ISSN (Print) 0975-685X
ISSN (Online) 0975-864X

J. Appl. Biosci., 50(2): 146-151, December, 2024
doi: 10.61081/joab/50v2i110

ORIGINAL ARTICLE

Screening of Essential Oils Against the Banana Wilt Pathogen *Fusarium* sp. from Arunachal Pradesh, India

Madhu Kamle[1], Chikanshi Sharma[2], Zeiwang Konyak[2], Rituraj Borah[2] and Pradeep Kumar[3]*
[1]Department of Biochemistry, University of Lucknow, Lucknow 226007, UP, India
[2]Applied Microbiology Laboratory, Department of Forestry, North Eastern Regional Institute of Science and Technology, Nirjuli 791109, Arunachal Pradesh, India
[3]Applied Microbiology Laboratory, Department of Botany, University of Lucknow, Lucknow 226007, UP, India

ABSTRACT

Fusarium wilt disease affects numerous commercially significant crops and is controlled by synthetic fungicides. The utilization of natural compounds derived from plant sources has been prioritized in the search for substitutes for fungicides. They are safer for the environment and less harmful. Therefore, in this present study inhibitory effect of sixteen essential oils, namely Lemon grass, clove, Lavender, Jasmine, peppermint, Citronella, Sandalwood, Cedarwood, Argan, Jojoba, White tea, Basil, Vanilla, Tea tree, rose and Eucalyptus essential oils were screened. The influence of four concentrations (1000, 1500, 2000, and 2500 ppm) of each essential oil was tested against the fusarium wilt of banana. The radial mycelium growth and mycelial growth inhibition percentages were calculated. Based on radial mycelium growth and growth inhibition After 7th day of proper incubation in controlled conditions, it was observed that clove, lemon grass, rose, vanilla, white tea, citronella, and jasmine were the most effective essential oils against the pathogenic fungus. There was 100% inhibition in the growth of phytopathogenic fungi at 1500 ppm concentration by clove essential oil. The essential oils of lemon grass rose, and citronella have been proven to limit the colony growth of *Fusarium oxysporum*, and 100% inhibition was observed at concentrations of 2000 and 2500 ppm after the 7th day of the incubation period.

KEY WORDS : Essential Oils, Banana Wilt, *Fusarium,* Disease management, Arunachal Pradesh

INTRODUCTION

Fusarium oxysporum is a soil-borne fungus found in air, water, and soil having pathogenic and non-pathogenic members. This fungus is pathogenic to human beings, plants, and animals also the causative agent of vascular wilt, a disease that damages a wide range of economically significant crops (Beckman, 1987). The pathogenic strains of *F. oxysporum* colonize the cortex and endodermis before entering the vascular system to infect hosts by invading the roots (Rodriguez-Galvez & Mendgen, 1995). The world's most valuable primary agricultural products include bananas and plantains, a variety of cooked bananas. In March 2015, a Cavendish (AAA) banana plantation in Tully, North Queensland, Australia, detected an outbreak of the disease known as "Panama disease [Banana wilt]," which is caused by a fungus. According to molecular and vegetative compatibility group analyses, FOC [*Fusarium oxysporum* Schlecht. f. sp. cubense] belonged to the clonal population VCG 01213/16, also known as TR4 (Tropical race 4). In the Indo-Malayan area, this population is assumed to have evolved alongside its banana host (Ploetz, 2015). Soil-borne fungus TR4 reproduces only asexually and creates microconidia, macroconidia, and chlamydospores as survival structures. The pathogen produces chlamydospores with a long survival capacity, grows as hyphae in organic residues invades and survives as asymptomatic endophytes in a variety of non-host plants, and colonizes host tissue, all of which make it exceedingly difficult to control. The pathogen can also produce disease at low inoculum levels, can live in the soil at a certain depth, and the disease may have a protracted incubation period (Pegg *et al.,* 2019; Hennessy *et al.,* 2005; Pittaway *et al.,* 1999). The main method for preventing *Fusarium* wilts is to use synthetic

*Corresponding author email: pkbiotech@gmail.com ; Orcid id: https://orcid.org/0000-0002-0892-5425

fungicides. However, it has been discovered that these synthetic fungicides adversely impact humans and the environment. A hazardous fumigant used to treat *Fusarium* wilt is methyl bromide. Therefore, attempts in the search for different non-hazardous fungicides have been focused on plant-based natural components due to their lower toxicity and greater environmental compatibility. The use of essential oils extracted from plants as a fungus-controlling agent is one of the alternative methods. Research on essential oils revealed that they are strong antifungal agents and could be developed as efficient fungus-control agents.

Essential oils are accountable for the flavor and aroma that are associated with spices, perfumes, and herbs. EOs are intricate compounds made up of multi-component combinations including hundreds of different components. They derive from terpenes & associated oxygenated compounds chemically. These ingredients all exhibit antifungal, antiviral, antioxidant, insecticidal, and antibacterial effects (Burt, 2004). Numerous essential oils and the ingredients in them have been studied for their potential antimicrobial effects on various bacterial and fungal strains (Bakkali *et al.,* 2008). The antimicrobial activity of essential oils has been the subject of numerous investigations for several years. The largest class of compounds present in essential oils is terpenes. Isoprene units with five carbons create this group of compounds. Terpenes can be used as building blocks for larger, more complex compounds to create linear-chained substances having one or more ring structures. Terpenes come in a variety of groups, although sesquiterpenes and monoterpenes are the most significant in essential oils. These two chemical groups give EOs their distinctive scent (de Groot & Schmidt, 2016). Numerous essential oils have been reported to have antifungal properties, according to previous studies. For example, clove oil antifungal activity showed minimal inhibitory concentration against, *Aspergillus niger, Alternaria alternata, Phomopsis viticola, Colletotrichum gloeosporioides, Lasiodiplodia theobromae* and *Rhizopus stolonifera* (Sukatta *et al.*, 2008). According to Milanovi *et al.*, lemongrass EOs inhibited the growth of 74 isolates that were isolated from 14 different species of the *Candida*, *Rhodotorula*, *Debaryomyces*, *Kluyveromyces*, and *Yarrowia* genera.

Table 1: MIC for selected essential oils against *F. oxysporum*

MIC of oils against fungi	
Essential oils	*Fusarium sp.*
Clove	1500 ppm
Citronella	2000 ppm
Lemon grass	2000 ppm
Rose	2000 ppm

Lucas *et al.* (2012) verified that EOs from citronella, clove, lemongrass, eucalyptus, and tea trees had a direct toxic effect on *Xanthomonas vesicatoria*. The study's objective was to evaluate sixteen essential oils from various plant species for their *in vitro* antifungal efficacy on the *Fusarium* wilt of banana.

MATERIALS AND METHODS

Plant material collection and essential oils

Banana plants showing symptoms of Panama disease were collected from plantations near NERIST, Nirjuli, Arunachal Pradesh. For pathogen isolation, roots from five banana plantlets were carefully dug up, placed in plastic bags, and transported to the laboratory. Sample processing commenced within four hours of collection to ensure sample integrity and accurate isolation results. Total of sixteen therapeutic-grade essential oils was purchased including Lemon grass, clove, Lavender, Jasmine, peppermint, Citronella, Sandalwood, Cedarwood, Argan, Jojoba, White tea, Basil, Vanilla, Tea tree, rose and Eucalyptus which were extracted from *Cymbopogon citratus, Eugenia caryophyllata, Lavandula angustifolia, Jasminum grandiflorum, Mentha piperita, Cymbopogon nardus, Santalum album L, Juniperus virginiana, Argania spinosa, Simmondsia chinensis, Camellia sinensis*, *Ocimum Canum, Vanilla planifolia, Melaleuca alternifolia, Rosa damascene, Eucalyptus,* respectively.

Isolation and Identification of Pathogenic Fungi

The pathogens from surface-sterilized banana roots on potato dextrose agar (PDA) media were successfully isolated (Johnson & Curl, 1972). Randomly selected infected parts were gathered and surface sterilized using 4% sodium hypochlorite, 75% alcohol, and then sterilized distilled water. The infected roots were cut into small pieces, the contaminated sections were then placed in Petri plates containing sterilized media and cultured for 7-10 days at 27°C. Based on morphological, cultural, and microscopic properties, fungal pathogens were identified.

Antifungal Assay

The antifungal properties of the EOs were ascertained using PDA autoclaved and poured in sterile 9 cm diameter Petri dishes. All the essential oils (at concentrations of 1000ppm, 1500ppm, 2000ppm and 2500ppm prepared by adding Tween 20 surfactant) were mixed individually in the media before pouring. Test fungus discs (5 mm diameter) were cut from a seven-day-old culture's periphery, and they were aseptically inoculated into the center of each Petri plate in the treatment and control sets. The Petri plates were kept in the incubator chamber for six days at 27 ± 1°C. The test fungal colony diameters in the treatment and control sets were measured in mutually perpendicular

Table 2: Radial growth of *F. oxysporum* on selected essential oils during MIC

Period	Clove (*E. caryophyllata)*				
Days	1000ppm	1500 ppm	2000 ppm	2500 ppm	Control
3rd	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	23.00 ± 1.41
7th	9.00 ± 0.00	8.33 ± 0.57	8.00 ± 0.00	8.00 ± 0.00	42.00 ± 2.82
		Citronella (*C. nardus)*			
3rd	9.00 ± 0.00	9.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	23.00 ± 1.41
7th	17.33 ± 1.52	14.33 ± 2.30	11.00 ± 0.00	9.33 ± 0.57	42.00 ± 2.82
		Lemon grass (*C. citratus)*			
3rd	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	23.00 ± 1.41
7th	18.33 ± 1.15	9.66 ± 0.57	8.00 ± 0.00	8.00 ± 0.00	42.00 ± 2.82
		Rose (*R. damascene*)			
3rd	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	23.00 ± 1.41
7th	18.33 ± 1.15	9.66 ± 0.57	8.00 ± 0.00	8.00 ± 0.00	42.00 ± 2.82

directions and were recorded using the formula below in terms of percent mycelial inhibition (Grover & Moore, 1962).

Percentage of mycelial inhibition = dc – dt / dc × 100

Where: dc = mean colony diameter of control sets
dt = mean colony diameter of treatment sets

Minimum Inhibitory Concentration (MIC)

Experimental work was done to determine the MIC at which the oil displayed absolute fungitoxicity (total suppression of test fungi growth) using PDA autoclaved and poured in sterile 9 cm diameter Petri dishes. All the essential oils (at concentrations of 1000, 1500, 2000, and 2500 ppm), prepared were mixed individually in the media before pouring. Test fungus discs (5 mm diameter) were cut from a seven-day-old culture's periphery, and they were aseptically inoculated into the center of each Petri plate in the treatment and control sets. The Petri plates were kept in the incubator chamber for six days at 27 ± 1°C. The test fungal colony diameters in the treatment and control sets were measured.

RESULTS

Isolation and identification of pathogenic fungi

The pathogen isolates from the roots were identified as *F. oxysporum* based on colony morphology and microscopic features, as described by Nelson *et al.* (1983). Pure cultures were grown on the PDA medium to determine their growth rate and colony pigmentation. Cultures were incubated at 30°C for 7th days in the dark after which colony diameter was measured and colony color determined.

Evaluation of essential oils against fungi

Sixteen different essential oils were tested to see how they affected the growth of phytopathogenic fungi *Fusarium* wilt of banana. To assess the impact, oil concentrations of 1000 ppm were used. Control was also kept in place simultaneously by inoculating a culture disc onto the medium without using any oil. After the third, fifth, and seventh day of incubation in proper condition, the colony diameter was measured in mm and the data was recorded. From the experimental data, it was practically observed that essential oils of clove, and lemongrass showed maximum inhibition or highest antifungal activity with 100% inhibition of mycelial growth of *Fusarium sp.* on the third, fifth, and seven days of incubation period. Oils of rose and citronella were 100% effective and showed inhibition of *Fusarium sp.* till 5 days of incubation period and on the seventh day growth was observed. Eos of vanilla, jasmine, and white tea essential oils showed maximum inhibition till five days of incubation but after the fifth day, mycelial growth was observed (Fig. 1). The radial growth of the fungus was analyzed with the data collected by measuring the growth of the fungus or reduction in colony diameter (Fig. 2). Each test was carried out in triplicate.

The result demonstrated that the % of mycelial growth inhibition is significantly $p < 0.05$ affected by essential oil concentrations and incubation time. Mycelia growth decreased as essential oil concentration increased but increased as the incubation period increased. Clove, Lemongrass, Rose, and citronella essential oils had substantial fungistatic activity against species examined with MIC values (Fig. 3).

Antifungal Assay for Selected EOs

The growth of the tested fungus was effectively inhibited by clove oil essential oil at concentrations of 1500 ppm. 100% inhibition of *F. oxysporum* was seen

up to the 7th day. However, throughout the subsequent incubation phase, growth was seen on the control plate. The growth of fungus or reduction in colony diameter correlated with the amount of oil present. At concentrations of 1500, 2000, and 2500 ppm, the action of the oil was significantly prohibitive, with 100% inhibition seen up to day 7th.

The growth of fungus or reduction in colony diameter correlated with the amount of oil present for the rose. At the concentration of 2000 ppm of rose essential oil, *Fusarium* showed 100% inhibition up to the 7th day. However, a small amount of growth was observed after incubation period in plates containing 1000 and 1500 ppm concentration of essential oils. At decreasing oil concentrations, a fungal colony's diameter increased, although it was always smaller than the control.

Phytopathogenic fungi were inhibited from growing when Lemon grass and Citronella essential oil were present in higher concentrations. After 7 days of incubation, 100% inhibition of *Fusarium* wilt of banana at a concentration of 2000 ppm was observed. The growth of fungus or reduction in colony diameter correlated with the amount of oil present. However, a small amount of growth was observed after seven days of incubation period in plates containing 1000ppm and 1500ppm concentrations of oils. Even while the fungal colony's diameter increased at lower oil concentrations, it was always smaller than the control.

White tea, Jasmine, and vanilla essential oil also prevent phytopathogenic fungi from growing. When oil was present in higher concentrations, it had an inhibitory effect on *F. oxysporum*. The growth of fungus or reduction in colony diameter correlated with the amount of oil present. While the fungus colony at 2500 ppm concentration was initially reduced, during consecutive incubation periods the growth of the colony increased, but it always remained less than the control.

Lavender, peppermint, sandalwood, cedarwood, argan, jojoba, basil, tea tree, and eucalyptus essential oils had no inhibitory effect on the growth of the fungal colony. The growth was essentially identical to the control even at a higher oil concentration of 2500 ppm. Hence, this plant-based essential oil has no impact on the growth of a fungal colony.

Minimum Inhibitory Concentration (MIC)

Experiments were conducted to determine the lowest inhibitory concentration (total suppression of test fungi growth) at which the oil displayed absolute fungitoxicity for most potent EOs, as mentioned in Table 1. Different oil concentrations were made by dissolving the necessary

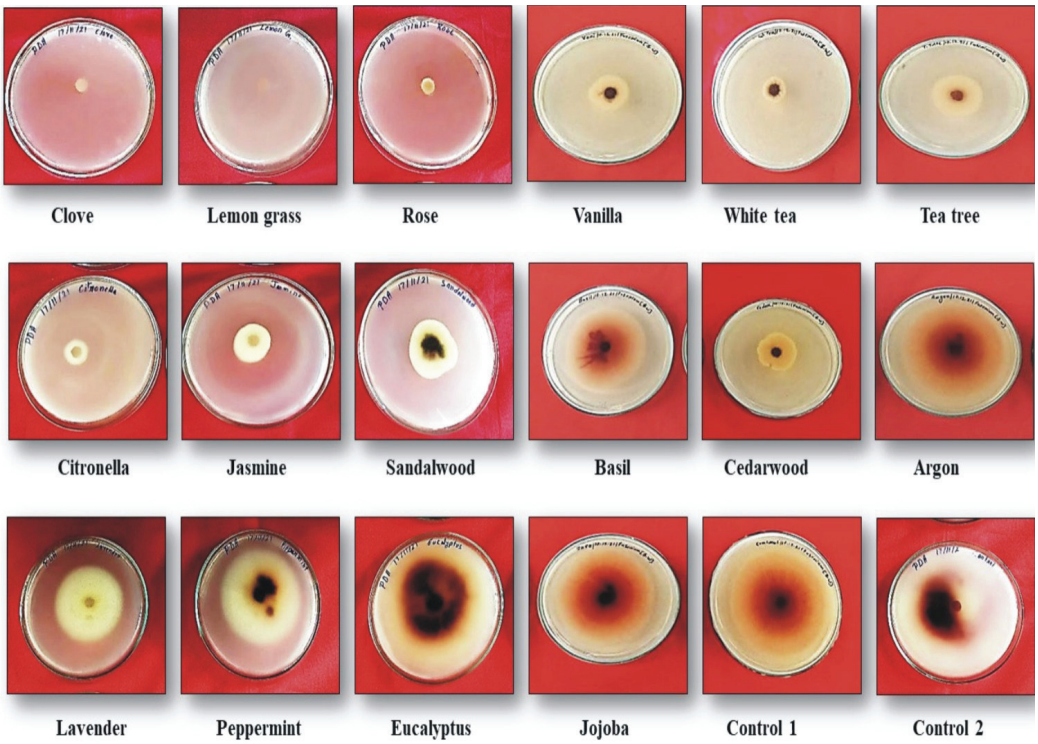

Fig. 1. Screening of *Fusarium* sp. mycelium growth on 1000 PPM concentration of essential oils after 7th day of incubation.

amount of each oil individually in aqueous Tween-80 solution before pouring it into plates prepared with potato dextrose agar medium. The control plate was kept with no essential oil applied on it. As per normal, the prepared plates were aseptically inoculated upside-down with the test fungus assay disc in the center of the Petri plates for the treatment and control sets. The petri plates were kept in the BOD incubator at 27°C for six days. On the third and seventh day, the fungal colony diameters (mm) of the treatment and control sets were measured in directions that were perpendicular to one another, and the percentage of inhibition was computed (Table 2).

STATICAL ANALYSIS

Each test was carried out in triplicate. The mean value and standard error of the mean were used to express the results. P-values less than 0.05 were regarded as significant.

DISCUSSION

Each EO showed either complete or partial effectiveness against *F. oxysporum*. The growth of the tested fungi was seen to be completely inhibited by clove essential oil at 1500ppm, 2000ppm, and 2500ppm concentrations on three, five, and seven days of incubation period. *F. oxysporum* exhibited 100% inhibition up to the seventh day at a concentration of 2000 ppm of rose essential oil. However, following the incubation time, growth was seen on plates with essential oil concentrations of 1000 ppm and 1500 ppm. Banana Fusarium wilt was completely inhibited at a dosage of 2000ppm after 7 days of proper incubation by essential oils of lemongrass and citronella. Findings show that the fungi toxic and fungistatic properties of the essential oils of *E. caryophyllata*, *C. citrates*, *R. damascene*, and *C. nardus* are effective against pathogenic fungi even at lower concentrations. At higher concentrations, the essential oils of *J. grandiflorum*, *C. sinensis,* and *V. planifolia* (white tea, jasmine, and vanilla respectively) suppress the growth of pathogenic fungus. In the case of White tea, the fungus colony at 2500ppm concentration was initially diminished; nevertheless, throughout subsequent incubation periods, the colony's growth increased while consistently being lower than the control. The growth of fungus or reduction in colony diameter correlated with the amount of oil present. At a concentration of oil of 2500 ppm, there was 100% inhibition on jasmine essential oils up to the fifth day. A small amount of growth was observed after another incubation period. All the sixteen essential oils varied in concentration and type, and each had a different effect on the tested fungi. The susceptibility of the fungal isolate to various essential oils showed distinct variations. In a study treatment of oil combinations with an aqueous emulsion of clove oil effectively reduced the occurrence of the tomato Fusarium wilt disease (La Torre *et al*., 2016). Sharma *et al.* (2017) reported that four essential oils viz., clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), mint (*Mentha* × *piperita*) and eucalyptus (*Eucalyptus globulus*) examined showed the moderate to high *in vitro* antifungal activity against *F. oxysporum* f. sp. *lycopersici* 1322. Both inhibition of mycelial growth and fungal spore germination bioassay results showed that the pathogen varied in its susceptibility to essential oils and it is a dose-dependent activity. Clove oil proved most effective against *F. oxysporum* f. sp. *lycopersici* 1322, followed by lemongrass. In similar study by Rana *et al.* (2011) for *S. aromaticum* (L.) essential oil *in vitro* against the *Fusarium moniliforme* NCIM 1100, Fusarium *sp.* MTCC284, *Aspergillus* sp., *Mucor* sp., *Trichophyton rubrum*, and *Microsporum gypseum*. They reported that clove oil has potent antifungal properties against all the tested pathogens. Girish & Fatima (2019) reported that five essential oils namely viz., basil oil, camphor oil, cinnamon oil, lavender oil, and rose oil possess antifungal potential under laboratory conditions and could be used for the eco-friendly management of *Phomopsis azadirachtin*. Hassan *et al.* (2022) examined the antifungal properties of EOs from four aromatic plants: *C. citratus,*

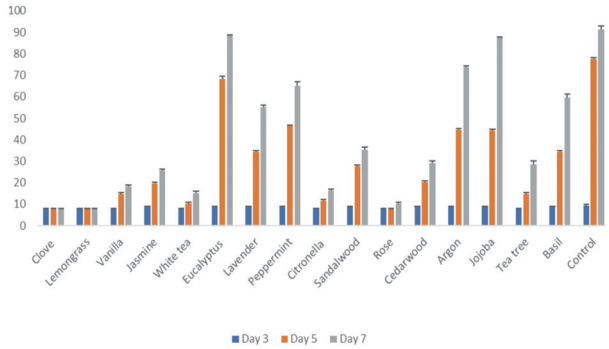

Fig. 2. Radial growth of *Fusarium* sp. against each EOs.

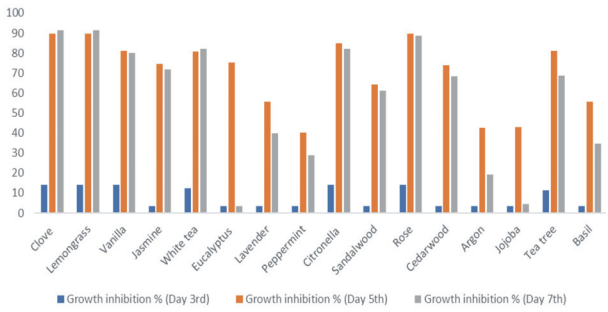

Fig. 3. Radial growth inhibition rate (%) of essential oils against *F. oxysporum.*

Salvia officinalis, Rosmarinus officinalis, and *Lavandula dentate* against *Fusarium sp.* f. sp. *albedinis* mycelial growth They observed that the essential oils of lavender and lemon grass exert a strong antifungal activity on the fungus at a concentration of 2.5 g/L for the essential oil of lavender and at a concentration of 1g/l for the essential oil of lemon grass. On the other hand, the two essential oils of sage and rosemary proved to have a lesser impact on the tested strain. It is thought that EOs' lipophilic nature makes it easier for lipid bilayer fungal membranes to penetrate them, which disrupts membranes (Lambert *et al.,* 2001; Ultee *et al.* 2000). These results demonstrate the ability to combine several plant essential oils to increase their antifungal activity and crop protection effectiveness.

CONCLUSION

Our findings showed that the seven plant essential oils including clove, lemongrass, rose, vanilla, white tea, citronella, and jasmine examined in this study effectively inhibited *F. oxysporum* conidial germination and mycelial growth *in vitro*.

Author Contributions

Conceptualization, P.K.; writing– original draft preparation, C.S., Z.K., R.B.; Writing– review and editing, P.K. and M.K.; supervision, P.K., and M.K. All authors have read and agreed to the published version of the manuscript.

Funding

Not applicable.

Acknowledgments

The authors are grateful to their respective authorities, departments, institutions, and universities for their support and cooperation in this research.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chem. Toxicol.,* 46(2): 446-475.

Beckman, C.H. (1987). The nature of wilt diseases of plants. American Phytopathological Society.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods – a review. *Interna. J. Food Microbiol.,* 94(3): 223-253.

de Groot, A.C., & Schmidt, E. (2016). Essential oils, part III: Chemical composition. *Dermat.,* 27(4): 161-169.

Hennessy, C., Walduck, G., Daly, A., & Padovan, A. (2005). Weed hosts of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in northern Australia. *Australasian Plant Pathol.,* 34(1): 115-117.

Johnson, L.F., & Curl, E.A. (1972). Methods for research on the ecology of soil-borne plant pathogens. Burgess Publishing Company.

Lambert, R.J., Skandamis, P.N., Coote, P.J., & Nychas, G.J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol, and carvacrol. *J. Appl. Microbiol.,* 91: 453-462.

Lucas, G.C., Alves, E., Pereira, R.B., Perina, F.J., & Souza, R.M.D. (2012). Antibacterial activity of essential oils on *Xanthomonas vesicatoria* and control of bacterial spot in tomato. *Pesquisa Agropecuária Brasileira,* 47: 351-359.

Rana, I.S., Rana, A.S., & Rajak, R.C. (2011). Evaluation of antifungal activity in essential oil of *Syzygium aromaticum* (L.) by extraction, purification, and analysis of its main component eugenol. *Brazilian J. Microbiol.,* 42(4): 1269-1277.

Pegg, K.G., Coates, L.M., O'Neill, W.T., & Turner, D.W. (2019). The epidemiology of Fusarium wilt of banana. *Front. Plant Sci.,* 10: 1395.

Pittaway, P.A., Nasir, N., & Pegg, K.G. (1999). Soil receptivity and host-pathogen dynamics in soils naturally infested with *Fusarium oxysporum* f. sp. *cubense,* the cause of Panama disease in bananas. *Australian J. Agricul. Res.,* 50(4): 623-628.

Ploetz, R.C. (2015). Fusarium wilt of banana. *Phytopathology,* 105(12): 1512-1521.

Rodriguez-Galvez, E., & Mendgen, K. (1995). The infection process of *Fusarium oxysporum* in cotton root tips. *Protopl.,* 189(1-2): 61-72.

Sukatta, U., Haruthaithanasan, V., Chantarapanont, W., Dilokkunanant, U., & Suppakul, P. (2008). Antifungal activity of clove and cinnamon oil and their synergy against postharvest decay fungi of grape in vitro. *Agricul. Nat. Res.,* 42(5): 169-174.

Ultee, A., Slump, R.A., Steging, G., & Smid, E.J. (2000). Antimicrobial activity of carvacrol towards *Bacillus cereus* on rice. *J. Food Prot.,* 63: 620-624.

Nelson, P.E., Toussoun, T.A., & Marasas, W.F.O. (1983). *Fusarium* species: An illustrated manual for identification. University Park, PA: Pennsylvania State University Press.

Hassan, B., Zouheir, C., Redouane, B., Mohammed, C., Mustapha, E.M., & Elbachir, H. (2022). Antifungal activity of the essential oils of *Rosmarinus officinalis*, *Salvia officinalis*, *Lavandula dentata*, and *Cymbopogon citratus* against the mycelial growth of *Fusarium oxysporum* f. sp. *Albedinis. Arabian J. Medi. Arom. Plants,* 8(1): 108-133.

Sharma, A., Rajendran, S., Srivastava, A., Sharma, S., & Kundu, B. (2017). Antifungal activities of selected essential oils against *Fusarium oxysporum* f. sp. *lycopersici* 1322, with emphasis on *Syzygium aromaticum* essential oil. *J. Biosci. Bioengineer.,* 123(3): 308-313.

Grover, R.K., & Moore, J.D. (1962). Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa. Phytopathol.,* 52: 876-879.