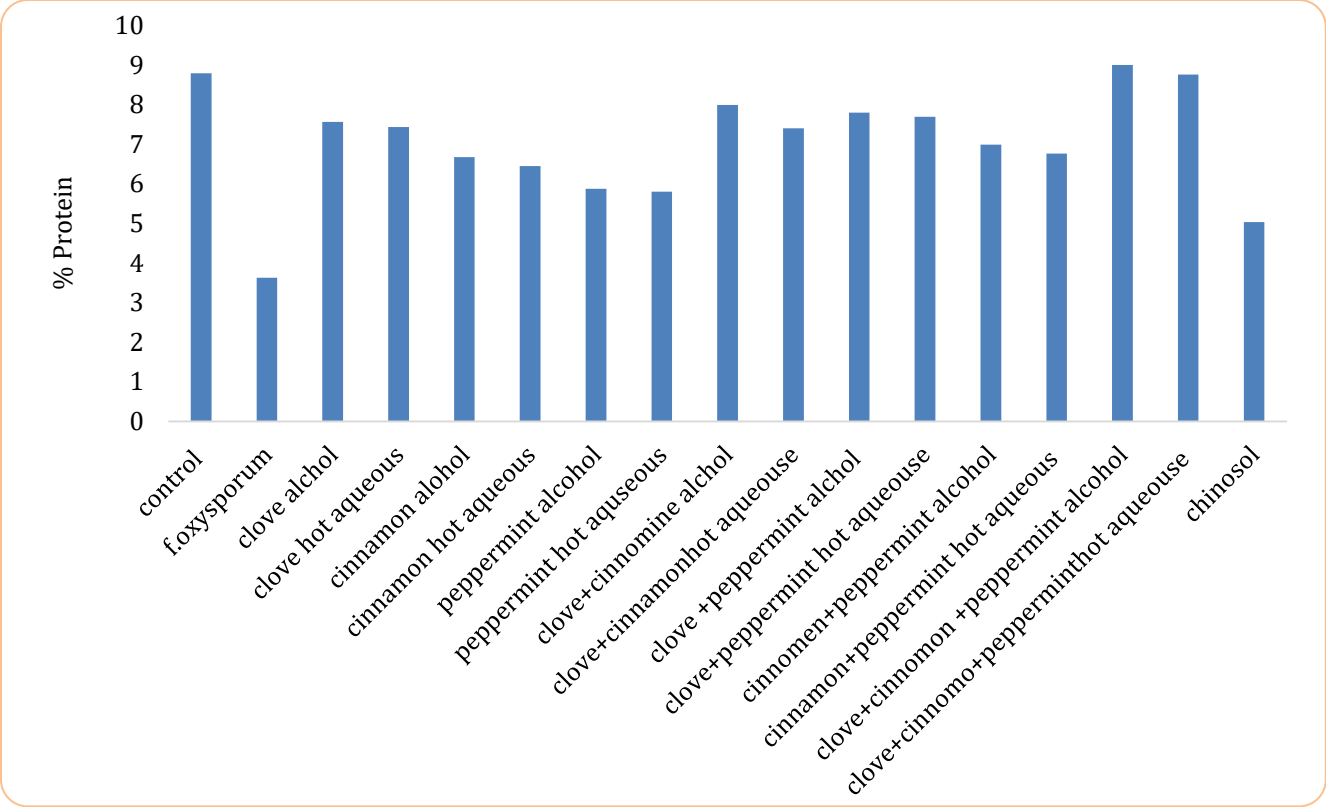


Figure 3. Effect of selected plant extracts on total protein content. LSD at P ≤ 0.05 = 0.51.

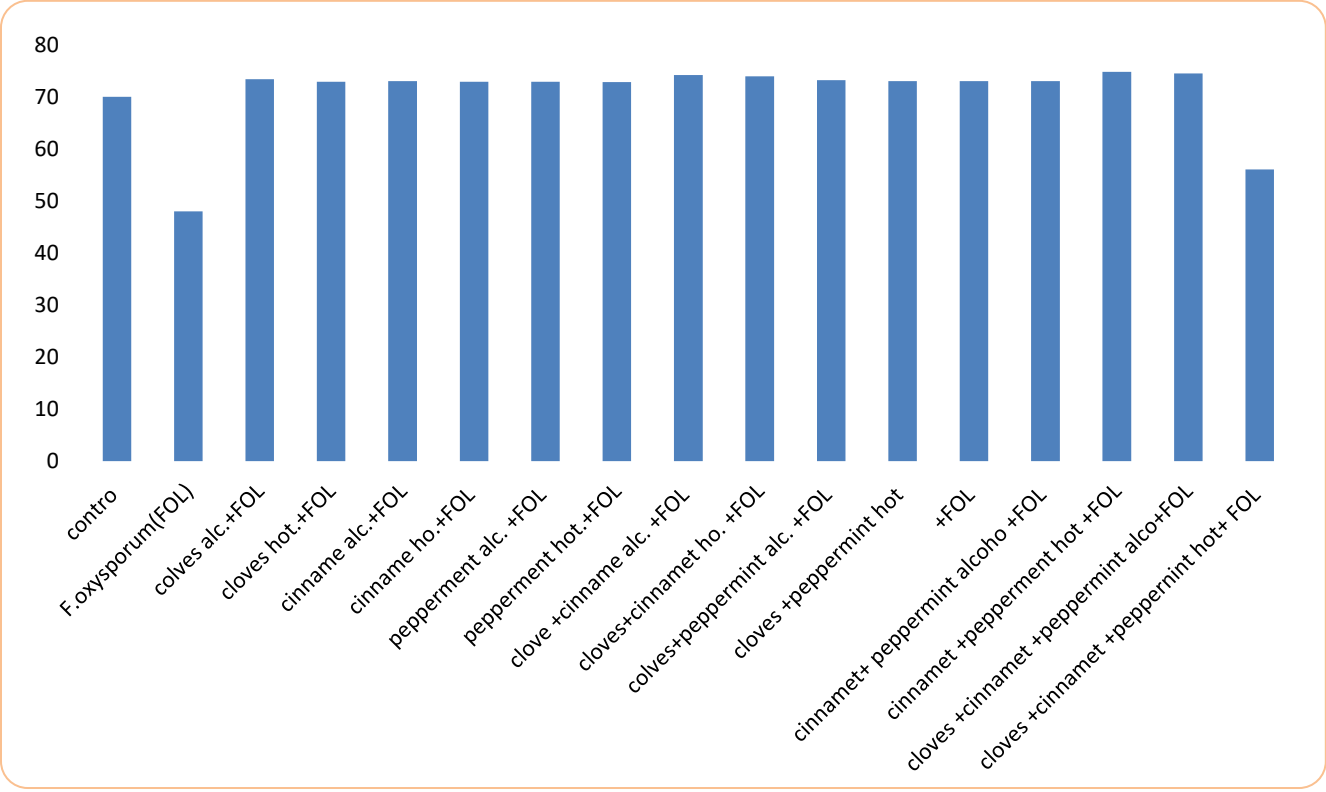


Figure 4. Effect of plant extracts on chlorophyll content of eggplant under greenhouse conditions. LSD (0.05) = 0.43.

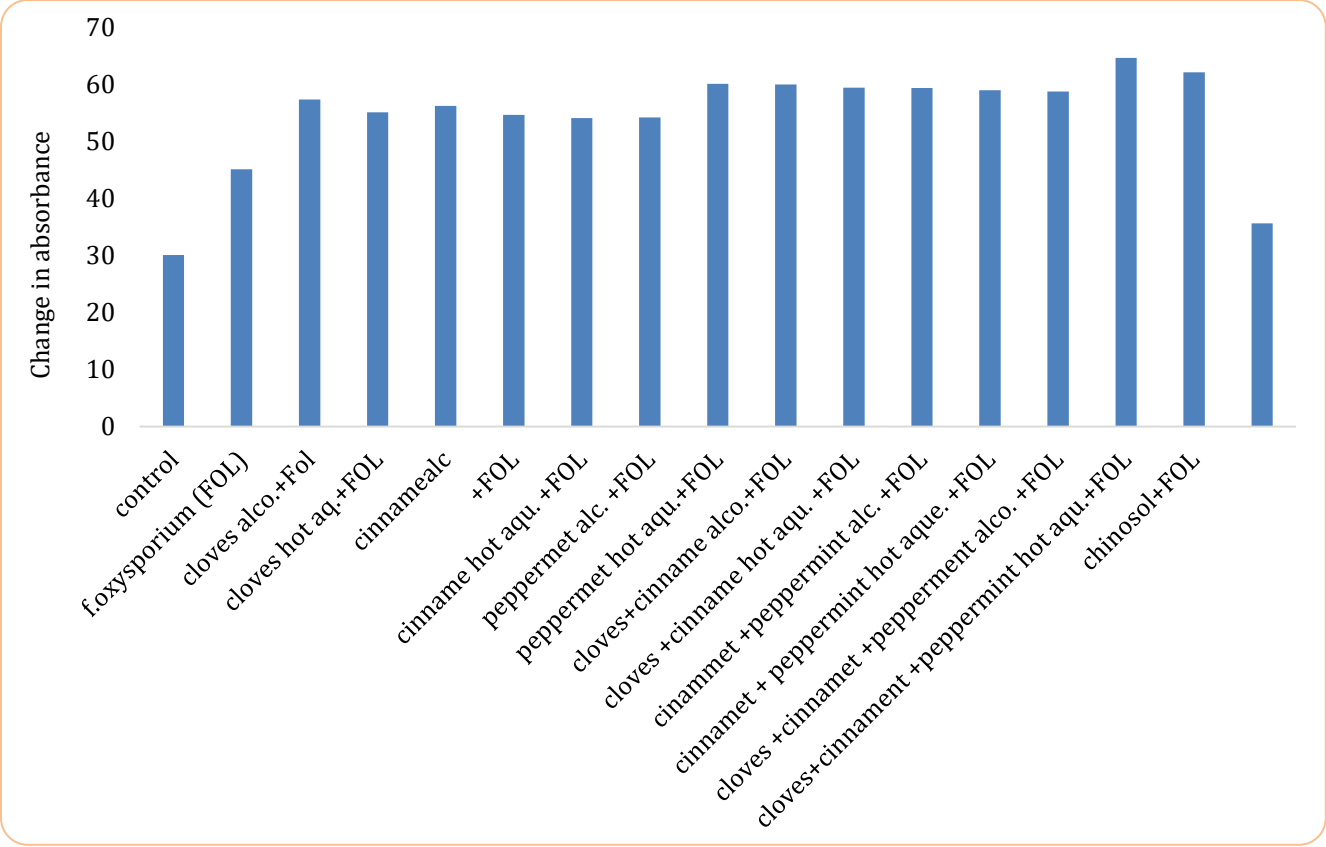


Figure 5. PPO activity expressed as changes in absorbance min^{-1} g^{-1} fresh tissue weight 15 days after pathogen inoculation under different treatments ($LSD_{0.05}$ = 0.93).

Recommendations

The effectiveness of these botanical extracts when used in combinations should be better assessed under greenhouse and multi-site field conditions to confirm the performance under different environments in the future. For large-scale commercialization, it is crucial to standardize the extraction process, formulation stability, shelf life and application rates. The synergistic effects of bioactive constituents of clove, cinnamon and peppermint should be explored through metabolomic and molecular methods to understand their mechanisms of fungicidal activity and induction of host resistance. The application of these botanical products in combination with other approaches of integrated disease management programs involving resistant cultivars, biological control agents and cultural practices is suggested to achieve sustainable control of *F. oxysporum*. Last but not the least, economic costs, phytotoxicity, environmental safety, and their impacts on beneficial soil biota must be thoroughly evaluated before these botanical products are recommended for commercial use.

Author Contributions

BSAA and AH conceived and designed the study. IA conducted the field investigations and carried out the experimental work. JK and BSAA provided overall supervision, ensuring methodological rigor and analytical consistency throughout the study. BSAA and AH also served as co-supervisors, providing critical guidance on the experimental design, data interpretation, and refinement of the research at various stages of the project. BSAA, IA, AH, and JK contributed to the preparation of the original manuscript draft, including organizing the manuscript structure, compiling the results, and developing the initial interpretations. All authors subsequently critically reviewed and revised the manuscript, verified the accuracy of the data and analyses, contributed to its intellectual content, and approved the final version for publication.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

Policy Addressed

The study provides scientific evidence supporting policies focused on sustainable agriculture, reduced pesticide dependency, environmental conservation, and the development of botanical-based disease management technologies. The successful application of plant extracts against Fusarium wilt highlights their potential role in future eco-friendly crop protection programs.

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