COMPARATIVE ANALYSIS OF ANIFUNGAL ACTIVITY OF FOUR ESSENTIAL OILS AGAINST DIFFERENT FUNGI

[1]Geethanjali R, [2]Aathiya Vanekar, [3]Pratibha K.Y, [4]Marhoob Banu,

[1]Assistant Professor, [3]Professor [2,4]Research Scholar
Department of Botany, Maharani Cluster University,
Palace Road, Bengaluru, Karnataka, India, 560001
Corresponding author: Marhoob Banu

Abstract- **The present study was conducted with the main purpose to compare the antifungal activity of four essential oils viz., Clove oil (*Syzygium aromaticum*), Eucalyptus oil (*Eucalyptus globulus*), Neem oil (*Azadirachta indica*) and Rose oil (*Rosa rubiginosa*) at concentrations of 20%, 40%, 60%, 80% and 100% respectively on *Aspergillus fumigatus, Aspergillus niger* and *Fusarium oxysporum* associated with *Sorghum vulgare* and *Zea mays* by poisoned food technique. Screening of fungi on *Sorghum vulgare* and *Zea mays* seeds was done by agar plate method and the fungi studied were *Aspergillus fumigatus, Aspergillus niger* and *Fusarium oxysporum*. Potato dextrose agar was prepared and well grown colonies of fungi were inoculated. Pure cultures of fungi were obtained by subculturing the previous culture and incubated at 27 ± 2 °C for 48 hours. Fungus was suspended in sterile distilled water and adjusted to a standard inoculum size to 1-2 x 106 CFU/mL. Five different concentrations of essential oils were added to sterile potato dextrose agar at concentration of 500 ppm in the hood of laminar air flow and 0.1 ml of fungal suspension was used to inoculate treated PDA petriplates with a sterile non-toxic cotton swab. Acetone at 500 ppm was added as control. The treated plates with essential oils and control were incubated at 27 ± 2 °C for 7 days. After incubation the treated plates were observed for zone of inhibition around wells and also for complete inhibition. Zone of inhibition were measured and recorded in millimetres.**

Keywords: Agar plate method, Antifungal activity, Poisoned food technique, *Sorghum vulgare*, *Zea mays*.

INTRODUCTION

Fungi are known to cause more than nineteen thousand diseases in plants.[1] Fungal pathogens are dangerous for plants and they are known to cause the death of the plants. They also cause the loss of crop harvests. Fungi can also affect the quality of crops, causing toxin accumulation within the plants. The toxins produced by these fungi are dangerous for both humans and animals.[2] Presently, there are numerous range of fungicides which are used to control plant diseases. However, it has been reported that fungicides may have negative effects on plant physiology, especially on photosynthesis. The change in photosynthesis might lead to a reduction in photoassimilate production which lead to decrease in both growth and yield of crop plants.[3] Frequent use of chemical fungicide is also the threat to the natural environment, mainly soil, by promoting the addition and movement of toxic substances in ecosystems.[4] The toxicity of chemical fungicides to humans is generally considered to be low, but fungicides can irritate the skin and eyes.[5] Because of the problems related with the use of chemical fungicides considerable attention has been paid in the recent years for using more consumer and nature-friendly protectants in the seed treatment.[6] Plants contain a wide spectrum of antimicrobial compounds which are effective antifungal agents. Among them essential oils have become major seed-decontamination substitutes to chemical seed protectives.[6] Essential oils, being strong fungicidal agents, are also biodegradable and show low toxicity to humans and animals.[7,8] There are many studies which have documented the antifungal properties of essential oils.[10-17] In the present study we aimed to evaluate and compare the antifungal efficacy of four essential oils viz., Clove oil (*Syzygium aromaticum*), Eucalyptus oil (*Eucalyptus globulus*), Neem oil (*Azadirachta indica*) and Rose oil (*Rosa rubiginosa*) at concentrations of 20%, 40%, 60%, 80% and 100% respectively on *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum* by poisoned food technique.

MATERIALS AND METHODS

Collection of samples

Zea mays and *Sorghum vulgare* seeds were collected from Monsoon seed company, Bengaluru, Karnataka, India

Isolation of seed-borne fungi from maize seeds

The standard Agar plate method was used for the isolation of fungi from seeds.[9] The seeds were first surface sterilized with sterile distilled water and then with 0.2% sodium hypochlorite solution for 2 minutes and again rinsed with sterile distilled water and dried for almost 1 minute in the hood of laminar air flow. 20 mL of sterilized Potato Dextrose Agar medium was poured in to the sterilized petriplates of 90 mm diameter. After solidification, 25 sterilized *Zea mays* seeds were placed onto the PDA medium. The inoculated plates were incubated for a week at 27 ± 2°C. After incubation period, the seeds were observed for infection and the fungi occurring on seed in the plates were identified based on sporulation characteristics with the help of stereoscopic binocular microscope. After identification *Aspergillus niger* was sub-cultured onto PDA plates and maintained as a pure culture at 4°C for further studies.

Antifungal activity

The antifungal activity of four essential oils viz., Clove oil (*Syzygium aromaticum*), Eucalyptus oil (*Eucalyptus globulus*), Neem oil (*Azadirachta indica*) and Rose oil (*Rosa rubiginosa*) at concentrations of 20%, 40%, 60%, 80% and 100% respectively was studied on *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum* by poisoned food technique. Different concentrations of essential oils were prepared using acetone. *Aspergillus niger* was adjusted to a standard inoculum size to 1-2 x 106 CFU/mL using sterile distilled water. Five different concentrations of essential oils were added to sterile potato dextrose agar at concentration of 500 ppm in the hood of laminar air flow and 0.1 ml of fungal suspension was used to inoculate treated PDA petriplates with autoclaved non-toxic cotton swab. Acetone at 500 ppm was added as control. The treated plates with essential oils and control were incubated at 27 ± 2 °C for 7 days. After incubation period the treated plates with essential oils were observed for zone of inhibition around wells and also complete inhibition. Zone of inhibition was measured and recorded in millimetres (mm).

RESULTS AND DISCUSSION

Out of all the four oils, clove oil at all concentrations inhibited the growth of *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum*. Eucalyptus oil at all concentrations showed the zone of inhibition for *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum*. Neem oil showed zone of inhibition for *Aspergillus fumigatus* and *Fusarium oxysporum* but it did not inhibited the growth of *Aspergillus niger.* Rose oil at all concentrations completely inhibited the growth of *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum* (Table 1, 2 and 3).

Mohamed shohayeb et al., has studied the antifungal activity of rose oil against *Aspergillus*. Results revealed that rose oil possessed antifungal activity against *Aspergillus*.[10] Similarly in the present study rose oil completely inhibited the growth of *Aspergillus fumigatus* and *Aspergillus niger*. From the findings of Rana, Inder Singh et al., antifungal activity of clove oil against *Aspergillus* was revealed.[11] Similar results were obtained in the present study where clove oil exhibited antifungal activity against *Aspergillus fumigatus* and *Aspergillus niger.* From the findings of Rini pujiarti et al., antifungal activity of eucalyptus oil against *Aspergillus* was revealed.[12] Same results were obtained in the present study where eucalyptus oil showed inhibitory activity against *Aspergillus fumigatus* and *Aspergillus niger*. M Ashfaq ahmed et al., had studied the antifungal activity of neem oil against *Aspergillus.* Results revealed that neem oil inhibited the growth of *Aspergillus*.[13] These results are partially in contrast and partially in accordance to our result where neem oil was not effective against *Aspergillus niger* while it was effective in inhibiting the growth of *Aspergillus fumigatus.* Sharma A, Rajendran S, Srivastava A, Sharma S, Kundu B et al., has studied the antifungal activity of clove oil against *Fusarium oxysporum*. Results revealed that clove oil possessed antifungal activity against *Fusarium oxysporum*.[14] Similar to the results obtained from that study, in the present study clove oil inhibited the growth of *Fusarium oxysporum*. From the findings of Gakuubi MM, Maina AW, Wagacha JM et al., antifungal activity of eucalyptus oil against five *Fusarium* species was revealed.[15] Similarly, in the present study eucalyptus oil exhibited antifungal activity against *Fusarium oxysporum.* From the findings Adepoju, Adeyinka & Femi-Adepoju, Abiola & Ogunkunle, Tunde et al., antifungal activity of neem oil against *Fusarium* was revealed.[16] Similar results were obtained in the present study where neem oil showed inhibitory activity against *Fusarium oxysporum*. Girish K, Chaithra TS, Fathima SK et al., had studied the antifungal activity of rose oil against *Fusarium.* Results revealed that rose oil inhibited the growth of *Fusarium*.[17] These results are in accordance to our result where rose oil was effective in inhibiting the myecelial growth of *Fusarium oxysporum*.

TABLE 1: Inhibitory activity of essential oils against *Aspergillus fumigatus*

Concentration	Eucalyptus oil	Neem oil	Clove oil	Rose oil
100%	41 mm	31 mm	Complete inhibition	Complete inhibition
80%	35 mm	_	Complete inhibition	Complete inhibition
60%	28 mm	_	Complete inhibition	Complete inhibition
40%	36 mm	35 mm	Complete inhibition	_
20%	30 mm	33 mm	43 mm	Complete inhibition
Control	Maximum growth	Maximum growth	Maximum growth	Maximum growth

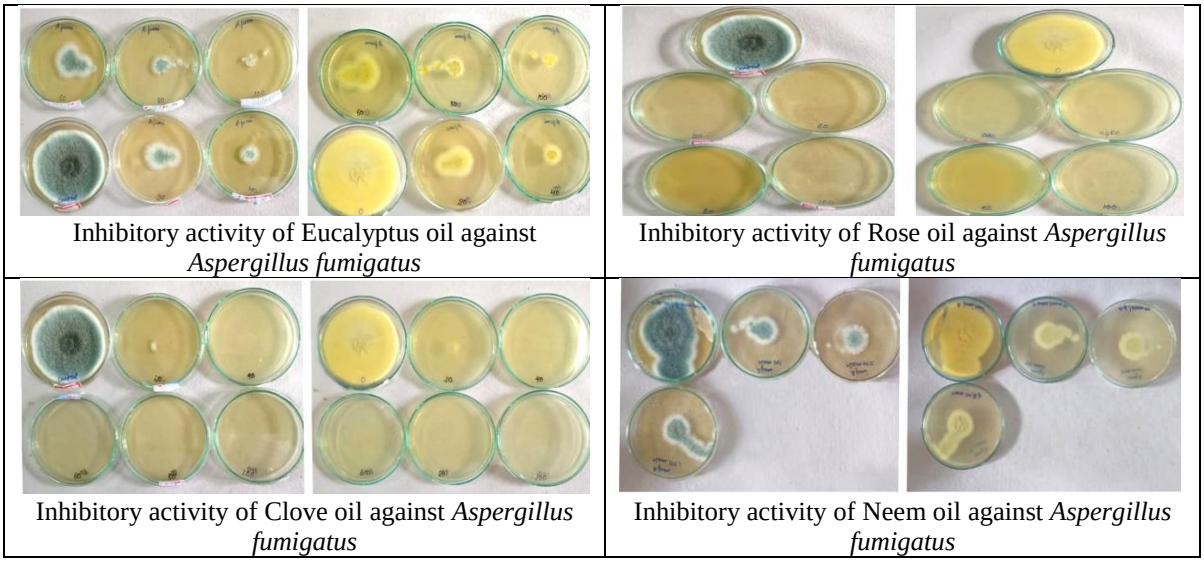
TABLE 2: Inhibitory activity of essential oils against *Aspergillus niger*

Concentration	Eucalyptus oil	Neem oil	Clove oil	Rose oil
100%	28 mm	_	Complete inhibition	Complete inhibition
80%	_	Negative inhibition	Complete inhibition	Complete inhibition
60%	28 mm	_	Complete inhibition	Complete inhibition

40%	_	Negative inhibition	Complete inhibition	Complete inhibition
20%	35 mm	Negative inhibition	Complete inhibition	_
Control	Maximum growth	Maximum growth	Maximum growth	Maximum growth

TABLE 3: Inhibitory activity of essential oils against *Fusarium oxysporum*

Concentration	**Eucalyptus oil**	**Neem oil**	**Clove oil**	**Rose oil**
100%	39 mm	Complete inhibition	Complete inhibition	Complete inhibition
80%	_	_	Complete inhibition	Complete inhibition
60%	35 mm	Complete inhibition	Complete inhibition	Complete inhibition
40%	34 mm	35 mm	Complete inhibition	Complete inhibition
20%	34 mm	35 mm	Complete inhibition	Complete inhibition
Control	Maximum growth	Maximum growth	Maximum growth	Maximum growth



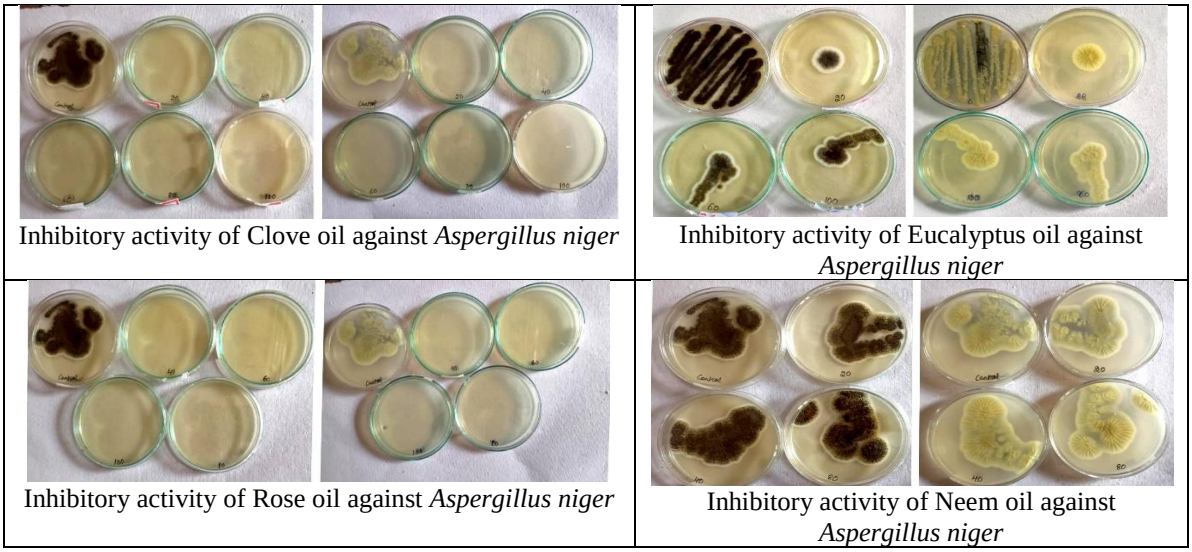
Inhibitory activity of Eucalyptus oil against *Aspergillus fumigatus*

Inhibitory activity of Rose oil against *Aspergillus fumigatus*

Inhibitory activity of Clove oil against *Aspergillus fumigatus*

Inhibitory activity of Neem oil against *Aspergillus fumigatus*

FIG 1: Inhibitory activity of essential oils against *Aspergillus fumigatus*



Inhibitory activity of Clove oil against *Aspergillus niger*

Inhibitory activity of Eucalyptus oil against *Aspergillus niger*

Inhibitory activity of Rose oil against *Aspergillus niger*

Inhibitory activity of Neem oil against *Aspergillus niger*

FIG 2: Inhibitory activity of essential oils against *Aspergillus niger*

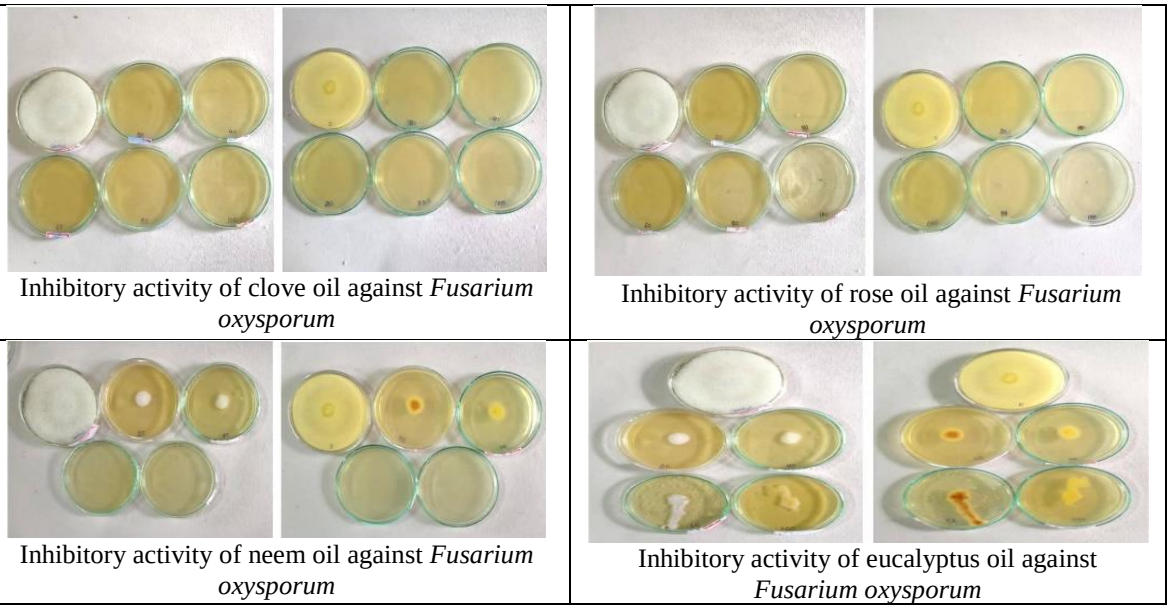


Fig 3: Inhibitory activity of four essential oils against *Fusarium oxysporum*

CONCLUSION

Results obtained from this study confirm the effective fungitoxicity of four essential oils viz., Clove oil (*Syzygium aromaticum*), Eucalyptus oil (*Eucalyptus globulus)*, Neem oil (*Azadirachta indica*) and Rose oil (*Rosa rubiginosa*) at concentrations of 20%, 40%, 60%, 80% and 100% respectively on *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium oxysporum* associated with *Sorghum vulgare* and *Zea mays* by poisoned food technique. Although significant antifungal activity was shown by all the four essential oils, clove oil and rose oil demonstrated better activity in all parameters studied than neem oil and eucalyptus oil. These essential oils could be recommended for *Sorghum vulgare* and *Zea mays* seeds treatment to maintain seed quality during storage. However, it is vitally important that more studies should be carried out to better understand manner of action of these oils against the pathogenic fungi.

REFERENCES:

1. Jain, Archana et al. “A review of plant leaf fungal diseases and its environment speciation.” Bioengineered vol. 10,1 (2019): 409-424. Doi:10.1080/21655979.2019.1649520
2. Marcianò D, Mizzotti C, Maddalena G and Toffolatti S (2021) The Dark Side of Fungi: How They Cause Diseases in Plants. Front. Young Minds. 9:560315. Doi: 10.3389/frym.2021.560315
3. Petit AN, Fontaine F, Vatsa P, Clément C, Vaillant-Gaveau N. Fungicide impacts on photosynthesis in crop plants. Photosynth Res. 2012 Mar;111(3):315-26. Doi: 10.1007/s11120-012-9719-8. PMID: 22302592.
4. Baćmaga, Małgorzata et al. “The effect of the Falcon 460 EC fungicide on soil microbial communities, enzyme activities and plant growth.” Ecotoxicology (London, England) vol. 25,8 (2016): 1575-1587. Doi:10.1007/s10646-016-1713-z
5. Potential Health Effects of Pesticides, Eric S. Lorenz, https://extension.psu.edu/potential-health-effects-of-pesticides/#:~:text=Fungicides,irritation%2C%20sneezing%2C%20and%20coughing.
6. Anžlovar S, Likar M, Koce JD Antifungal potential of thyme essential oil as a preservative for storage of Wheat Seeds. Acta Botanica Croatica, 2017; 76(1): 64–71.
7. Krisch J, Tserennadmid R, Vagvölgyi C (2011) Essential Oils against yeasts and moulds spoilage. In: Mendez-Vilas A (Ed.) Science against microbial pathogens: communicating current research and technological advances – 2, Formatex Research Center, Badajoz, Spain. Pp 1135 –1142.
8. Sivakumar D, Bautista-Baños S (2014) A review on the use of essential oils for postharvest decay control and Maintenance of fruit quality during storage. Crop protection 64: 27 – 37
9. ISTA (International Seed Testing Association) (1993) International rules for seed testing. Seed Science and Technology 21 (Suppl.): 1 – 75.
10. Shohayeb, Mohamed & Saleh, El-Sayed & Bazaid, S.A. & Maghrabi, Ibrahim. (2014). Antibacterial and antifungal activity of Rosa damascena MILL. Essential oil, different extracts of rose petals. Global Journal of Pharmacology. 8. 1-7. 10.5829/idosi.gjp.2014.8.1.81275.
11. Rana, Inder Singh et al. “Evaluation of antifungal activity in essential oil of the Syzygium aromaticum (L.) by extraction, purification and analysis of its main component eugenol.” Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology] vol. 42,4 (2011): 1269-77. Doi:10.1590/S1517-83822011000400004
12. PUJIARTI, Rini; HADI NURJANTO, Handojo; SUNARTA, Sigit. Antifungal Activity of Eucalyptus Oil Against Aspergillus niger and Fusarium oxysporum. AGRIVITA, Journal of Agricultural Science, [S.l.], v. 40, n. 1, p. 55-62, dec. 2017. ISSN 2477-8516.
13. M. Ashfaq Ahmed*, Gayathri R, Vishnupriya V, Anti-fungal Activity of Neem Oil, Int. J. Pharm. Sci. Rev. Res., 39(1), July – August 2016; Article No. 38, Pages: 200-202

14. Sharma A, Rajendran S, Srivastava A, Sharma S, Kundu B. Antifungal activities of selected essential oils against Fusarium oxysporum f. sp. lycopersici 1322, with emphasis on Syzygium aromaticum essential oil. J Biosci Bioeng. 2017 Mar;123(3):308-313. doi: 10.1016/j.jbiosc.2016.09.011. Epub 2016 Nov 18. PMID: 27876218.
15. Gakuubi MM, Maina AW, Wagacha JM. Antifungal Activity of Essential Oil of Eucalyptus camaldulensis Dehnh. against Selected Fusarium spp. Int J Microbiol. 2017;2017:8761610. doi: 10.1155/2017/8761610. Epub 2017 Jan 3. PMID: 28127308; PMCID: PMC5239988.
16. Adepoju, Adeyinka & Femi-Adepoju, Abiola & OGUNKUNLE, Tunde. (2014). ANTIFUNGAL ACTIVITIES OF SEED OIL OF NEEM (Azadirachta indica A. Juss.). ANTIFUNGAL ACTIVITIES OF SEED OIL OF NEEM (Azadirachta indica A. Juss.). Vol.3(1):106-109. 106-109
17. Girish K, Chaithra TS, Fathima SK (2021) Effect of essential oils on seed borne Fusarium sp., and seed quality of sesame (Sesamum indicum L.). Journal of Tropical Life Science 11 (1): 101 – 109. doi: 10.11594/jtls.11.01.13.