***Original Research Article***

***In vitro* evaluation of essential oils against *Fusarium oxysporum* f.sp*. lycopersici* causing wilt disease of tomato**

ABSTRACT

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| --- |
| **Aims:** An *in vitro* experiment was conducted to evaluate the bioefficacy of ~~some~~ commercially available essential oils against the pathogen responsible for causing wilt disease in tomatoes.**Study design:** The experimental ~~design used~~ was laid out in Completely Randomized ~~Block~~ Design (CRD). ~~(CRBD).~~**Place and Duration of Study:** The experiment was conducted in the Department of Plant Pathology, Biswanath College of Agriculture, Assam Agricultural University, Biswanath Chariali, during the R~~r~~abi season of 2023-24.**Methodology:** There were six treatments with five replications. Five essential oils, viz. *~~viz~~*~~.,~~ clove, cinnamon, neem, sesame, and peppermint were evaluated against the pathogen at 0.1%, 0.5%, 1.0%, and 1.5% concentrations by following Poisoned Food Technique ~~Poison Food Technique~~ to find out the minimum inhibitory concentration (MIC) under *in vitro* conditions.**Results:** Amongst all the essential oils, clove, and cinnamon oil were found to be the most effective in inhibiting the maximum radial mycelial growth (100%) of the pathogen at all the concentrations, whereas or with ~~however~~, 0.1 % being the MIC for both ~~the~~ essential oils, showing maximum inhibition (100.00%) of the pathogen.**Conclusion:** The antimicrobial properties of clove and cinnamon essential oils can be exploited for the management of fungal wilt disease of tomatoes in the field, which will be an eco-friendly ~~ecofriendly~~ and sustainable alternative to chemical control. |

*Keywords: Tomato; wilt; essential oils; MIC; radial mycelial growth*

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most important and popular vegetable crops cultivated in the world (Kumar et al. 2018). It is classified as a vegetable from a nutritional perspective ~~for nutritional aspects~~ and is a good source of vitamin C and phytochemical compound lycopene. Tomato is treated as “protective food” universally and provides almost all the vitamins and minerals in quite a significant ~~fair~~ amount. India is the second largest producer of tomatoes, accounting for 10.51% of total production after China, followed by Turkey and the United States. According to FAOSTAT 2021, Tomatoes ranked as the most-produced vegetable with a production of 189 million tonnes from 5,167,388 hectares in 2021, with an average yield of 37.1 metric tonnes per hectare (Anon 2021b). In India, it occupies an area of about 854 thousand ha producing over 21,181,000 tonnes in 2021 and an estimated 20.34 million metric tonnes in 2022 with a productivity of 25.0 MT per hectare (Anon 2022a). Madhya Pradesh is the leading producer of tomatoes in India followed by Andhra Pradesh and Karnataka (Anon 2022b). In Assam, tomato is cultivated over 18.28 thousand ha with a total production of 396.24 thousand MT (Anon 2021a). India is the third largest exporter of tomatoes, following Italy and Turkey. It is an important crop having significant economic value, however; there are many constraints in its production leading to severe yield losses ~~causing severe yield loss~~. Amongst other factors, fungal infections caused by *Fusarium oxysporum* contribute to 25-55 per cent of crop losses all around the world (Devi et al. 2016). Fusarium wilt of tomato is one of the most destructive diseases of tomatoes caused by *FOL.*  This disease can result in yield loss up to 80 per cent when it occurs in a severe form.

Fungicides are generally used to control this disease; however, frequent and indiscriminate use of these chemicals may lead to environmental pollution and the development of more virulent strains. ~~development of new strains with more virulence,~~ ~~hence~~ Hence, the use of biocontrol and plant-based essential oils has been advocated as one of the promising alternative strategy to overcome these problems (Calo et al. 2015; Chouhan et al. 2017). In view of the above, the present study was undertaken to evaluate the effect of essential oils against *Fusarium oxysporum* f.sp. *lycopersici in vitro.*

2. material and methods

The present experiment was carried out during 2023-2024 in the Department of Plant Pathology, Biswanath College of Agriculture, Assam Agricultural University, Biswanath Chariali. Assam, India

**Isolation and purification of the pathogen:** The disease specimens were collected from the Horticultural orchard, Biswanath College of Agriculture, Biswanath Chariali showing typical symptoms of fungal wilt on the tomato plant. The samples were brought to the laboratory for detailed ~~critical~~ observation and investigation, such as symptoms, isolation, and description of the pathogen for further studies. The pathogen was isolated and purified using a tissue isolation technique (Ricker and Ricker, 1936).Throughout the investigation, the pure culture of the pathogen was maintained on Potato Dextrose Agar (PDA) slants by routine sub-culturing at regular intervals and preserving at 4ºC in a refrigerator. The stored culture was used after restoring it to an active state by keeping it at room temperature for all subsequent studies. The pathogenicity of the fungus responsible for ~~pathogen~~ ~~causing fungal~~ wilt in ~~of~~ tomato was confirmed by Koch’ s postulates.

**Identification of the isolated pathogen:** The isolated pathogen was identified based on the cultural and morphological characteristics. The pathogen was identified with the available relevant literature, ~~a~~ keys and monograph (Tamura 1952), and a CMI description. Identification of *Fusarium oxysporum* f.sp*. lycopersici* was done based on the spore morphology and colony characteristics, ~~characters~~ ~~of the fungus by~~ referring to the “Illustrated genera of Imperfect fungi” (Barnett and Hunter 1972).

**Table 1. List of essential oils used**

|  |  |  |
| --- | --- | --- |
| **Sl No.** | **Essential oils** | **Scientific name** |
| 1 | Clove | *Syzgium aromaticum* |
| 2 | Cinnamon | *Cinnamomum zeylanicum* |
| 3 | Neem | *Azardichta indica* |
| 4 | Sesame | *Sesamum indicum* |
| 5 | Peppermint | *Mentha piperata* |

**Evaluation of essential oils against the pathogen (*FOL):*** In the present study, the Poisoned Food Technique described by Nene and Thapliyal (2000) was used to evaluate the bioefficacy of EOs *viz.*, clove, cinnamon, sesame, neem, and peppermint, against the pathogen. The required quantity of essential oils (0.1%. 0.5%. 1.0% and 1.5%) was poured into conical flasks containing 100ml of PDA media. PDA without any essential oils served as the control. ~~PDA without any oils served as the control plates.~~ The treated PDA was poured into 9 cm petri plates at the rate of 20 ml per plate. After solidification, a 5 mm diameter fungal disc obtained from a 7 day old culture was taken using a cork borer and inoculated in the center of the Petri plate under aseptic conditions and incubated at 25±1℃ for 7 to 10 days.The experiment was conducted in Completely Randomized ~~Block~~ Design (CRD) with five replications.

The diameter of the pathogen colony was assessed upon complete coverage of the Petri plates by the mycelium in the control plates. The percent inhibition of the mycelial growth was calculated by following the formula given by Vincent (1927).

I= (C-T/C) 100

Where, I = Inhibition of mycelial growth (%)

C = Growth in control (mm)

T = Growth in treatment (mm)

The best effective concentrations of EOs were screened out by calculating the average radial mycelial growth and by comparing the percent inhibition of radial mycelial growth of the fungus over control.

**Statistical analysis:** The experimental data collected were analyzed statistically for significance differences by the normal statistical procedure adopted for completely randomized ~~block~~ design and interpretation of data carried out in accordance with Gomez and Gomez, 1984.The observed data was analyzed by OPSTAT package of programs (Sheoran 2006) after angular transformation. The treatment means were compared using ~~by~~ Duncan’s Multiple Range Test (DMRT).

3. results and discussion

**Identification of the isolated pathogen**

The morphological characteristics of associated fungal isolates were studied for identification of the pathogen.

**Morphological identification of the pathogen:** In culture, the colony of the fungus appears white and cottony in texture, gradually turning **pink** in colour, ~~and then it gradually turns pink in colour,~~ and at ~~in~~ later stages, the reverse side ~~view~~ of the colony becomes ~~culture turns~~ reddish ~~in colour~~. Conidial masses were observed in the cottony portion of the culture, which were oval to kidney-shaped, tapering, and septate with ~~in~~ three cells. The growth rate of the pathogen was slow to moderate.

Conidia are hyaline, oval to kidney shaped, measuring 15.00 to 20.00 µm in length and 4-7 µm in width. Microconidia, which are oval to kidney-shaped, form false heads on short monophialides. ~~Oval to kidney-shaped microconidia have false heads on short monophialides,~~ ~~and~~ ~~m~~Macroconidia are sickle-shaped, thin-walled, and delicate. Chlamydospores form~~ed~~ in chains. The hyphae resemble those of aspergillus, with septate hyaline hyphae that are 3-8 microns in diameter and typically branch at acute angles.

The fungal isolate was identified as *Fusarium oxysporum* f.sp. *lycopersici* after comparing with standard literature and by referring to the “Illustrated genera of Imperfect fungi” (Barnett and Hunter 1972).

**Performance of pathogenicity test:** The pathogenicity of the pathogen causing fungal wilt of tomato was confirmed using ~~by~~ Koch’s postulates. Garden soil was ~~first~~ sterilized in an autoclave at 15 lbs pressure (1210C) for two successive days. Earthen pots were filled up with five kilograms ~~kgs~~ of sterilized soil and inoculated by mixing the freshly prepared Fusarium inoculums (multiplied on sand maize medium) @ 50g/kg soil (Muthusamy 1972). Tomato seedlings were planted in each pot and maintained properly by regular watering and constantly observed for the development of symptoms. The tomato plants exhibited typical fungal wilt symptoms, and the fungus was again isolated from the infected plant, purified, and maintained in PDA slants. The re-isolated fungus exhibited similar cultural characteristics to the original isolate, confirming its pathogenicity. ~~The fungus reproduced a similar kind of cultural characteristics.~~

**Effect of essential oils against *Fusarium oxysporum* f. sp. *lycopersici:*** The result presented in ~~the~~ Table 2 revealed that all the essential oils showed inhibitory activity against *F. oxysporum* f*.*sp. *lycopersici in vitro* at 0.1 per cent concentration. Amongst all, clove and cinnamon recorded the highest inhibition (100%) of radial mycelial growth of the pathogen over control, followed by peppermint (48.41%) and neem (3.75%) ~~with 48.41% and 3.75 %~~ inhibition. ~~of radial mycelial growth, respectively~~. The sesame oil recorded the lowest inhibition (1.19 %) against the pathogen *in vitro* (Fig. 1).

At 0.5% concentration (*Table 3*), the essential oils significantly inhibited the radial mycelial growth of *F. oxysporum* f. sp. *lycopersici*. ~~The essential oils significantly inhibited the radial mycelial growth of~~ *~~Fusarium oxysporum~~* ~~f.sp~~*~~. lycopersici~~* ~~at 0.5% concentration (Table 3).~~ Clove and cinnamon (colony diameter of 0 mm) were ~~found~~ the most effective, showing 100% inhibition of radial mycelial growth over the control. ~~against~~ *~~F. oxysporum~~* ~~f.sp~~*~~. lycopersici~~* ~~with the highest inhibition (100 %) of radial mycelial growth of the pathogen over control,~~ These were followed by peppermint (Colony diameter of 43.36 mm; inhibition 51.28%) and neem (Colony diameter of 83.66 mm; inhibition 6.00%) ~~with 51.28%, and 6.00 % inhibition of radial mycelial growth of~~ *~~F. oxysporum~~*~~f.sp~~*~~. lycopersici~~* ~~over control respectively~~ (Fig. 2).

Data presented in Table 4 showed that at 1.0% concentration, all the essential oils significantly inhibited the radial growth of *F. oxysporum* f.sp*. lycopersici*. ~~the~~ The highest inhibition was recorded in ~~being the~~ clove and cinnamon (colony diameter: 0 mm, inhibition: 100%) ~~(Colony diameter 0 mm), with 100 % inhibition~~ followed by peppermint (colony diameter: 39.17 mm, inhibition: 55.98%) ~~Colony diameter of 39.17 mm)~~, and neem (colony diameter: 79.66 mm, inhibition: 10.49%) (Fig. 3). ~~(Colony diameter of 79.66 mm) with 55.98 % and 10.49% inhibition of radial mycelial growth of the fungus over control respectively (Fig. 3).~~

~~In respect to~~ Regarding percent inhibition at 1.5% concentration, the data presented in Table 5 revealed that clove and cinnamon oils had ~~showed~~ the maximum inhibitory effect against *F. oxysporum* f.sp*. lycopersici* as compared to other oils. Clove and cinnamon oils recorded the highest 100% inhibition ~~(100%)~~ of radial mycelial growth of *FOL* over the control. This was followed by peppermint and neem with 61.04 % and 14.82 % inhibition, respectively (Fig. 4).

**Table 2. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 0.1 % concentration**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Radial Mycelial growth****(mm)** | **Inhibition of mycelial growth****(%)** |
| T1: Clove | 0.00e | 100.00 |
| T2: Cinnamon | 0.00e | 100.00 |
| T3: Sesame | 87.94b | 1.19 |
| T4: Neem | 85.66c | 3.75 |
| T5: Peppermint | 45.91d | 48.41 |
| T6: Control | 89.00a | 0.00 |
| SEd(±) | 0.37 |
| CD (P=0.05) | 0.78 |

*Data are the mean of five replications*

**Fig 1. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp.*lycopersici* at 0.1 % concentration**

**Table 3. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 0.5 % concentration**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Radial Mycelial growth****(mm)** | **Inhibition of mycelial growth****(%)** |
| T1: Clove  | 0.00e | 100.00 |
| T2: Cinnamon  | 0.00e | 100.00 |
| T3: Sesame  | 86.94b | 2.31 |
| T4: Neem  | 83.66c | 6.00 |
| T5: Peppermint  | 43.36d | 51.28 |
| T6: Control | 89.00a | 0.00 |
| SEd(±) | 0.35 |
| CD (P=0.05) | 0.74 |

*Data are the mean of five replications*

**Fig 2. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 0.5 % concentration**

**Table 4. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 1% concentration**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Radial Mycelial growth****(mm)** | **Inhibition of mycelial growth****(%)** |
| T1: Clove  | 0.00e | 100.00 |
| T2: Cinnamon  | 0.00e | 100.00 |
| T3: Sesame  | 85.90b | 3.48 |
| T4: Neem  | 79.66c | 10.49 |
| T5: Peppermint  | 39.17d | 55.98 |
| T6: Control | 89.00a | 0.00 |
| SEd(±) | 0.47 |
| CD (P=0.05) | 0.99 |

*Data are the mean of five replications*

**Fig 3. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 1% concentration**

**Table 5. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum*f.sp*. lycopersici*at 1.5% concentration**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Radial Mycelial growth****(mm)** | **Inhibition of mycelial growth****(%)** |
| T1: Clove  | 0.00e | 100.00 |
| T2: Cinnamon  | 0.00e | 100.00 |
| T3: Sesame  | 83.77b | 5.87 |
| T4: Neem  | 75.81c | 14.82 |
| T5: Peppermint  | 34.67d | 61.04 |
| T6: Control | 89.00a | 0.00 |
| SEd(±) | 0.35 |
| CD (P=0.05) | 0.73 |

*Data are the mean of five replications*

**Fig 4. effect of Essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at 1.5% concentration**

The data presented in Table 6 showed that all the essential oils significantly influenced the radial mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* at all the concentrations (0.1%, 0.5 %, 1.0%, and 1.5%). Irrespective of the concentrations, all the essential oils significantly reduced the radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici in vitro*. Out of the five essential oils evaluated, clove and cinnamon recorded the lowest mean radial growth (0 mm) at all the concentrations (0.1%, 0.5%, 1.0%, 1.5%) followed by peppermint and neem with mean radial growth of 40.77mm and 81.19 mm, respectively (Fig 5).

With respect to the percent inhibition of mycelium, the data presented in Table 7 showed that oils of clove and cinnamon were found to be most effective resulting highest mean inhibition (0%) of radial mycelial growth of *FOL* over control in all four concentrations (0.1%, 0.5 %, 1.0%, 1.5%) which was followed by peppermint and neem with a mean radial inhibition of mycelial growth of 54.17 % and 8.76 % respectively over control. However, the effect of aqueous extract increased with the increase of concentration which was reflected at 1.5% concentrations showing the highest inhibition (100%) of mycelial growth of *FOL* exhibited by oils of clove and cinnamon followed by peppermint and neem with 61.04 % and 14.82 % inhibition, respectively, over control (Fig 6).

**Table 6. Effect of essential oils on radial mycelial growth of *Fusarium oxysporum* f.sp*. lycopersici* at different concentrations (0.1, 0.5, 1.0 and 1.5 %) *in vitro***

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Radial mycelial growth (mm)** | **Mean** |
|  | **Concentrations (%)** |  |
|  | **0.1** | **0.5** | **1.0** | **1.5** |  |
| T1: Clove | 0 | 0 | 0 | 0 | 0.00 |
| T2:Cinnamon | 0 | 0 | 0 | 0 | 0.00 |
| T3:Sesame | 87.94 | 86.94 | 85.90 | 83.77 | 86.14 |
| T4: Neem  | 85.66 | 83.66 | 79.66 | 75.81 | 81.19 |
| T5: Peppermint  | 45.91 | 43.36 | 39.17 | 34.67 | 40.77 |
|  | **Essential oil** **(EO)** | **Concentration** **(C)**  | **Interaction****(EO×C)** |
| **SEd(±)** | 0.19 | 0.17 | 0.39 |
| **CD( p=0.05)** | 0.39 | 0.35 | 0.78 |
| **CV(%)** | 1.48 |

**Fig 5. Effect of essential oils on radial mycelial growth of *FOL* at different concentrations (0.1, 0.5, 1.0 and 1.5 %) *in vitro***

**Table 7. Effect of essential oils on percent inhibition of mycelial growth of *Fusarium oxysporum*f.sp*. lycopersici*at different concentrations (0.1, 0.5, 1.0 and 1.5 %)**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Inhibition of mycelial growth over control (%)** | **Mean** |
| **Concentrations (%)** |
| **0.1** | **0.5** | **1.0** | **1.5** |
| T1: Clove | 100(89.96) | 100(89.96) | 100(89.96) | 100(89.96) | 100.00(89.96) |
| T2:Cinnamon | 100(89.96) | 100(89.96) | 100(89.96) | 100(89.96) | 100.00(89.96) |
| T3:Sesame | 1.19(6.26) | 2.31(8.74) | 3.48(10.75) | 5.87(14.02) | 3.21(10.32) |
| T4: Neem  | 3.75(11.16) | 6.00(14.17) | 10.49(18.89) | 14.82(22.64) | 8.76(17.21) |
| T5: Peppermint  | 48.41(40.08) | 51.28(45.73) | 55.98(48.43) | 61.04(51.37) | 54.17(47.39) |
|  | **Essential oil (EO)** | **Concentration (C)**  | **Interaction (EO×C)** |
| **SEd(±)** | 0.22 | 0.2 | 0.44 |
| **CD( p=0.05)** | 0.44 | 0.4 | 0.89 |
| **CV(%)** | 1.37 |

*Data within the parenthesis are arcsine transformed values*

**Fig 6. Effect of essential oils on percent inhibition of mycelial growth of *FOL* at different concentrations (0.1, 0.5, 1.0 and 1.5 %) *in vitro***

**Discussion:**The antifungal effect of five essential oils was carried out *in vitro*at four concentrations (0.1%, 0.5%, 1.0%, and 1.5%) against *Fusarium oxyxporum* f.sp.*lycopersici* causing wilt of tomatoes by following the Poison Food Technique (Nene and Thapliyal 2000).

Essential oils exhibited varied levels of radial mycelial growth and inhibition of radial mycelial growth at all four concentrations. The inhibitory effect of oils showed dose-dependent activity on the test pathogen. Clove and cinnamon oil-treated plates recorded the least mycelial growth (0.00 mm) of *FOL,* whereas sesame oil recorded the highest mean radial mycelial growth (86.14 mm**)** at all concentrations. Maximum inhibition (100.00%) of radial mycelial growth of *FOL* over control was obtained in the clove and cinnamon oil plates irrespective of all concentrations evaluated, whereas, sesame oil recorded the lowest mean inhibition of radial mycelial growth (3.21%) of *FOL* in all concentrations (Table 6 and 7).

The maximum inhibition of radial mycelial growth of *FOL* obtained in the clove oil-treated plate in all the concentrations may be due to the presence of a high concentration of eugenal, an antiseptic phenolic compound. Clove oil is reported to change phytopathogenic hyphae (Baratta et al. 1998). The highest inhibition of radial mycelial growth of *FOL* found in the cinnamon oil treated plate may be due to the presence of antimicrobial compounds such as cinamaldehyde, linalool, and eugenol (Ranasinghe et al. 2002).

As a potent antifungal agent, clove oil could be used as biofungicide for the control of *FOL* in both preventive and therapeutic manners (Sharma et al*.* 2017). The results of the present investigation are in agreement with the findings of Rodrigues *et al.* 2018, Unnithan et al. 2016, Jagana et al. 2018, and Idris et al. 2015.

4. Conclusion

The results of the *in vitro* experiment revealed that clove and cinnamon oil were the most effective against *Fusarium oxysporum* f.sp. *lycopersici* by recording the least mycelial growth (0.00 mm) and exhibiting complete inhibition (100 %) of radial mycelial growth at all the concentrations, however,0.1 % was identified as the MIC ~~being the MIC~~ for both the oils showing maximum inhibition (100 %). The antimicrobial properties of clove and cinnamon essential oils can be exploited for the management of the fungal wilt disease of tomatoes in the field, which will be an ecofriendly and sustainable alternative to chemical control.

Consent (whereever applicable)

Consent from all the authors were taken before submitting this manuscript.

Ethical approval (whereever applicable)

This manuscript is ethically approved by all the authors.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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