PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(4): 580-590
https://doi.org/10.14719/pst.4837

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RESEARCH ARTICLE

Exploring the antifungal activity of clove oil (*Syzygium aromaticum*) against wilt disease caused by *Fusarium equiseti* in bitter gourd (*Momordica charantia* L.)

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OPEN ACCESS

ARTICLE HISTORY
Received: 28 August 2024
Accepted: 29 September 2024
Available online
Version 1.0 : 14 October 2024
Version 2.0 : 17 October 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Ragul S, Vanitha S, Harish S, Johnson I, Irene Vethamoni P, Senthil A. Exploring the antifungal activity of clove oil (*Syzygium aromaticum*) against wilt disease caused by Fusarium equiseti in bitter gourd (*Momordica charantia* L). Plant Science Today. 2024; 11(4): 580-590. https://doi.org/10.14719/pst.4837

Abstract

This study investigates the isolation and characterization of *Fusarium equiseti*, a fungal pathogen causing wilt in bitter gourd. It explores the antifungal activity of essential oils, particularly clove oil, as a natural management approach. The pathogen was isolated from wilt-infected bitter gourd stems and it was confirmed as *F. equiseti* morphologically by the presence of three-septate falcate macroconidia and globose microconidia and also confirmed molecularly by PCR amplification of the ITS region (PP501044.1). Among the tested essential oils, clove oil demonstrated the highest mycelial growth inhibition (100%) at a concentration of 0.5%, followed by peppermint, wintergreen and tea tree oils. In comparison, neem oil exhibited the least inhibition (15.54%). GC-MS analysis of clove oil revealed 38 compounds, where eugenol is the predominant compound that played a crucial role in antifungal activity. Additionally, metabolic analysis revealed several enriched pathways in *F. equiseti* during its interaction with clove oil, particularly those involved in lipid metabolism and energy production. Pot culture experiments confirmed the efficacy of clove oil in reducing disease severity and incidence in bitter gourd, achieving a 66.99 % reduction when combined with soil application.

Furthermore, clove oil-treated plants showed increased activity of defense-related enzymes, including peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), suggesting the induction of systemic resistance. This study highlights the potential of clove oil as an eco-friendly alternative to synthetic fungicides for managing *F. exquisite*-induced wilt in bitter gourd. Future research should focus on optimizing clove oil application methods and understanding its interactions with fungal pathogens at the molecular level to enhance its efficacy in sustainable disease management.

Keywords

F. equiseti; clove oil; KEGG; metaboanalyst; bitter gourd

Introduction

Bitter gourd (*Momordica charantia L.*), belonging to the Cucurbitaceae family, is widely grown in tropical and subtropical regions. This vegetable crop is primarily used in food and medicine and has many bioactive compounds like *Momordicin charantin* (1). Bitter gourd cultivation faces constraints due to significant diseases, including wilt, powdery mildew, leaf

spot and viral infections. Among the diseases, *Fusarium sp*. causes severe damage yield losses of up to 40% and reduces fruit market value (2). *F.equiseti is associated with bitter gourd and* causes severe leaf spots and wilt, with a 37% incidence in Pakistan (3) and wilt in tomatoes, leading to a 60% yield loss in Mexico (4). It also causes wilt in chickpeas, with a 45% disease incidence, significantly reducing vegetable yield (5).

Essential oils are effectively used to manage plant pathogens, with some exhibiting both fungicidal and bactericidal activity. *Eucalyptus camaldulensis Dehnh.* (river red gum) is effective in controlling *Fusarium* species (6). *Cymbopogon nardus (citronella oil)* is used to manage tomato fruit rot. Likewise, *Origanum vulgare (oregano* and *Thymus vulgaris (thyme)*, as well as *Rosmarinus officinalis*, are effective against TOLCNDV in cucurbits (7). *Thymus serpyllum* and *Origanum vulgare* subsp. *hirtum* essential oils are employed to control bacterial wilt in tomatoes. *Hyssopus officinalis* L. effectively reduces the severity of *Alternaria* species, which cause rot disease in carrots (8). Clove oil was extracted from leaves and air-dried buds of spice crops. *Syzygium aromaticum is primarily used in the perfumery and pharmaceutical industries (9). It is* rich in volatile and phenolic compounds, provides therapeutic effects against various ailments and has potent antifungal activity (10). Clove oil has demonstrated significant antifungal properties, effectively controlling agricultural diseases caused by fungi like *Fusarium oxysporum* and *Aspergillus niger* in tomato (11). Beyond its antifungal capabilities, clove oil has also inhibited bacterial pathogens (12).

This study focuses on isolating and characterizing *F. equiseti* from wilt-infected bitter gourd and assessing the antifungal activity of essential oils, with a particular emphasis on clove oil. The research aims to elucidate the interaction between clove oil and F. equiseti at the biochemical level by integrating molecular techniques and metabolomic profiling. Additionally, pot culture experiments were conducted to evaluate the practical application of clove oil in managing wilt disease in bitter gourd. The findings are intended to contribute to developing sustainable, plant-based disease management strategies that reduce dependence on synthetic chemicals and support eco-friendly agriculture.

Materials and Methods

Isolation of pathogen from wilt associated with bitter gourd

Symptomatic samples of wilt-infected bitter gourd stem were collected from Santhegoundapalyam village in Coimbatore district (lat N 10.72'', long E 77''.) The infected stem portion was excised using a sterilized scalpel up to 1cm long, washed with 1% sodium hypochlorite and washed with sterilized water and air-dried. The sterilized tissue was placed on potato dextrose agar (PDA) plates under laminar air flow chamber condition, and plates were incubated in controlled conditions at 25-28°C for seven days. After seven days, the morphological characteristics of the fungus were studied by observing the cultural and conidial characteristics. Conidial septation was captured using a compound microscope (Labomed camera model LX 400) with an image analyzer at 400x magnification (13).

Molecular characterization of *F. equiseti*

The CTAB method was employed to extract genomic DNA from *Fusarium equiseti* mycelium. Tissue was pulverized in liquid nitrogen and subjected to CTAB buffer extraction (2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 0.2% β-mercaptoethanol). After incubation at 65°C for 30 minutes, DNA was extracted using chloroform alcohol (24:1), precipitated with isopropanol, and cleaned with 70% ethanol before resuspension in TE buffer. The ITS region was then amplified with ITS1 and ITS4 primers through PCR, conducted over 35 cycles of denaturation (95°C), annealing (55°C) and extension (72°C). PCR products were analyzed by electrophoresis on a 1.5% agarose gel, stained and visualized under UV light. The purified PCR products were sequenced and identification was confirmed as Fusarium equiseti through NCBI BLAST analysis. A phylogenetic tree was generated by combining the obtained sequences with GenBank sequences and conducting multigene analysis in MEGA 11.0 (14). The maximum likelihood method was used with 1000 bootstrap iterations and bootstrap values above 50% were presented.

Antifungal activity assay

The antifungal activity of clove oil, peppermint oil, wintergreen oil, tea tree oil and neem oil against *F.equiseti* was evaluated using the poisoned food technique. Five concentrations of each essential oil (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were mixed with 2% Tween 80 and gently placed on a rotary shaker. The prepared essential oils (v/v) were incorporated into sterilized, cooled PDA media, while a PDA medium containing only 2% Tween 80 was used as the control. A completely randomized design with four replicates was adopted; mycelial growth and mycelial growth inhibition were measured after seven days and calculated using the formula (15) below,

$$MGI = \frac{(dc-dt)}{dc} \times 100$$

MGI= mycelial growth inhibition

dc - mean colony diameter of control

dt - mean diameter colony of treatment set

Metabolomics analysis of pathogen, clove oil and their interaction

Clove oil demonstrated the highest inhibition of *F. equiseti* mycelial growth in a poisoned food assay. PDB broth was inoculated with clove oil and *F. equiseti*, while control broths contained either clove oil or *F. equiseti* alone. A 1 ml sample was extracted using ethyl acetate and subjected to GC-MS analysis to determine the metabolites responsible for this inhibition. This analysis was performed with a Shimadzu GC system (QP 2020) featuring an Rxi-5 MS column and an Elite-1 column (100% Dimethyl Polysiloxane,

30 m x 0.25 mm ID) at the CPMB lab, TNAU, Coimbatore. Helium was the carrier gas at a 1 ml/min flow rate. The oven temperature was initially maintained at 110°C for 2 minutes and reached 280°C in 9 minutes, with the injector set to 250° C. The total run time was 45 minutes. The components were identified by comparing the mass spectra with the NIST library (Version 2.0, 2005), using Turbo mass -5.1 software (16).

Based on the results of GC-MS, the metabolite variation among *F. equiseti* alone and clove oil alone and clove oil amended *F. equiseti* was assessed using R software Heat map visualization. The GCMS result data file was prepared in txt format and data integrity and missing value imputation were checked and normalized. After normalization of data, further analysis in Metaboanalyst 6.0. MetEA (Metabolite Enrichment Analysis) was used to identify the enrichment of metabolites for these three different treatments (17).

Pot culture Experiment

The pot culture experiment was conducted under glasshouse conditions with six treatments and four replicates arranged in a completely randomized design. *F. equiseti* spores 1 × 10^8 spores/ml 2% (v/w) were artificially inoculated into the soil. Commonly cultivated bitter gourd variety (CO1) seedlings were grown in the TNAU orchard. After 15 days, the seedlings were dipped in an emulsified concentrate (2% Tween 80) with 0.5% clove oil 30 minutes before transplanting. After 35 days, 0.5% clove oil was applied, and carbendazim (0.1%) was used as a seedling dip and soil application. Inoculated and uninoculated plants were maintained, and all pots were kept at 25-30°C under a 12-hour light/dark cycle. Disease incidence and severity were recorded at 15-day intervals until crop harvest (18).

Treatment	Treatment detail
T1	Seedling dip with clove oil (0.5%)
T2	Seedling dip with carbendazim (0.1%)
T3	T1+ Soil application of clove oil (0.5%)
T4	T2+ Soil application of carbendazim (0.1%)
T5	Inoculated control
T6	Uninoculated control

$$\text{Percent disease severity} = \frac{\text{Number of infected leaves x grade obtained}}{\text{Total number of leaves x maximum grade}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{Number of Infected Plant}}{\text{Total number of plant observed}} \times 100$$

Disease scale (0-5)	Symptoms observed
0	Leaf discolouration
1	One leaf yellowing
2	More than one leaf yellowing
3	50 - 75% leaf wilting
4	More than 75% leaf wilting
5	Dead seedling

Sample preparation for enzyme analysis

Leaf material (0.5 g) was blended with 2 ml of a 0.1 M sodium phosphate buffer at a pH of 6.5 and maintained at four °C. After homogenization, the resulting mixture was centrifuged at 10,000 rpm for 2 minutes. The supernatant was devoid of solid debris and was collected and utilized as the enzyme source for estimating plant defence enzymes, including phenylalanine ammonia-lyase (PAL), peroxidase (PO) and polyphenol oxidase (PPO).

Peroxidase Activity (PO)

The PO activity was measured using a 100 µl aliquot of enzyme extract and mixed with 1.5 ml of 0.05 M pyrogallol and 0.5 ml of 1 % hydrogen peroxide. The absorbance at 420 nm was measured every 30 seconds for three minutes at room temperature (28 ± 2 °C). A heat-treated enzyme sample was used as the control. The enzyme activity was measured by estimating the change in absorbance of the resulting mixture per minute per gram of fresh tissue. Such measurements were made periodically 30, 45, 60 and 75 days after germination (19).

Polyphenol Oxidase Activity (PPO)

The PPO activity was evaluated using the reaction mix containing 100 µl of enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). The reaction was started by adding 200 µl of 0.01 M catechol. The activity change was monitored by measuring absorbance at 495 nm every 30 seconds for three minutes. The enzyme activity was measured as a change in absorbance per minute per gram of fresh tissue. PPO activity was also recorded 30, 45, 60 and 75 days after germination (20).

Phenylalanine Ammonia-Lyase Activity (PAL)

The PAL activity was assessed by mixing the 200 µl of enzyme extract with 1.3 ml of water and 0.5 ml of borate buffer. To begin the reaction, 1 ml of 12 mM L-phenylalanine was added and incubated at 32 ± 2 °C for an hour. The reaction was stopped by adding 0.5 ml of 2 N HCl, with a blank acting as a control. The enzyme activity was calculated as µmol of cinnamic acid per minute per gram of fresh tissue. The absorbance was measured at 290 nm. PAL activity was also measured 30, 45, 60 and 75 days after germination (21).

Statistical Analysis

The graphs and charts were generated using R software. A Completely Randomized Block Design (CRD) and the least significant difference were implemented for the *in vitro* studies. The data were statistically analyzed using WASP - Web Agri Stat Package V2.0.

Results and Discussion

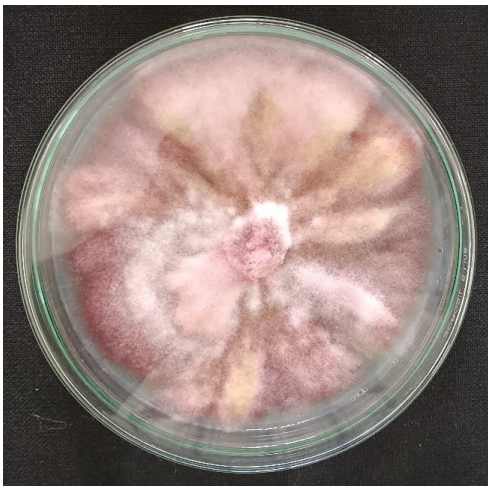
Isolation and characterization of *F. equiseti infecting bitter gourd*

The pathogen was isolated from wilt-infected bitter gourd stems collected from Santhegoundapalayam village, Coimbatore. The isolate showed a pink colour with orange, fluffy colonies. Microscopic analysis revealed oval-shaped microconidia and three septate, sickle-shaped macroconidia in the isolates. These observations are similar to *F. equiseti, causing wilt in chilli*, which exhibited white fluffy growth with an orange colour and 1-2 septate, hyaline macroconidia (14). The isolated pathogen was confirmed as *F. equiseti* through PCR amplification with ITS1 and ITS4 primers, producing a 560 bp band. The sequence was submitted to NCBI (PQ111513.1). Phylogenetic analysis showed that the isolate is closely related to *F. equiseti* isolated from leaves and roots in bitter gourd AR 4 (MW880179.1) and PAK 5 (MH054915.1) from Pakistan (3). Likewise, *F. equiseti* causing wilt in dragon fruit was confirmed using multigene phylogenetic construction of rDNA ITS region (22). This molecular confirmation is essential, as it provides a reliable method for identifying *Fusarium* species beyond morphological observations, which can sometimes be ambiguous due to the morphological plasticity of *Fusarium spp.* (23) (Fig 1,2,3).

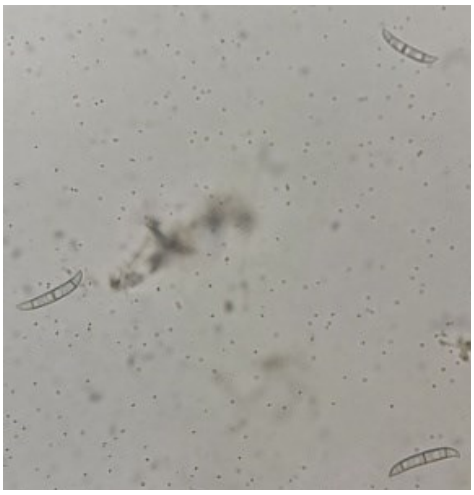
Antifungal activity of essential oils against *F. equiseti*

Among the tested essential oils, clove oil exhibited the highest mycelial growth inhibition (MGI) against *F. equiseti*, achieving up to 100% inhibition at 0.5% concentration, followed by peppermint oil (61.62 %), wintergreen oil (39.16 %) and tea tree oil (27.10 %). The least mycelial growth inhibition was observed with neem oil (15.54 %) against *F. equiseti.* This confirms the high antifungal potency of clove oil, consistent with earlier studies on its effectiveness against other plant pathogens. The antifungal activity reported in clove oil at 5000 ppm shows maximum mycelium growth inhibition against *F. equiseti* in cereals (24). In another study, the antifungal activity of *Piper auritum* oil at 5 mg/mL concentration mycelial inhibition (90.00 %) on the seventh day is also consistent with our findings (25) (Table 1) (Fig 4, 5).

Fig. 1. Collection of wilt infected bitter gourd samples from Santhegoundapalayam village in Coimbatore district



Colony character of *F.equiseti*



Conidial character of *F.equiseti*

Fig. 2. Morphological and microscopic characters of *F.equiseti*

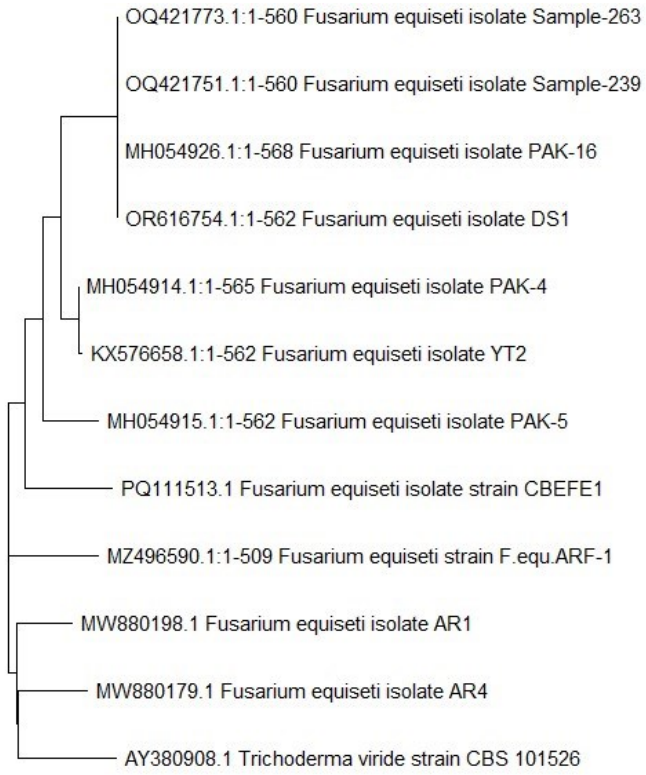


Fig. 3. Phylogenetic analysis of *Fusarium equiseti* based on showing the relationship of *F.equiseti* to another *Fusarium* spp. The phylogenetic tree was constructed based on internal transcribed spacer sequences using MEGA ver 11.

Fig. 4. *In vitro* efficacy of different essential oils (0.5%) against *F.equiseti*

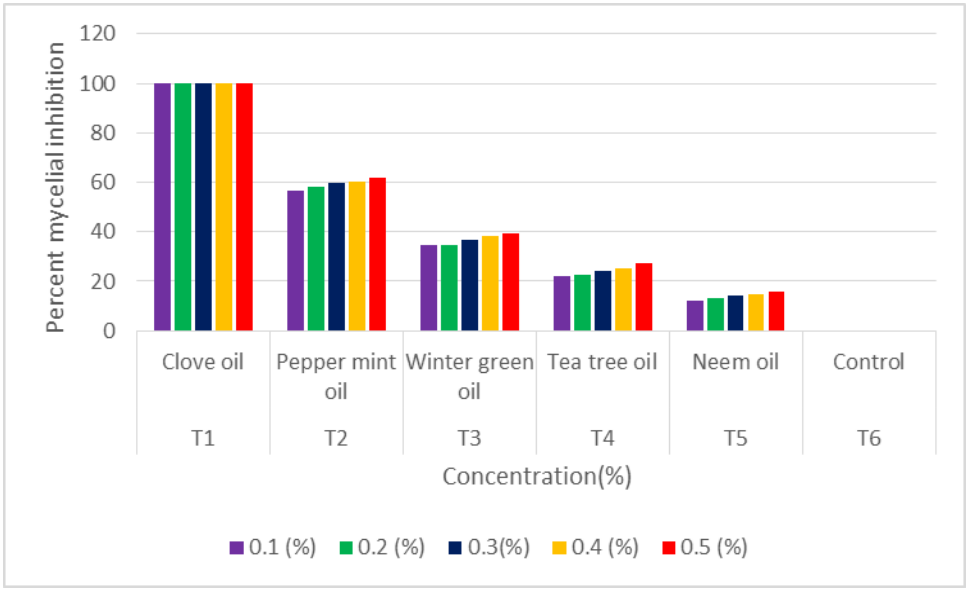


Fig. 5. Effect of different essential oils against the mycelial growth of *F.equiseti*. Each bar of the chart shows the mean diameter of the mycelial growth

Table 1: *In vitro* efficacy of different concentration of essential oils against *F. equiseti.*

S.no	Treatment detail	Mycelial growth (cm)					Percent mycelial growth inhibition (%)				
		0.1(%)	0.2 (%)	0.3(%)	0.4(%)	0.5(%)	0.1 (%)	0.2 (%)	0.3(%)	0.4 (%)	0.5 (%)
T1	Clove oil	0.00^{e} (0.00)	0.00^{e} (0.00)	0.00^{e} (0.00)	0.00^{e} (0.00)	0.00^{e} (0.00)	100.00^{a} (0.00)	100.00^{a} (0.00)	100.0^{a} (0.00)	100.00^{a} (0.00)	100.00^{a} (0.00)
T2	Peppermint oil	3.90^{d} (0.00)	3.76^{d} (0.04)	3.60^{d} (0.01)	3.56^{d} (0.16)	3.45^{d} (0.11)	56.67^{b} (0.893)	58.28^{b} (0.94)	59.97^{b} (2.62)	60.50^{b} (1.75)	61.62^{b} (0.63)
T3	Wintergreen oil	5.90^{c} (0.07)	5.87^{c} (0.06)	5.70^{c} (0.04)	5.58^{c} (0.08)	5.48^{c} (0.03)	34.44^{c} (0.979)	34.73^{c} (0.98)	36.62^{c} (1.54)	38.06^{c} (2.46)	39.16^{c} (0.23)
T4	Tea tree oil	7.00^{b} (0.10)	6.98^{b} (0.11)	6.84^{b} (0.07)	6.74^{b} (0.04)	6.56^{b} (0.00)	22.22^{d} (0.88)	22.50^{d} (0.74	23.96^{d} (2.5)	25.17^{d} (2.00)	27.10^{d} (0.59)
T5	Neem oil	7.90^{a} (0.10)	7.84^{a} (0.09)	7.72^{a} (0.06)	7.69^{a} (0.01)	7.60^{a} (0.02)	12.22^{e} (0.69)	12.94^{e} (0.57)	14.20^{e} (1.14)	14.61^{e} (1.35)	15.54^{e} (0.04)
T6	Control	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	CD (p = 0.05)	0.195	0.198	0.138	0.150	0.153	2.07	1.970	2.190	2.060	0.528

- Values of the mean of four replicates
- Values in parentheses are arcsine transformed values
- In a column, the mean followed by a common letter is not significantly different at the 5% level by least significant difference

Metabolomic analysis of *F.equiseti* and clove oil (Bipartite Interaction)

GC-MS profiling of clove oil alone identified eugenol (9.10%), humulene (5.39%), levomenol (0.98%), and eucalyptol (0.63%). Eugenol was the dominant metabolite and likely contributed to the significant inhibition of *F. equiseti*. Jiang *et al.* (26) reported that eugenol, a major component of clove oil, plays a crucial role in antifungal activity against *Fusarium* species. In addition, clove oil, containing 80.86% eugenol, has also been evaluated in pulse crops against significant pathogens and it was found to be effective against *Fusarium avenaceum* and *Didymella pisi* (27). To explore the pathogenic metabolites of *F. equiseti* associated with sporulation and infection, 23 compounds were identified, such as squalene (8.88%), cyclohexanol (7.37%), n-hexadecanoic acid (6.99%), 1-pentadecene (2.58%), propionic acid (2.38%), 1-nonadecene (1.92%) and fumaric acid (0.5%). A previous report on GCMS analysis of *F. equiseti* isolated from *Tridax procumbens L.* evaluated many secondary metabolic compounds. The primary chemical compounds identified were hexitol, pentadecanoic acid ethyl ester, 2, 3-dimethyl fumaric acid, 2(3H)-furanone, dihydro-4-hydroxy- and 2-propenamide, N-(1-cyclohexyl ethyl) (28). The interaction between *F. equiseti* and clove oil resulted in the upregulation of benzyl alcohol (49.70 %), eugenol (38.45%), hydrazine, 1, 1-dimethyl- (0.58%) and bis (2-ethylhexyl) phthalate (1.79%). This suggests that several fungal metabolites related to the suppression of pathogens were upregulated, indicating that the antifungal mechanism of clove oil likely targets the inhibition of fungal development. Similar studies shown that during the interaction of eugenol derivative compound 4 (2-(4-allyl-2-methoxyphenoxy)-3-chloronaphthalene-1,4-dione) against *Fusarium spp.,* reported that leakage of cell membrane, cell disintegration, spore germination and reduce growth of fungal hyphae and showed cytotoxicity (29) (Fig 6) (Tables S1, S2, S3).

Metabolite enrichment analysis was conducted to predict the pathways during the interaction between clove oil and *F. equiseti*. KEGG pathway analysis for clove oil indicated pathways involved in ascorbate and alternate metabolism, galactose metabolism and inositol phosphate metabolism in modulating defence. Jibrin *et al.* (30) reported that eugenol targets 12 metabolic pathways. Among these, pathways such as glutathione metabolism, aminoacyl-tRNA biosynthesis, pyrimidine metabolism, arginine and proline metabolism and glycine, serine and threonine metabolism were particularly effective in reducing bacterial wilt infection in tomato plants. Moreover, 10 ten distinct metabolic pathways were identified in *F. equiseti*, which helps in sesquiterpenoid and triterpenoid biosynthesis, arginine biosynthesis, the citrate cycle, alanine, aspartate and glutamate metabolism, pyruvate metabolism, fatty acid elongation, steroid biosynthesis, tyrosine metabolism, fatty acid degradation and biosynthesis which are critical for virulence and ability to survive in the host plant. In alignment with our findings, ten differentially accumulated metabolic pathways were detected in *F. equiseti,* the causal agent of root rot in soybeans. These pathways were found to play a role in the pathogen's infection process in soybean (31). The interaction between clove oil and *F. equiseti* was associated with pathways such as propanoate metabolism, unsaturated fatty acid biosynthesis, fatty acid elongation, degradation, biosynthesis and drug metabolism. These pathways suggest that essential oil may disturb lipid synthesis and energy production. This aligns with previous studies indicating that crucial oils interfere with key fungal metabolic pathways, which reduces their virulence and ability to cause disease. These findings provide evidence for the potential of essential oils as alternatives to chemical fungicides in integrated disease management strategies (32) Fig (7) (Table S4).

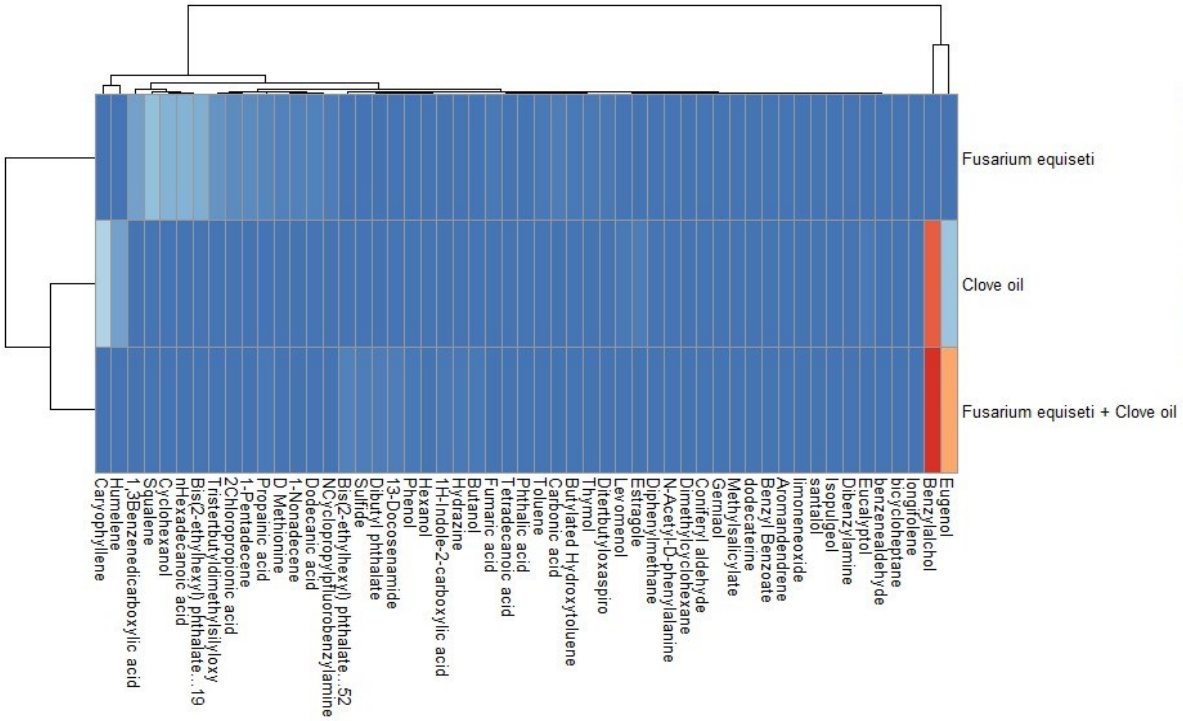


Fig. 6. Heatmap analysis - Bipartite interaction of *F.equiseti* and clove oil. A heat map was generated to measure metabolites associated with the interaction between clove oil and a pathogen. The ratio of precursors for each metabolite was calculated, and metabolites with a p-value < 0.01 were selected. In the heat map, red indicates a high ratio and blue indicates a low ratio. Benzyl alcohol and eugenol showed high ratios, while caryophyllene and humulene exhibited low ratios.

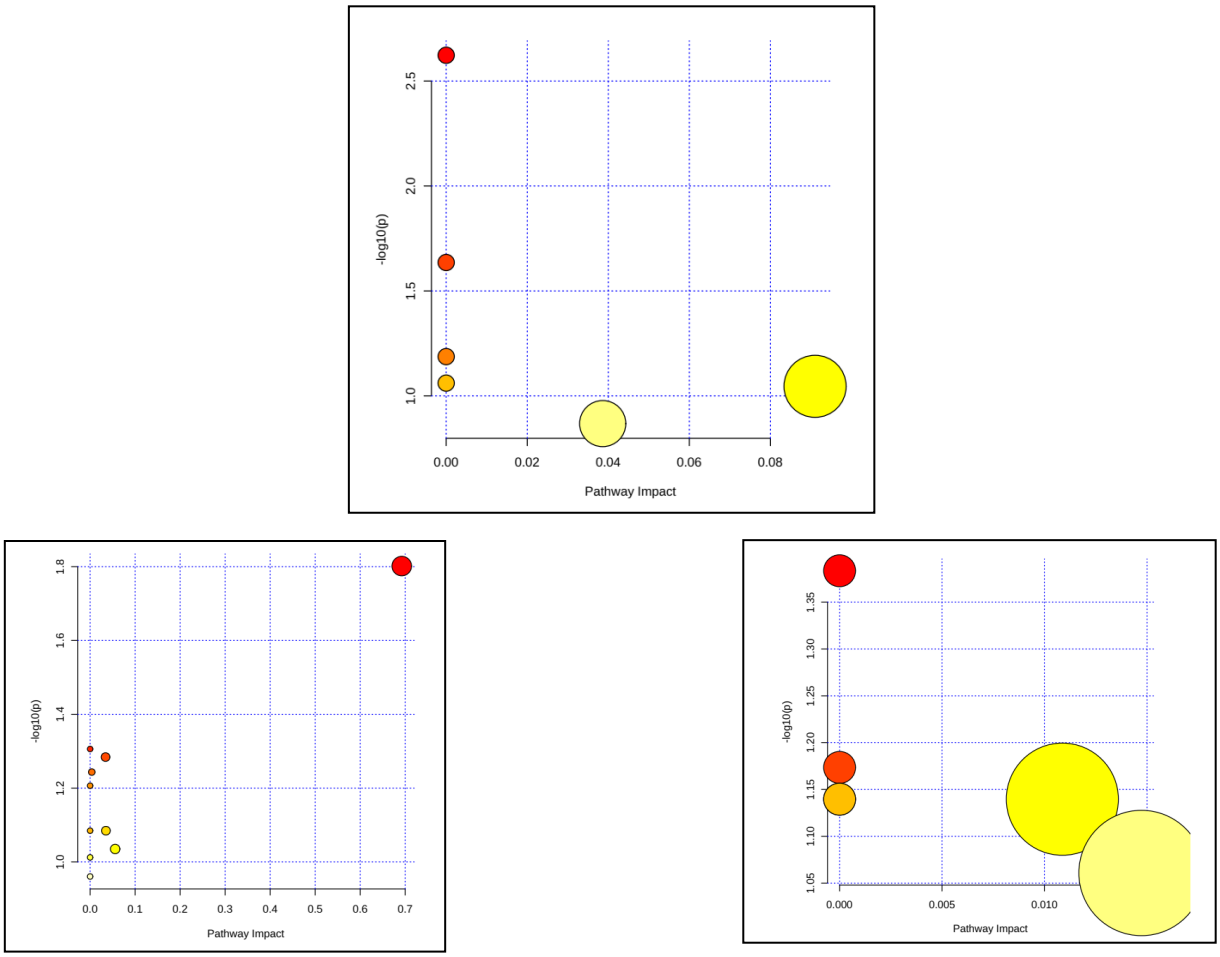


Fig 7: KEGG pathway enrichment analysis between pathogen and their interaction a. The clove oil enrichment pathway - the log p < 0.05 = red colour circle shows the highest peak (2.8%), i.e. ascorbate and aldarate metabolism pathway and white colour show lowest peak 0.1% i.e. inositol phosphate metabolism pathway. b. The *F.equseti* pathway were demonstrated. Log p < 0.05=red colour circle shows the highest ratio (1.8%), i.e. phenylalanine metabolism (1.4%), pyruvate metabolism and (1.2%), nitrogen metabolism and white colour (0.3%) shows tyrosine metabolism pathway in lowest ratio. c. The interaction result were shown- log p < 0.05= red colour circle showing propanate metabolism (1.65%) and the lowest ratio shows in white colour shows fatty acid elongation, degradation and biosynthesis. All matched pathways were plotted in circle colour and circle size based on p and pathway impact values.

Pot culture experiment

The pot culture experiment revealed significant disease severity and incidence reductions in bitter gourd plants treated with clove oil. The clove oil (0.5%) seedling dip (T1) showed percent of 54.45% disease reduction over control and when combined with soil application (T3), it further increased to 66.99%. This was comparable to the chemical fungicide carbendazim (0.1%) treatment (T2 and T4), which reduced disease over control levels of 72.21%. Similarly, Thabet *et al*., (33) investigated the impact application of clove oil at varying concentrations (0-4%) through seedling dip and soil application which has also shown to achieve disease reduction over control (72.00%) for *Fusarium* wilt of tomato in greenhouse conditions. These findings provide evidence for the potential of essential oils as alternatives to chemical fungicides in integrated disease management strategies Table (2) Fig (8, 9).

Fig 8: Efficacy of different treatments on *F.equiseti* wilt incidence under pot culture studies

Table 2: Evaluation of 0.5% clove oil against *F. equiseti* in bitter gourd under glasshouse conditions

Treatment number	Treatment detail	Percent Disease incidence (%)	Disease reduction over control (%)
T1	Seedling dip with clove oil (0.5%)	33.85[a] (0.13)	54.45[d] (0.53)
T2	Seedling dip with carbendazim (0.1%)	30.63[b] (0.28)	58.79[c] (0.93)
T3	T1 + Soil application of clove oil (0.5%)	24.53[c] (0.07)	66.99[b] (0.66)
T4	T2 + Soil application of carbendazim (0.1%)	20.65[d] (0.05)	72.21[a] (0.88)
T5	Inoculated control	98.15 (0.00)	98.15 (0.00)
T6	Uninoculated control	74.33 (0.05)	74.33 (0.10)
	CD P = (0.05)	0.39	1.87

- Values of the mean of four replicates
- Values in parentheses are arcsine transformed values
- In a column, the mean followed by a common letter is not significantly different at the 5% level by Duncan's multiple range test.

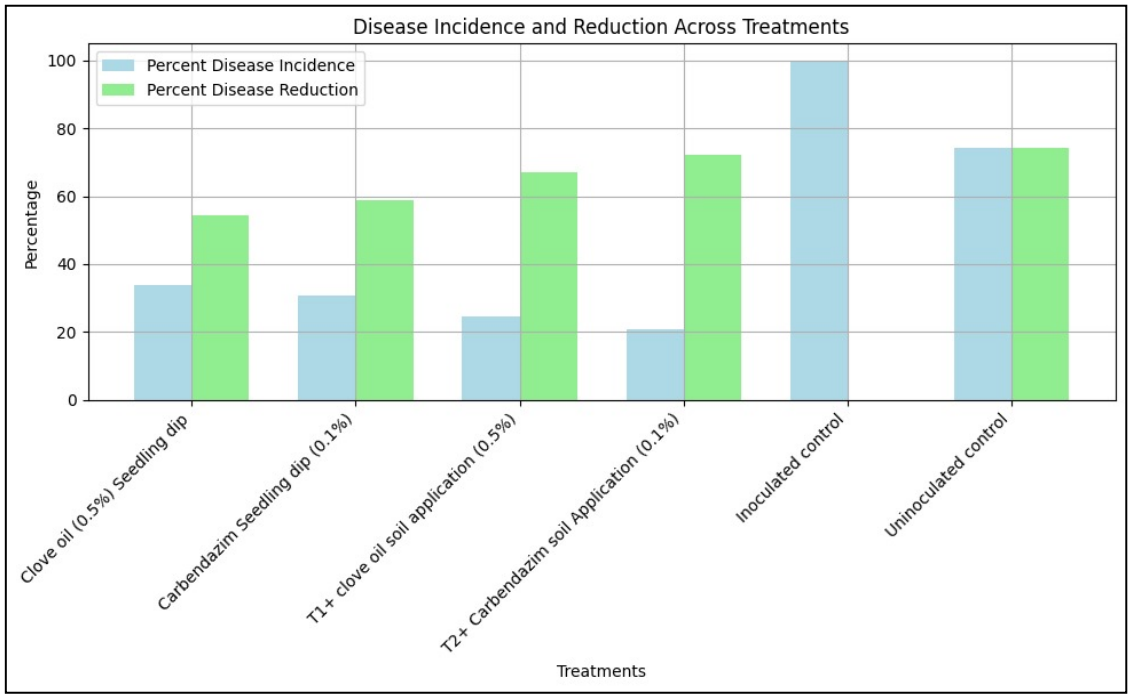


Fig. 9: Efficacy of different treatments on *F. equiseti* wilt incidence under pot culture studies.

Peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) activity

The clove oil-treated plants (T3) showed increased enzymatic activity compared to control plants. Peroxidase (PO) activity increased significantly at 3.75 (min /mg) in the 75th day after germination, correlating with reduced disease severity. This increase in PO activity suggests an induced systemic resistance (ISR) response triggered by clove oil, which enhances the plant's defense mechanisms. Similarly, polyphenol oxidase (PPO) activity also increased in clove oil-treated plants, with peak activity at 1.73 (min / mg) 75th days after germination. Higher PPO activity is associated with lignin deposition, which strengthens the cell walls of plant and limits pathogen invasion. Phenylalanine ammonia-lyase (PAL) activity was also elevated in clove oil treatments, especially at 2.47 (min/ mg) in 75th days after germination. PAL plays a crucial role in the phenylpropanoid pathway, leading to synthesizing compounds like phytoalexins and lignin, contributing to the plant's defense response. The increased PAL activity in clove oil-treated plants provides further evidence of ISR being activated as part of the plant's defense mechanism against *F.equiseti*. A similar plant growth promotion activity was observed with clove oil against *Alternaria* sp. in tomato plants. On the 7th day post-infection, PO, PPO, and CAT activities significantly increased to 4.495, 1.280, and 1.820 units/mL, respectively, for each application. These findings align with our results, where clove oil effectively promoted plant growth and reduced disease infection during pathogen attack (34) Fig (10).

Conclusion

This study concludes that *F. equiseti* is a major pathogen causing wilt disease in bitter gourd. In recent days essential oil is a novel implementation in eco-friendly crop protection techniques to reduce the use of synthetic chemicals. The present study revealed that eugenol, the predominant compound in clove oil, showed antifungal activity against F.equiseti and can be used as an ecofriendly product to manage wilt disease in bitter gourd. However, further research is needed to standardize that natural compound's dosage and application method. Understanding the interactions between essential oil metabolites and pathogens will help develop targeted and eco-friendly disease management strategies.

Acknowledgement

We want to express gratitude to the Centre for Plant Protection science and PG Student lab for funding this research project and my special thanks are extended to the Chairman, Head of the Department (Plant Pathology), Director (CPPS) and advisory members for their valuable feedback and constructive suggestions on the manuscript.

Authors' contributions

Dr. S. Vanitha experimented and analyzed the data. Dr. S. Harish guided the research, secured funding and approved the manuscript. I. Johnson contributed ideas and reviewed the manuscript. Dr. P. Irene Vethamoni imposed the experiment and assisted with editing and revising. Dr.

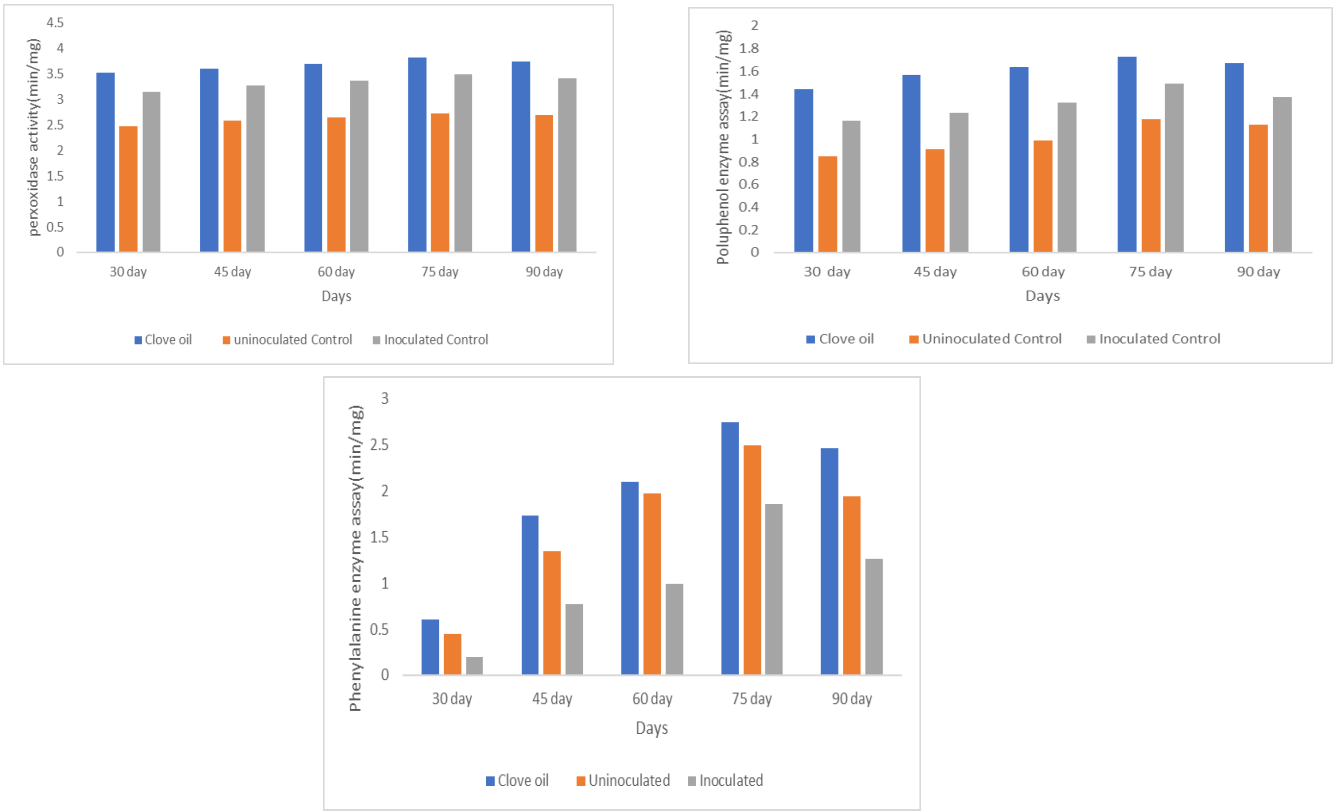


Fig 10: Estimation of plant defense-related enzymes of bitter gourd under clove oil treatment compared with inoculated and uninoculated controls pot culture study (a) peroxidase, (b) polyphenol oxidase, (c) phenylalanine lyase activity.

A. Senthil helped summarize and revise the manuscript. The research complies with ethical standards.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) not used AI tools and the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

1. Goo KS, Ashari S, Basuki N, Sugiharto AN. The bitter gourd *Momordica charantia* L.: morphological aspects, charantin and vitamin C contents. J Agri Vet Sci. 2016;9(10):76-81. https://doi.org:/10.9790/23800910017681
2. Lal D, Dev D, Kumari S, Pandey S, Aparna, Sharma N, et al. Fusarium wilt pandemic: current understanding and molecular perspectives. Funct Integr Genomics.2024;24(2):41.https://doi.org/10.1007/s10142-024-01319-w
3. Rehman AU, Rauf A, Ali A, Taimoor Shakeel M, et al. First report of Fusarium equiseti causing leaf spots of Bitter gourd (Momordica charantia) in Pakistan. Plant Disease. 2023; 107(2): 584. https://doi.org/10.1094/PDIS-04-22-0786-PDN
4. Beltran M, Delgado JC, Valdivia AG, Hernandez A, Garcia AM. First report of Fusarium equiseti causing root and crown rot in tomato in Mexico. Plant Disease.2023; 107(8):2542.https://doi.org/10.1094/PDIS-10-22-2494-PDN
5. Hazzat NE, Adnani M, Msairi S, Alaoui MA, et al. Fusarium equiseti as one of the main fusarium species causing wilt and root rot of chickpeas in Morocco. Acta Mycologica. 2023;58.
6. Gakuubi MM, Maina AW, Wagacha JM. Antifungal activity of essential oil of Eucalyptus camaldulensis Dehnh. against selected Fusarium spp. International Journal of Microbiology.2017;(1). https://doi.org/10.1155/2017/8761610.
7. Ziedan ES. Application of essential oils and their volatile components as green and eco-friendly bio-fumigation pesticides for controlling various plant diseases. African Mediterrean Agricultural Journal. 2024; (142):33-72 A review.
8. Chrapačienė S, Rasiukevičiūtė N, Valiuškaitė A. biocontrol of carrot disease-causing pathogens using essential oils. Plants (Basel). 2021; 10(11). https://doi.org/10.3390/plants10112231.
9. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, et al. *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 2020; 10(2):202. https://doi.org/10.3390/biom10020202 .
10. Pinto E, Vale SL, Cavaleiro C, Asalgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and dermatophyte species. Journal of Medical Microbiology. 2009; 58(11): 1454-462. https://doi.org/10.1099/jmm.0.010538-0 .
11. Muñoz Castellanos L, Amaya Olivas N, Ayala-Soto J, De La O Contreras CM, Zermeño Ortega M, et al. *In Vitro* and *In Vivo* antifungal activity of clove (*Eugenia caryophyllata*) and pepper (*Piper nigrum* L.) essential oils and functional extracts against *Fusarium oxysporum* and *Aspergillus niger* in tomato (*Solanum lycopersicum* L.). Int J Microbiol. 2020;10(1). https://doi.org/10.1155/2020/1702037 .
12. Nuñez L, Aquino MD. Microbicide activity of clove essential oil (Eugenia caryophyllata). Braz J Microbiol. 2012;43(4):1255-60. https://doi.org/10.1590/S1517-83822012000400003 .
13. Moparthi S, Perez-Hernandez O, Burrows ME, Bradshaw MJ, et al. Identification of *Fusarium* spp. Associated with chickpea root rot in montana. Agriculture. 2024; 14(7):974. https://doi.org/10.3390/agriculture14070974
14. Hami A, Rasool RS, Khan NA, Mansoor S, Mir MA, et al. Morpho-molecular identification and first report of Fusarium equiseti in causing chilli wilt from Kashmir (Northern Himalayas). Scientific Reports. 2021;11(1):3610. https://doi.org/10.1038/s41598-021-82854-5
15. Muñoz Castellanos L, Amaya Olivas N, Ayala-Soto J, De La O Contreras CM, et al. *In vitro* and *in vivo* antifungal activity of clove (Eugenia caryophyllata) and pepper (*Piper nigrum* L.) essential oils and functional extracts against *Fusarium oxysporum* and *Aspergillus nige*r in tomato (*Solanum lycopersicum* L.). International Journal of Microbiology. 2020; (1). https://doi.org/10.1155/2020/1702037
16. Hemalatha R, Nivetha P, Mohanapriya C, Sharmila G, et al. Phytochemical composition, GC-MS analysis, *in vitro* antioxidant and antibacterial potential of clove flower bud (Eugenia *caryophyllus*) methanolic extract. Journal of Food Science and Technology. 2016;53:1189-98. https://doi.org/10.1007/s13197-015-2108-5
17. Costa JP, Nasser VG, Macedo WR, Santos MF, Silva GH. The biostimulant potential of clove essential oil for treating soybean seeds. Agriculture. 2024;14(7):1202.https://doi.org/10.3390/agriculture14071202
18. Moutassem D, Belabid L, Bellik Y, Ziouche S, Baali F. Efficacy of essential oils of various aromatic plants in the biocontrol of Fusarium wilt and inducing systemic resistance in chickpea seedlings. Plant Protection Science. (2019);55(3);202-17. https://doi.org/10.17221/134/2018-PPS .
19. Hammerschmidt R, Nuckles E, Kuc J, Association of enhanced peroxidase activity with induced systemic resistance of cucumber *to Colletotrichum lagenarium*. Physiological Plant Pathology. 1982; 20(1): 73-82. https://doi.org/10.1016/0048-4059(82)90025-X.
20. Mayer A, Harel E, Ben R. Assay of catechol oxidase-a critical comparison ofmethods.Phytochemistry.1966;5(4):783-789. https://doi.org/10.1016/S0031-9422(00)83660-2.
21. Dickerson, D, Pascholati S, Hagerman AE, Butler R. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiological plant pathology. 1984; 25(2): 111-23. https://doi.org/10.1016/0048-4059(84)90050-X
22. Singh AR, Sharma SK, Dayal V, Dutta SK, et al. Fusarium equiseti as the pathogen causing stem rot disease of red-fleshed dragon fruit (*Hylocereus polyrhizus*) in India. Crop Protection. 2023;173:106380. https://doi.org/10.1094/PDIS-04-22-0786-PDN
23. O'Donnell K. Molecular phylogeny of the *Nectria haematococca-Fusarium solani species* complex. Mycologia.2000;92(5):919-38. https://doi.org/10.1080/00275514.2000.12061237
24. Grzanka M, Sobiech Ł, Danielewicz J, Horoszkiewicz-Janka J, et al. Impact of essential oils on the development of pathogens of the Fusarium genus and germination parameters of selected crops. Open Chemistry. 2021;19(1):884-93. https://doi.org/10.1515/chem-2021-0079
25. Chacón C, Bojórquez-Quintal E, Caamal-Chan G, Ruíz-Valdiviezo VM, et al. *In Vitro* Antifungal Activity and Chemical Composition of *Piper auritum* Kunth Essential Oil against *Fusarium oxysporum* and *Fusarium equiseti*. Agronomy. 2021;11(6):1098. https://doi.org/10.3390/agronomy11061098
26. Jiang H, Zhong S, Schwarz P, Chen B, Rao J. Chemical

composition of essential oils from leaf and bud of clove and their impact on the antifungal and mycotoxin inhibitory activities of clove oil-in-water nanoemulsions. Industrial Crops and Products. 2022; 187. https://doi.org/10.1016/j.indcrop.2022.115479

27. Parikh L, Agindotan BO, Burrows ME. Antifungal activity of plant-derived essential oils on pathogens of pulse crops. Plant Disease. 2021;105(6):1692-701. https://doi.org/10.1094/PDIS-06-20-1401-RE

28. Laosinwattana C, Manichart N, Thongbang M, Wichittrakarn P, et al. The effect of natural herbicide from Fusarium equiseti crude extract on the aquatic weed water hyacinth (Eichornia crassipes (Mart.) Solms). Scientific Reports.2024;14(1):19542. https://doi.org/10.1038/s41598-024-70694-y

29. Maximino SC, Dutra JAP, Rodrigues RP, Gonçalves RCR, et al. Synthesis of eugenol derivatives and evaluation of their antifungal activity against *Fusarium solani* f. sp. *piperis*. Current Pharmaceutical design. (2020);26(14),1532-542. https://doi.org/10.2174/1381612826666200403120448

30. Jibrin MO, Liu Q, Garrett TJ, Jones JB, Zhang S. Potential and metabolic pathways of eugenol in the management of *Xanthomonas perforans*, a pathogen of bacterial spot of tomato. Int J Mol Sci. 2022;23(23). https://doi.org/10.3390/ijms232314648 .

31. Wang S, Zhang X, Zhang Z, Chen Y, et al. Fusarium-produced vitamin B6 promotes the evasion of soybean resistance by Phytophthora sojae. Journal of Integrative Plant Biology. 2023;65(9):2204-17. https://doi.org/10.1111/jipb.13505

32. El-Nagar A, Elzaawely AA, El-Zahaby HM, Xuan TDet al. Benzimidazole derivatives suppress fusarium wilt disease via interaction with *ERG6* of *Fusarium equiseti* and activation of the antioxidant defense system of pepper plants. Journal of Fungi. 2023; 9(2):244. https://doi.org/10.3390/jof9020244

33. Thabet M, Khalifa W. Antifungal activities of clove oil against root rot and wilt pathogens of tomato plants. Am. Eurasian J Agric Environ Sci. 2018;18:105-14.

34. Ali AA, Saleh RA, Khalifa NA. Effect of biocides and essential oil nanoemulsions against tomato early blight disease under greenhouse and field conditions. New Valley Journal of Agricultural Science. 2023;3(10). https://doi.org/10.21608/nvjas.2023.242291.1257