



# Evaluation of Aromatic Oils as Natural Antifungal Agents Against Plant Pathogens: An *In vitro* and Pot Condition Study

R.S. Mishra <sup>a\*</sup> and Vipul Kumar Pandey <sup>a</sup>

<sup>a</sup> Department of Medicinal and Aromatic Plant, College of Horticulture and Forestry, A.N.D. University of Agriculture & Technology, Kumarganj, Ayodhya-224229, India.

## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

The essential oil of aromatic plants is often possessing antimicrobial features which have an important role in the defense of the plants. Aromatic oil have consist monoterpenes, sesquiterpenes and their oxygenated derivatives, such as alcohol, ketones, acids, phenols, ethers, esters, etc. Different aromatic plants oil viz; Eucalyptus, Basil, lemon grass, Palma Rosa, clove, Citronella, Orange, Neem and, Mint Oil, have been evaluated because it is safe alternative to chemicals and have a limitless ability to synthesize secondary metabolites (eg. phenols, flavones, flavonoids, alkaloids, tannins) which operate as the plant's defense mechanism against pathogenic microorganisms. These oils have the ability to delay or inhibit the growth of pathogenic fungi including *Pythium spp.*, *Rhizoctonia spp.*, *Fusarium spp.*, *Alternaria spp.* etc. Highest per cent

\*Corresponding author: E-mail: [dramsumanmishra@gmail.com](mailto:dramsumanmishra@gmail.com);

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mycelia growth inhibition of *Fusarium oxysporum f.sp. asparagi* was recorded in *Citrus sinensis* at 264 hours after inoculation. Mycelium growth of *Pythium aphanidermatum* was not recorded @ 2.0 percent concentration of aromatic oils viz, *Cymbopogon nardus*, *Cymbopogon citratus*, *Syzygium aromaticum*, *Mentha piperita*, and *Azadirachta indica* + *Mentha piperita* and *Eucalyptus globulus* (00 mm) after 264 hours. The lowest mycelial growth was found in *Cymbopogon martini* + *Cymbopogon citratus* 19.75mm. and highest growth observed in control 45.00mm after 264 hours inoculation. In *Rhizoctonia solanai* maximum percent growth inhibition was found in *Syzygium aromaticum* 93.84% at 264 hours after inoculation as compare to control. In pot condition, minimum percent mortality of *Asparagus racemosus* disease caused by *Fusarium oxysporum f.sp. asparagi* was found in *Azadirachta indica* + *Mentha piperita* 17.49%, followed by *Cymbopogon nardus* 22.49% and *Syzygium aromaticum* 25.90% at 150 day after transplanting.

**Keywords:** Aromatic oil; *Asparagus racemosus*; *Fusarium oxysporum f.sp. asparagi*; *Pythium aphanidermatum*.

## 1. INTRODUCTION

The essential oil of aromatic plants is secondary metabolites which often possess antimicrobial features and, as such, have an important role in the defense of the plants (Hyldgaard et al., 2012). Due to their antifungal, antiviral and insecticidal characteristics, they are a desirable source of alternative methods for plant disease management (Bassolé & Juliani, 2012). Essential Oil can also have a significant role in prolonging the food shelf-life and overcoming losses caused by storing agricultural products (Farzaneh et al., 2015; Prakash et al., 2015). Aromatic oil have consist monoterpenes, sesquiterpenes and their oxygenated derivatives, such as alcohol, ketones, acids, phenols, ethers, esters, etc. (Fornari et al., 2012; Ishaq et al., 2024). It has contained more than 60 different ingredients, although two or three are present in high concentration and others are present only in traces (Bakkali et al., 2008). Their antimicrobial effect (Queiroga et al., 2007; Manzoor et al., 2019) and their interactions may lead to antagonistic effect on plant pathogenic fungi (Bassolé & Juliani, 2012; Palfi et al., 2019).

Therefore, interest has been increased in the possibility of using natural products such as plant extracts or essential oils as biological agents of plant protection and plant disease control (Shrishti et al., 2014). In the present study, the different aromatic plants oil viz; *Eucalyptus*, *Basil*, *lemon grass*, *Palma Rosa*, *clove*, *Citronella*, *Orange*, *Neem* and, *Mint Oil*, have been evaluated because it is safe alternative to chemicals and have a limitless ability to synthesize secondary metabolites (eg. phenols, flavones, flavonoids, alkaloids, tannins) which operate as the plant's defence mechanism against pathogenic microorganisms. These oils

have the ability to delay or inhibit the growth of pathogenic or toxin producing fungi including *Pythium sp.*, *Rhizoctonia sp.*, *Fusarium sp.*, *Alternaria sp.* etc. However, the inhibition of mycelial growth and spore germination of fungi depends on the concentration of essential oils and their frequencies. Keeping in view of the importance of crop and seriousness of the disease, present investigation was undertaken to test the efficacy of essential oil of aromatic plants against the fungal pathogens *in vitro*

## 2. MATERIALS AND METHODS

Nine aromatic oils were used for the evaluation of efficacy against *Fusarium oxysporum*, *Alternaria alternata*, *Pythium spp* and *Rhizoctonia solanai* using the food poison technique *in-vitro*.

### 2.1 To Test the Efficacy of Aromatic Oil Against Fungal Pathogens *In Vitro*

Important plant pathogenic fungi viz, *Fusarium*, *Alternaria*, *Pythium*, and *Rhizoctonia* were collected from the field and maintained in the laboratory to test the efficacy of nine essential oil of aromatic plants *in vitro* viz., T1 *Cymbopogon nardus* oil @2%, T2 *Eucalyptus globulus* oil @2%, T3 *Azadirachta indica* oil @2%, T4 *Citrus sinensis* oil @2%, T5 *Syzygium aromaticum* oil @2%, T6 *Ocimum basilicum* oil @2%, T7 *Mentha piperita* oil @2%, T8 *Cymbopogon martini* oil @2%, T9 *Cymbopogon citratus* oil @2%, T10: *Cymbopogon martini* and *Cymbopogon citratus* oil @2%, T11 *Azadirachta indica* oil and *Mentha piperita* oil @2% and T12 Control on PDA media with four replication at Plant Pathological laboratory of medicinal and aromatic plants, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya, Uttar Pradesh,

India. The 2.0ml aromatic oils were mixed in separate 98ml autoclaved sterilized Potato Dextrose Agar (PDA) media to achieve the two percent concentrations of aromatic oil and carefully poured into four sterile Petri plates 20ml each for each treatment and allowed to solidify. Actively growing culture of *Fusarium oxysporum*, *Rhizoctonia solanai*, *Pythium aphanidermatum* and *Alternaria alternata* were inoculated in four Petri plate at the center with the help of cork borer (3mmdiameter) under aseptic condition in each treatment respectively. One set of PDA petriplate was maintained as control only pathogen was poured without any aromatic oil. The Petri plates were then placed in an incubator at room temperature ( $25\pm 2^{\circ}\text{C}$ ) for 11days and to record the radial mycelial growth (mm) at the interval of 72 hours, 120 hours,168 hours,216 hours and 264 hours and till the full growth of mycelium in control plate. Mycelium growth was measured in two directions at right angles with help of linear scale. The Percent mycelia growth inhibition was calculated with following formula;

$$\text{Percent growth Inhibition} = \frac{C-T}{C} \times 100$$

Where,

C=Radial mycelial growth in control plate

T=Radial mycelial growth in treatment plate

## 2.2 Evaluation of Aromatic Oils under Pot Condition

The experiment was conducted using earthen pots with 30cm diameter. Pots were filled with autoclaved 3.0kg soil pot<sup>-1</sup> (soil: FYM, 3:1 autoclaved at 1.045 kg / cm<sup>2</sup> for 2 hours on three consecutive days). Before adding the pathogen in pots, it was multiplied on sorghum grain sat  $25\pm 2^{\circ}\text{C}$  temperature for one week and served as the soil inoculums. The top 5cm layer of soil in each pot was thoroughly mixed with 20 g of inoculums, 7 days before transplanting of Asparagus (*Asparagus racemosus*). The crown and root rot susceptible genotype NDAS-101 was transplanted in the pots with three replications. The seedlings treatments were done with different Aromatic oils @2% concentration (volume/volume) (98 ml water and 2 ml oil) for 15 minutes. After the treatment, excess water of seedlings was drained and air dried prior to transplanting. Total 8 seedlings were maintained in each pot. After the transplanting, pots were

placed in green house. Foliar spray of the same Aromatic oil was done at 90 DAT. Percent mortality was observed at 30, 60,90, 120 and 150 DAT.

$$\text{Percent mortality} = \frac{\text{Total no.of plant in plot} - \text{Total no.of survival plant in plot}}{\text{Total no.of plant in plot}} \times 100$$

## 3. RESULTS AND DISCUSSION

Data presented in Table 1 revealed that all the aromatic oils have significantly inhibited the mycelial growth of all plant pathogens as compared to control. The cent percent mycelium growth inhibition was observed in *Cymbopogon nardus* oil, *Citrus sinensis* oil, *Syzigum aromaticum* oil, *cymbopogon martini* oil, *Cymbopogon martini* + *Cymbopogon citratus* and *Azadirachta indica* + *Mentha piperita* oil (100%), over the control after 264 hours of inoculation as compared to *Eucalyptus globulus* oil (75.00%), *Cymbopogon citratus* oil (53.89%), *Azadirachta indica* oil (23.88%) and lowest in *Ocimum basilicum* oil (26.66%). It is because of high level of eugenol present in aromatic oil which have strong inhibitory properties (Ranasinghe et al., 2002; Barrera- Necha et al., 2009). Gangavarapu and Palwai (2021) found that the *Cymbopogon citratus* and *Syzigum aromaticum* oil inhibit the 100% growth of *Fusarium oxysporum* at 1.0% concentration. Eugenol has proven to inhibit the activity of some enzymes. ATPase, histidine decarboxylase, amylase and protease may be important for cell killing at high eugenol concentration (Hyldgaard et al., 2012). Lie et al., (2017) found minimum mycelium growth at 1.5 % concentration of all tested essential oil. The inhibition of mycelial growth was increased with increase of essential oil concentration. Essential oils of thyme, clove, peppermint, tea, sage and basil completely inhibited the mycelial growth of *Fusarium*. Whereas in *Alternaria*, 264 hours after inoculation highest growth inhibition was recorded in *Citrus sinensis* 96.11% *Eucalyptus globulus* oil (83.33%), *Ocimumbasilicum*oil (26.81%), *Cymbopogon martini* oil (21.66%) and lowest in *Azardirachta indica* oil (15.55%). The Compounds of essential oils with broad spectrum activity are expected to provide protection against a range of plant pathogenic fungi that applied different stages of plant growth. Cinnamon oil, clove oil and essential oil components of citral, eugenol and geraniol were found to have broad spectrum antifungal activity

**Table 1. Evaluation of aromatic oils on per cent mycelial growth inhibition at 264 hours after inoculation**

Treatment	%Concentration.	Per cent mycelial growth inhibition			
		<i>Fusarium oxysporum</i>	<i>Rhizoctonia Solanai</i>	<i>Pythium aphanidermatum</i>	<i>Alternaria alternata</i>
T <sub>1</sub> <i>Cymbopogon nardus</i>	2	100 (90)	87.66 (69.45)	93.89(76.11)	100 (90)
T <sub>2</sub> <i>Eucalyptus globulus</i>	2	75 (60.29)	85.82 (68.00)	87.22 (69.36)	83.33 (65.93)
T <sub>3</sub> <i>Azadirachta indica</i>	2	23.88(29.24)	32.7 (34.85)	17.78 (24.90)	15.55 (23.16)
T <sub>4</sub> <i>Citrus sinensis</i>	2	100 (90)	82.10 (64.95)	88.33 (70.13)	96.11 (81.90)
T <sub>5</sub> <i>Syzigum aromaticum</i>	2	100 (90)	93.84 (75.76)	100 (90)	100 (90)
T <sub>6</sub> <i>Ocimum basilicum</i>	2	26.66 (31.06)	31.49 (34.12)	26.66 (30.94)	26.81 (31.09)
T <sub>7</sub> <i>Mentha piperita</i>	2	100 (90)	91.35 (72.91)	100 (90)	100 (90)
T <sub>8</sub> <i>Cymbopogon martini</i>	2	100 (90)	40.12 (39.27)	37.77 (37.89)	21.66 (27.66)
T <sub>9</sub> <i>Cymbopogon citratus</i>	2	53.89 (47.21)	80.84 (64.11)	100 (90)	100 (90)
T <sub>10</sub> <i>Cymbopogon martini + Cymbopogon ciratus</i>	2	100 (90)	74.08 (59.38)	56.11 (48.49)	16.11 (23.62)
T <sub>11</sub> <i>Azadirachta indica + Mentha piperita</i>	2	100 (90)	88.88 (70.52)	100 (90)	100 (90)
T <sub>12</sub> Control		0 0	0 0	0 (0)	00.00(00.00)
<b>CD (P=0.01)</b>		<b>4.002</b>	<b>3.49</b>	<b>4.74</b>	<b>3.78</b>

**Table 2. Evaluation of aromatic oil against percent mortality of asparagus plants under pot condition**

Treatments	Con. %	30DAP	60DAP	90DAP	120DAP	150DAP	Mean
T <sub>1</sub> <i>Cymbopogon nardus</i>	2.0	20.83 (26.89)	20.83 (26.89)	20.83 (26.89)	25.00 (29.47)	25.00 (29.47)	22.49(27.92)
T <sub>2</sub> <i>Eucalyptus globulus</i>	2.0	25.00 (29.98)	25.00 (29.98)	29.16 (32.57)	29.16 (32.57)	29.16 (32.57)	27.49(31.53)
T <sub>3</sub> <i>Azadirachta indica</i>	2.0	29.17 (32.57)	33.33 (35.16)	41.66 (40.15)	41.66 (40.15)	41.66 (40.15)	37.49(37.63)
T <sub>4</sub> <i>Citrus sinensis</i>	2.0	25.00 (29.98)	29.16 (32.57)	29.16 (32.57)	29.16 (32.57)	29.16 (32.57)	28.32(32.05)
T <sub>5</sub> <i>Syzigum aromaticum</i>	2.0	16.67 (23.79)	16.66 (23.79)	29.16 (32.57)	33.33 (35.16)	33.33 (35.16)	25.9.(30.09)
T <sub>6</sub> <i>Ocimum basilicum</i>	2.0	37.50 (37.74)	41.66 (40.15)	45.83 (42.57)	45.83 (42.57)	45.83 (42.57)	43.33(41.12)
T <sub>7</sub> <i>Mentha piperita</i>	2.0	33.33 (35.16)	37.50 (37.74)	37.50 (37.74)	37.50 (37.74)	37.50 (37.74)	36.66(37.22)
T <sub>8</sub> <i>Cymbopogon martini</i>	2.0	29.17 (32.57)	29.16 (32.57)	33.33 (35.16)	33.33 (35.16)	33.33 (35.16)	31.66(34.12)
T <sub>9</sub> <i>Cymbopogon flexuosus</i>	2.0	12.50 (20.69)	16.66 (23.79)	16.66 (23.79)	20.83 (26.89)	20.83 (26.89)	17.49(24.41)
T <sub>10</sub> <i>Cymbopogon martini +Cymbopogon ciratus</i>	2.0	8.33 (13.79)	12.50 (20.69)	20.83 (26.89)	25.00 (29.98)	25.00 (29.98)	18.32(24.26)
T <sub>11</sub> <i>Azadirachta indica + Mentha piperita</i>	2.0	16.67 (23.79)	20.83 (26.89)	16.66 (23.79)	16.66 (23.79)	16.66 (23.79)	17.49(24.41)
T <sub>12</sub> control		45.83 (42.57)	54.06 (47.33)	58.33 (49.80)	58.33 (49.80)	62.50 (52.21)	55.81(48.34)
<b>CD(P=0.05)</b>		<b>8.571</b>	<b>7.081</b>	<b>7.702</b>	<b>7.937</b>	<b>7.669</b>	
<b>SE(m)±</b>		<b>2.919</b>	<b>2.412</b>	<b>2.623</b>	<b>2.703</b>	<b>2.612</b>	
<b>CV%</b>		<b>17.356</b>	<b>13.27</b>	<b>13.478</b>	<b>13.509</b>	<b>12.977</b>	

DAP=Days after planting

and inhibited 14 phytopathogenic fungi tested in the disc diffusion assay or spore germination assay (Kishore et al., 2007; Lukanga Nakavuma et al., 2016). The essential oil of basil inhibit the growth of fungal pathogen at 10,8,6,4,2,1,0.5 and 0.25 percent concentration, the decrease in mycelial growth was correlated to the increase in the concentration of basil essential oil. The lowest 0.25% concentration inhibit mycelial growth of *A. alternata* (1.9%) and highest concentration 10 % inhibit mycelial growth 88.0% (Perveen et al.,2020). The highest growth inhibition of *Pythium* was recorded in *Citrus sinensis* 88.33% *Eucalyptus globulus* oil (87.22%), *Ocimum basilicum*oil (26.66%), *Cymbopogon martini* oil (56.11%) and lowest in *Azadirachta indica* oil (17.78%). The inhibitory and biocidal activity of these essential oil 500 ppm against mycelium of *R. solani* and *S. minor* (clove bud and origanum), and *Pythium spp.* was confirmed in bioassay.The concentrations less than 500 ppm were frequently inhibitory but not biocidal (McMaster et al., 2013). Basil, geranium, peppermint, pine and tea tree oils were biocidal to *R. solani* at the highest concentration of 2,500 ppm. Eugenol was also inhibitory and biocidal to *P. irregulare* at 1,000 and 2,500 ppm and *F. oxysporum* at 2,500 ppm. The biocidal activity of eugenol against *R. solani* and geraniol against *Pythium spp.* was confirmed in bioassays at 500 ppm. Pure component of geraniol was more effective than geranium oil in killing of mycelium in all cases. The similar efficacy of eugenol (clove bud) was against *R. solani*, thymol (thyme oil) on *Pythium sp.* In case of *Rhizoctonia*, maximum percent mycelial growth inhibition was recorded in *Syzigium aromaticum* oil followed by *Mentha piperita* oil, *Azadirachta indica* +*Mentha piperita* oil and *Cymbopogon nardus* oil. Khaledi et al., (2014) reported that essential oil of *Mentha piperita*, *Ocimum basilicum*, *Zingiber officinalis*, *F. vulgare* inhibit the mycelial growth of *Rhizoctonia solani* ranging from 100% to 43.38% at 2000 ppm concentration. Previous data of this study had revealed considerable inhibitory effect on mycelial growth of *Rhizoctonia solani* in vitro except the essential oils (Eos) of *Ocimum basilicum* and *Juniperus polycarpus*. Antifungal activity of the EOs of those plants observed in our research is parallel with other reports (Bansod & Rai 2008; Sharma & Sharma 2011). It might be due to the variation of chemical components present in each plant variety which the oil derived from it. Plant growth and the components of its essential oil (EO) may change with the region of collection, altitude, climatic

conditions of each season and also interaction of various micro-organisms with plant tissues. So, we should be careful to recommend any EO without sufficient sampling and repetition. In a recent study, essential oils derived from *C. citrates*, Lemongrass and *S. aromaticum* were investigated for their antimicrobial properties against a fungal pathogen in a dose dependent manner (Sowmya et al. 2021). Moreover, in a similar study by Naveenkumar et al., (2017), essential oils of *C. citratus*, *C. martini* and *P. graveolens* were found to be the most importantly effective, as they exhibited complete inhibition at a concentration of 0.02% against *F. oxysporum f.sp. niveum*.

### 3.1 Efficacy of Aromatic Oil on Percent Mortality of Asparagus under Pot Condition Due to Crown and Root Rot Caused by *Fusarium oxysporum f. sp. asparagi*

The data related to efficacy of aromatic oils under pot condition was presented in Table 2, It clearly indicates that percent mortality of *Asparagus racemosus* was significantly reduced in all treatments. The minimum percent mortality was found in *Cymbopogon martini*+*Cymbopogon citratus* oil (8.33%) followed by *Cymbopogon citratus* oil (12.50%), *Azadirachta indica* + *Mentha piperita* oil (16.67%), *Syzigium aromaticum* oil (16.67%), *Cymbopogon nardus* oil (20.83%), *Eucalyptus globulus* oil (25.00%), *Citrus sinensis* oil (25.00%), *Cymbopogon martini* oil (29.17%), *Azadirachta indica* oil (29.17%), *Mentha piperita* oil (33.33%) and *Ocimum basilicum* oil (37.50%). The highest percent mortality was found in controlled pot (45.83%) at 30 day after transplanting. In case of 60 day after transplanting, the minimum percent mortality was found in *Cymbopogon martini*+*Cymbopogon citratus* oil (12.50%) followed by *Cymbopogon citratus* oil (16.66%), the combination of *Azadirachta indica* + *Mentha piperita* oil (20.83%), *Syzigium aromaticum* oil (16.66%), *Eucalyptus globulus* oil (25.00%), *Citrus sinensis* oil (29.16%), *Cymbopogon martini* oil (29.16%), *Azadirachta indica* oil (33.33%), *Mentha piperita* oil (37.50%) and *Ocimum basilicum* oil (41.66%). The highest percent mortality was found in controlled pot (54.06%). Whereas 90 day after transplanting, the minimum percent mortality were found in *Cymbopogon martini*+*Cymbopogon citratus* oil (20.83%) followed by *Cymbopogon citratus* oil (16.66%), *Azadirachta indica* + *Mentha piperita* oil (20.83%), *Syzigium aromaticum* oil (29.16%),

*Eucalyptus globulus* oil (29.16%), *Citrus sinensis* oil (29.16%), *Cymbopogon martini* oil (33.33%), *Azadirachta indica* oil (33.33%), *Mentha piperita* oil (37.50%) and *Ocimum basilicum* oil (45.83%). The highest percent mortality was found in controlled pot (58.33%). 120 day after transplanting, the minimum percent mortality was recorded in *Cymbopogon martini*+*Cymbopogon citratus* oil (25.00%) followed by *Cymbopogon citratus* oil (20.83%), *Azadirachta indica* + *Mentha piperita* oil (16.66%), *Syzigium aromaticum* oil (33.33%), *Eucalyptus globulus* oil (29.16%), *Citrus sinensis* oil (29.16%), *Cymbopogon martini* oil (33.33%), *Azadirachta indica* oil (41.66%), *Mentha piperita* oil (37.50%) and *Ocimum basilicum* oil (45.83%). The highest percent mortality was observed in controlled pot (58.33%). At 150 day after transplanting, the minimum percent mortality was found in *Cymbopogon martini*+*Cymbopogon citratus* oil (25.00%) and maximum in controlled pot (62.50%). Experimental results of Gouda et al., (2009) and Shaheen et al., (2024) indicated that all essential oils were positively reduced the linear growth of soil borne fungal pathogen. The effect was increased by increasing the concentration of essential oils from 1000 to 2000ppm. The oils of *C. cyminum* and *S. aromaticum* were highly effective in reducing damping off and root rot as well as severity of rot diseases in sugar beet (Gouda et al., 2009). Sanap et al., (2020) six essential oils viz. Citronella oil, Clove oil, Neem oil, mustard oil, Eucalyptus oil and garlic oil were tested at 5% and 10% concentration each reduced the disease incidence of *Fusarium oxysporum* f. sp. *lycopersici* over untreated control (70.83%). But the per cent disease reduction of test pathogen increased with the increasing the concentrations of the essential oils. Sanap et al (2020) conducted pot experiment to know the efficacy of different essential oils as disease resistance inducer by root immersion method, @ 5% and 10% concentrations on incidence of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. All the essential oils tested exhibited wide range of wilt incidence, the average maximum incidence was recorded in untreated control (70.83%) followed by eucalyptus oil (43.74%), garlic oil (39.58%), mustard oil (18.33%) and clove oil (6.24%). The highest disease reduction was recorded with clove oil (94.12%), followed by citronella oil (88.23%), neem oil and mustard oil (82.35%), garlic oil (47.05%) and eucalyptus oil 41.16% at 10% concentration over untreated control. Essential oils found most effective in the order of merit were clove oil, citronella oil, neem

oil, mustard oil, garlic oil and eucalyptus oil. Similar results were obtained earlier by Hamidou et al (2024); Sharma et al., (2017); Sagitov et al. (2011) and Serife et al., (2013)

#### 4. CONCLUSION

Study has been concluded that the essential oil of *Cymbopogon nardus*, *Citrus sinensis*, *Syzigium aromaticum*, *cymbopogon martini*, *Cymbopogon martini* +*Cymbopogon citratus* and *Azadirachta indica* +*Mentha piperita* inhibited the cent percent mycelium growth of plant pathogens, which are applicable in field condition for the management of soil and foliar diseases.

#### 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 writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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