The Pharma Innovation Journal 2022; 11(9): 2928-2931

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 2928-2931
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www.thepharmajournal.com
Received: 14-06-2022
Accepted: 29-07-2022

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Efficacy of certain essential oils against leaf spot disease (*Alternaria alternata*) of Sarpgandha (*Rauvolfia serpentina* L.)

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Abstract
Sarpagandha (*Rauvolfia serpentina*) roots contains many important alkaloids, like Ajmalicine, Ajmaline, isoajmaline, rauvolfinine, reserpine, serpentine, rescinnamine, tetraphylicine, yohimbine and 3 epi-yohimbine. Different genera of fungi cause infection to the foliage and roots of Sarpagandha, makes responsible for decreased biochemical productivity. The foliar parts causing light brown to dark brown, roundish-oval to irregular spots were observed in plants. Microscopic observation of infected leaf samples revealed association of foliar pathogen namely *Alternaria alternata*. The pot experiment was conducted in the Department of Plant pathology, SHUATS, in kharif season 2021-22, it was analysed using C.R.D (Completely Randomized Design) with seven treatments and four replications of each treatment. The treatments were Control (Untreated) T_0, Bavistin (Treated check) T_6 (2%), Clove oil (2%) T_1, Neem oil (2%) T_2, Lemongrass oil (2%) T_3, Clove oil (1%) + Neem oil (1%) T_4, Clove oil (1%) + Lemongrass oil (1%) T_5 was taken thrice as foliar spraying accordingly. Observations were recorded at successive stage of plant growth on various parameters such as percent disease incidence, plant height and root mass. Evaluation *was* done for the effect of three essential oils, their combinations and one fungicide (treated check) *in vivo* against *Alternaria alternata* causing leaf spot of Sarpgandha. The evaluation results shows that all the treatments was found significantly reduced the disease incidence as compared to control. Increased the growth parameters was recorded. It was recorded in Neem oil minimum percent disease incidence (13.23%), followed by Clove oil+ Neem oil (14.2%), Clove oil + Lemongrass oil (20.3%), Clove oil (20.6%) and Lemongrass oil (20.6%). Whereas, Clove oil + Neem oil were recorded the maximum plant height (29.4 cm).

Keywords: Sarpgandha, *Alternaria alternata*, Bavistin, Neem oil, clove oil

Introduction
Sarpagandha (*Rauvolfia serpentina*) belongs to the family Apocynaceae; it is an evergreen, perennial shrub, erect of the height upto 0.6-1 meter and has cylindrical stems. Leaves are simple and opposite, more commonly arranged in whorls of 3 to 5 and bright green in colour, flowers are in irregular corymbose inflorescences with white and pink colour. Plants which are raised from stem cutting yield about 1-2 tones/ha and the plants from root cutting gives productivity of 3-4 tones/ ha (Pande *et al*., 2017) [4]. This plant is found in Bangladesh, Bhutan, China, Indonesia, India, Lao PDR, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand and Vietnam (De *et al*., 2010) [1]. It is found all over in India, mostly in Bihar, Bengal, Uttar Pradesh, and Maharashtra.

Its root contains many important alkaloids, like Ajmalicine, Ajmaline, Isoajmaline, Rauvolfinine, reserpine, serpentine, rescinnamine, tetraphyllicine, yohimbine and 3 epi-yohimbine (Pande *et al*., 2017) [4]. The juice of the leaves has been used as a remedy for opacity of the cornea and also to prevent inflammation (Thakur *et al*., 2016) [8].

Sarpagandha crop suffer from a number of leaf spot diseases caused by many fungal pathogens such as *Cercospora rauwolfia*, *Alternaria tenuis*, *Alternaria alternata*, *Macrophomina phaseolina* (Pande *et al.*, 2017) [4].

Puni and Harsh, 2009 observed that leaf spot is one of the most wide spread disease which appears usually in July and August. It is caused by *Alternaria alternata* (Fr.) Keissler. The symptoms of this disease appears as minute yellow spots which gradually increase in size, turns dark and the leaves fall off (Thakur *et al*., 2016) [9]. This pathogen mostly affects the foliar parts (Nagrale *et al*., 2013) [3]. Several methods are used for the control of the disease such as physical, chemical and biological. The excess use of fungicide is found harmful to the soil, humans and environment.

Hence, there is a need for adopting biological and eco-friendly methods of control.

The essential oils play a vital role in plant defence mechanisms against phytopathogenic microorganisms. Antimicrobial activity of clove oil has been reported to inhibit the growth of melds, yeasts and bacteria. Eugenol is the main component of clove oil (Sukatta *et al*., 2008) [7]. Neem oil has a great potential to control various phytopathogenic fungi and, therefore, has much prospect to be used as a good fungicide (Dubey *et al*., 2009) [2]. Lemongrass (*Cymbopogon citrates* L.) oil was reported to be antifungal against several plant pathogens. The antagonistic activity of these essential oils was studied at different concentrations (1%, 2% and 4%) on Alternaria sp. (Ragupathi *et al*., 2020) [5]. With this in view the present work was designed to study the efficacy of different essential oils against *Alternaria alternata*.

Material and Methods

The present study was conducted under the pot condition. Pot experiment was laid out in Completely Randomized Design at the Department of Plant Pathology, SHUATS, Prayagraj, during the kharif season of 2021-2022. Prayagraj is located at 25.45°N 81.84°E in the southern part of the Uttar Pradesh at an elevation of 98 meters (322 ft.) and stands at the confluence of two, the Ganges and Yamuna.

Sarpgandha was propagated by seeds; seeds were sown in well prepared nursery beds. The nursery bed is from ground level prepared by mixing with FYM and Cow dung. Seeds were sown by line sowing method at the depth of 1 to 3 cm further light irrigation were given, germination of the seed started after one week of sowing. Pots were prepared by adding FYM and cow dung to it. About 20 days old seedlings were transplanted in pots. Transplanting was done in 28 pots with 4 replications and 7 treatments with control. Healthy seedlings were planted to pot carefully teasing apart the tangled mass of root.

The culture media used in experiment was prepared according to standard formula. For isolating and culturing of pathogen (*Alternaria alternata*) potato Dextrose Agar (PDA) medium was used.

Isolation of the pathogen: The infected leaf which showed typical symptoms of disease was used for the isolation of pathogen. The standard tissue isolation procedure was followed to isolate the pathogen. The infected parts was surface sterilized with 0.1% mercuric chloride (HgCl2) solution for 3 seconds and washed separately three times in sterilized distilled water to remove the traces of mercury if any and then transferred to sterilized petri-plates for removal of moisture. 3 petri plates were kept in Laminar Air flow chamber and % lactic acid was added to each plate. Then PDA media was poured in all the petri plates. The petri plates was incubated in BOD at temperature 25 ± 2^0C and observed periodically for the growth of pure colonies. The pure colonies which developed from the bits were transferred to PDA slants and incubated for 15 days. Then such slants were used to study characters.

The cultures of the fungus was sub-cultured on PDA slants and kept in laboratory at 28±1 °C for 15 days. Such mother culture slants was preserved at 4°C in refrigerator. Further, these cultures will be sub-cultured once in a month and used for future purpose.

Spore suspension was prepared in the laboratory. Sterile distilled water was added to petriplates which contained 10 days old *A. alternata* culture. Then the surface of the agar was scraped with the help of a sterilized spatula to remove hyphae and spores. The contents of few plates were poured into a beaker through muslin cloth to remove agar and also the large fragments of mycelium. The spore concentration was checked with the help of a Hemocytometer and then adjusted it to approximately 5 x 10^5 spores per ml of *A. alternata.* The spore suspension (5 x 10^5 per ml) of *Alternaria alternata* was sprayed twice to leaves and roots at first week and second week of plant age. Twenty-four plants were inoculated with the pathogen except the control plants (Haque *et al*., 2022) [10]. After 20 days of transplanting, leaves of sarpgandha showing typical symptoms of dark coloured spots with concentric rings caused by *Alternaria alternata* were collected from experimental pots in the Department of Plant Pathology, SHUATS, Naini, Prayagraj, Uttar Pradesh.

Identification of *Alternaria alternata* was done as soon as it is isolated in a pure form, the microscopic slide was prepared and put it on the stage of microscope and the morphological characters were noted.

Morphology of the pathogen is observed as mycelium initially hyaline, becoming pale brown to olive- brown, branched, Septate, 2-7µm wide. Conidiophores are straight and Flexous similar to mycelium in colour, Septate, unbranched or occasionally branched, erect, broader towards the distal end, on the host single or fasciculate, emerging through stomata, Amphigenous, geniculate or straight, length variable, between septa 20-63 x 9-18 µm. Conidia are obclavate to obpyriform or ellipsoid, short conical beak at the tip, or beakless, surface is smooth. Several vertical and 8 transverse septa are present.

Bavistin (2%) was used as treated check to compare the effect of essential oil in. Three essential oils, namely Neem, Clove and Lemon grass were purchased from market. The essential oils were applied by dissolving in 0.5 ml of 0.1% Tween 80 before use. 2% concentration of each essential oil was prepared. The spraying was done immediately after the appearance of the disease. Spraying of essential oils was done at an interval of 15 days. The readings for disease incidence were taken at 30, 60, and 90 DAT.

Percent disease incidence (PDI) was calculated using the following formula: (Pande *et al*., 2017) [4].

$$\text{PDI} = \frac{\text{Number of infected leaves}}{\text{Total no. of leaves in Plant}} \times 100$$

Results

The results presented in Table 1 and Figure 1, revealed that all the treatments were significant and reduced the infection of *Alternaria alternata* on leaf of Sarpgandha as compared to control.

The disease incidence at 30 DAT, after foliar spraying of essential oils and fungicide in treatment T_6 (3.9%), T_2 (8.9%), T_4 (12.5%) were significantly reduced the infection of *Alternaria alternata* on leaf of Sarpgandha as compared with control T_0 (25.6%). Among the treatments (T_6, T_4, T_2), (T_2, T_4, T_1, T_5, T_3), (T_1, T_5, T_3, T_0) are found non- significant from one another.

The disease incidence at 60 DAT, after foliar spraying of essential oils and fungicide in treatment T_6 (9.6%), T_2

(12.8%), T_4 (14.8%) were significantly reduced infection of *Alternaria alternata* on leaf of Sarpagandha as compared with control T_0 (36.2%). Among the treatments (T_6, T_2, T_4), (T_2, T_4, T_3, T_5), (T_3, T_5, T_1), are found non-significant from one another.

The disease incidence at 90 DAT, after foliar spraying of essential oils and fungicide, in treatment T_6 (13.0%), T_4 (15.3%), T_2 (18%), T_1 (23.7%), T_3 (25.9%), T_5 (26.1%), were significantly reduced infection of *Alternaria alternata* on leaf of Sarpgandha on comparison with control T_0 (42.2%). Among the treatments (T_6, T_4, T_2, T_1, T_3) are found non-significant from one another.

Table 1: Effect of treatments on incidence of *Alternaria alternata* of Sarpagandha at 30 DAT, 60 DAT, 90 DAT

S. No	Treatments	Disease incidence (%)		
		30 DAT	60 DAT	90 DAT
T0	Control	25.6	36.2	42.2
T1	Clove oil	15.3	23	23.6
T2	Neem oil	8.9	12.8	18
T3	Lemongrass oil	18.2	17.8	25.8
T4	Clove oil + Neem oil	12.5	14.8	15.3
T5	Clove oil + Lemongrass oil	16	19	26
T6	Bavistin	3.9	9.6	13
	F- test	S		
	S. Ed (±)	2.08		
	C.D (5%)	4.53		

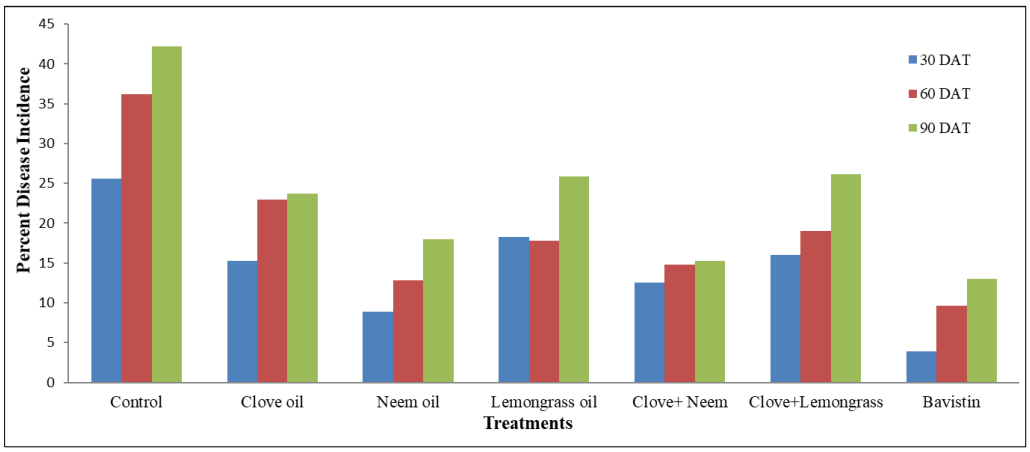

Fig 1: Effect of treatments on Percent Disease Incidence of *Alternaria alternata* causing leaf spot of Sarpgandha at different intervals of time

The results of Table 2 and Figure 2 revealed that all the treatments significantly increased the plant height. At 30 DAT, after foliar spraying of essential oils and fungicide, in treatment (T_0, T_3, T_5) significantly reduced the plant height of Sarpgandha as compared with T_4 (23.4), T_2 (23.5) and T_6 (24.8). Among the treatments (T_6, T_2), (T_2, T_4, T_1), (T_1, T_5, T_3, T_0) are found non- significant from one another.

The plant height (cm) at 60 DAT, after foliar spraying of essential oils and fungicide, in treatment (T_0, T_3, T_5, T_1) significantly reduced the plant height of Sarpgandha as compared with T_4 (24), T_2 (24.2), and T_6 (25.4). Among the treatments (T_6, T_2, T_4), (T_4, T_1), (T_1, T_5, T_3, T_0) are found non-significant from one another.

The plant height (cm) at 90 DAT, after foliar spraying of essential oils and fungicide, in treatment (T_0, T_3, T_5, T_1), significantly reduced the plant height of Sarpgandha as compared with T_2 (38.3), T_4 (41.0), T_6 (44.5). Among the treatments (T_6, T_4, T_2, T_1), (T_4, T_2, T_1, T_5, T_3), (T_1, T_5, T_3, T_0) are found non- significant from one another.

Table 2: Effect of treatments on plant height (cm) of Sarpgandha at 30 DAT, 60 DAT, 90 DAT

S. No	Treatments	Plant Height (cm)		
		30 DAT	60 DAT	90 DAT
T0	Control	20.5	20.8	24.3
T1	Clove oil	21.5	22.3	33.8
T2	Neem oil	23.5	24.2	38.3
T3	Lemongrass oil	20.6	21.3	29.6
T4	Clove oil + Neem oil	23.4	24	41
T5	Clove oil + Lemongrass oil	21	21.6	31.7
T6	Bavistin	24.8	25.4	44.5
	F- test	S		
	S. Ed (±)	1.78		
	C.D (5%)	5.50		

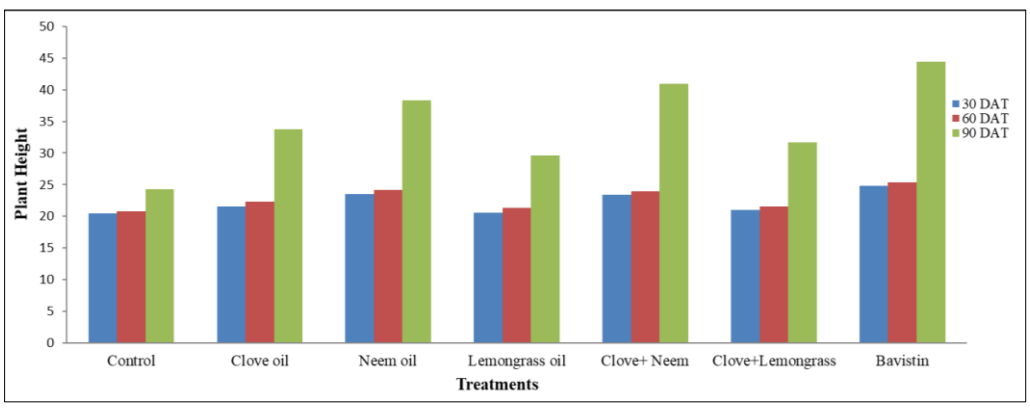

Fig 2: Field efficacy of treatments on the plant height (cm) of Sarpgandha at different intervals of time

Discussion

Application of chemicals is one of the most effective and widely recommended methods of disease control but is not economical and eco-friendly. Essential oils are being used as an alternative to chemicals. Use of essential oils can reduce the use of chemicals. It is cheap, eco-friendly and easy method for the management of the disease. The use of chemicals has been discouraged in the present system of management of any disease. But as a treated control Bavistin (2%) was taken and found effective against *Alternaria alternata* with lowest disease incidence percentage (8.83%).

The fungicidal spectrum of Clove oil (2%) has already been investigated by Beg *et al*. (2002) against *Alternaria alternata*. In his study, he reported that clove oil (0.05%) has fungi static properties. The efficacy of neem oil has been investiagted by Sitara *et al*. (2008); she reported the antifungal activity of neem oil @ 0.5, 0.1 and 0.15%. The efficacy of Lemongrass oil against *Alternaria alternata* has also been effective, it was investigated by Rajagopal *et al*. (2009), he reported antifungal efficacy of lemongrass oil (0.5%). But in the present study all these three essential oils showed inhibition of growth of pathogen (20.63%, 13.23%, 20.63%) respectively.

Eco- friendly management has encouraged the plant protection specialists to go for use of essential oils for the management of diseases. Also this can avoid the pollution of air, water and soil. With this idea, three plant essential oils which are previously known for their antifungal nature were evaluated against *Alternaria alternata*. The essential oils used were Clove oil, Neem oil, Lemongrass oil and the combination of these three.

Conclusion

In the present study management of Alternaria leaf spot disease was done with treatments of three essential oils *viz*., Clove oil, Neem oil, and Lemongrass oil. Neem oil @ 2% recorded minimum percent disease incidence. Whereas, Clove oil @ 1% + Neem oil @ 1% recorded the maximum plant height.

Acknowledgements

The authors are thankful to the SHUATS University official, Department of the Plant Pathology for their continuous support during my thesis work. NAI, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, and for government of Uttar Pradesh for providing all necessary facilities and support.

References

1. Dey Abhijit, De JN. *Rauvolfia serpentina* (L). Benth. ex Kurz.-A Review. Asian Journal of Plant Sciences. 2010;9(6):285-298.
2. Dubey RC, Kumar H, Pandey RR. Fungitoxic Effect of Neem Extracts on Growth and Sclerotial Survival of Macrophomina phaseolina *in vitro*. Journal of American Science. 2009;5(5):17-24.
3. Nagrale Dipak T, Gaikwad Anil P, Sharma L. Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler blight of gerbera (Gerbera jamesonii H. Bolus ex J.D. Hook). Journal of Applied and Natural Science. 2013;5(1):171-178.
4. Pande SK, Chaubey AN, Singh V, Pal K. Eco-friendly management of Cercospora leaf spot (*Cercospora rauvolfia*) of Sarpgandha. Plant Archives. 2017;17(1):546-548.
5. Ragupathi KP, Renganayaki1 PR, Sundareswaran S, Mohan S, Kamalakannan A. Evaluation of essential oils against early blight (*Alternaria solani*) of Tomato. International Research Journal of Pure & Applied Chemistry. 2020;21(24):164-169.
6. Refai Mohamed, El-Yazid Heidy Abo. A guide for description of dematiaceous fungi fungi of medical importance, diseases caused by them, diagnosis and treatment. Department of Microbiology, Faculty of Veterinary Medicine, Cairo University; c2014.
7. Sukatta U, Vichai H, Walairut C, Uraiwan D, Panuwat S 2008. Antifungal Activity of Clove and Cinnamon Oil and Their Synergistic against Postharvest Decay Fungi of Grape *in vitro*. Kasetsart Journal (Nat. Sci.). 2014;42:169-174.
8. Thakur S. Application of phylloplane fungi to manage the Leaf spot of *Rauwolfia serpentina* caused by *Alternaria alternata*. International Journal of Life Sciences Scientific Research. 2016;2(2):163-172.
9. Thakur S, Harsh NSK. Biocontrol Potential of Phylloplane Fungi against *Alternaria Alternata* Causing Leaf Spot in *Rauwolfia serpentina in vitro*. International Journal of Scientific research. 2016;5(2).
10. Haque E Md, Parvin SM. *In vitro* and *in vivo* Pathogenicity of A. alternate to Sugar beet Assessment of sensitivity to Fungicides. Bio Rxiv; c2022.