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Evaluation of Essential Oils against Early Blight (*Alternaria solani*) of Tomato

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Authors' contributions

This work was carried out in collaboration among all authors. Author KPR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KPR and SS managed the analyses of the study. Authors SMK and AK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The fungicidal effect of some essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass, Thyme and Clove were evaluated against *Alternaria solani* causing early blight of tomato resulting in severe yield loss. All essential oil treatments showed the significantly varied antifungal activity against the pathogen. Among the in-vitro screened essential oils percent growth inhibition of pathogen was higher in Thyme oil (80%) and it was followed by Clove oil and Eucalyptus oil. Plant biometric observations *viz.,* seed germination (84%) and seedling vigor (1747) was maximum with lower percent disease index (13%) was recorded in Thyme oil treated seeds and seedlings of tomato cultivar PKM 1.

Keywords: Essential oils; Alternaria solani; lemongrass; thyme; clove.

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is one of the most important vegetables. The fungus *Alternaria solani* (Ellis and Martin) Jones and Grout is the causal agent of early blight disease, is a major pathogen of tomato causing considerable yield loss. Species of the genus *Alternaria* are cosmopolitan, surviving both as saprophytes as well as weak parasites. The genus is characterized by the formation of polymorphous conidia either singly or in short or longer chains and provided with cross, longitudinal as well as oblique septa and having longer or short beaks. The spores of these polyphagus fungi occur commonly in the atmosphere and also in soil. The telomorphs (sexual stage) were known in a very few species and placed in the genus *Pleospora,* in which sleeper-shaped, muriform ascospores were produced in bitunicate asci [1]. To reduce the use of chemical antifungals, the study of natural products exhibiting antifungal activity has become extremely important since plants have physiological properties or defense mechanisms allow them to metabolize active compounds generating protection against insects and preventing pathogens invasion. [2] Essential oils properties such as antioxidant and antimicrobial are known for a long time and hence widely used in traditional medicines and food industries. [3] The plant essential oils have potential to be developed as potent fungicides in organic farming. [4] Recently, there has been increasing interest in essential oils and their components as possible substitutes for conventional synthetic pesticides due to concern over ecosystem pollution and pesticide resistance in pests and pathogens. There are several previous studies on fungicidal properties of various essential oils [5]. Lemongrass (*Cymbopogon citrates* L.) oil was reported to be antifungal against several plant pathogens. Carnation, caraway, thyme, peppermint, geranium and several other essential oils also have been found to have inhibitory effects against the mycelial growth of several plant pathogens under *in vitro* conditions. [6]. Under *in vitro* conditions, all concentrations of melaleuca essential oil reduced the mycelial growth of *A. alternata.* [7]. The best antifungal activity of essential oils were observed in species of Mentha and Thymus. [8]. The most effective antifungal effect on both *Fusarium oxysporum* and *Botrytis cinerea* was in the essential oil of thyme. [9]. *Alternaria solani* can infect every part of the plant (causing foliage blight, fruit lesions and stem collar rot) and can damage during all stages of plant development [10]. So management of this disease is very necessary. Use of resistant varieties is the ultimate control of this disease. Wide use of chemical fungicides often leads to serious environmental problems besides affecting the health of ecosystem. So, it is necessary to minimize the use of chemicals for controlling disease. Hence, the attempt has been made to evaluate at both *in-vitro* and *in-vivo* conditions of some predominant native essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass*,* Thyme and Clove against *Alternaria solani*, as it will be use full in formulating eco-friendly management strategies for early blight of tomato.

2. MATERIALS AND METHODS

The pathogen was isolated and pathogenicity was proved. Essential oils efficacy at both *in-vitro* and *in-vivo* conditions in the seeds and seedlings of tomato cultivar PKM 1 was studied by following below methodologies.

2.1 Isolation of Pathogen and Pathogenicity Test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and sub culture was used for pathogenicity test by following Koch's postulate. The pathogenicity test was carried by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After inoculation, the symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease. Thus pathogenicity on tomato was confirmed. [11]

2.2 *In-vitro* Efficacy of Essential Oils on Mycelial Growth of *Alternaria solani* by Poisoned Food Technique

Bio-efficacy of essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass*,* Thyme and Clove were evaluated *in vitro,* against *Alternaria solani*. The antagonistic activity of these essential oils were studied at different concentrations (1%, 2% and 4%) on *A. solani* by poisoned food technique. The efficacy of essential oils was tested against *A. solani* for radial growth inhibition on the Potato dextrose

agar medium using poisoned food technique under in vitro condition. Twenty ml of poisoned medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of essential oils. *Alternaria solani* mycelial disc of 5 mm taken from the periphery of 14 days old colony was placed in the centre of Petri incubated at 27 ± 1°C for 14 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and an average value was recorded. Percent inhibition of mycelial growth of the fungus was calculated by using the formula by Vincent [12].

$$\text{Percentage of inhibition} = \frac{A - B}{A} \times 100$$

Where, A = Growth of the pathogen in the absence of essential oil
B = Growth of the pathogen in the presence of essential oil

2.3 *In-vivo* Efficacy of Bio-Control Agents on *Alternaria solani*

Seeds were treated with essential oils at 4% concentration for 1h and dried back to original moisture content. Pin prick method of *Alternaria solani* inoculation was followed. The leaves were injured with sterilized pin and the mycelial disc of pathogen was placed over the injured leaf portion and covered with moist cotton and incubated in the moist chamber. The plants were sprayed frequently with water for 2 days. After 48 h, the plants were sprayed with different essential oils. The plant biometric observations *viz.,* Germination %, Root length, Shoot length, Vigour index was recorded and the per cent disease index (PDI) was calculated by using following formula proposed by Wheeler [13].

$$\text{PDI} = \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/ leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

2.4 Germination (%)

Seeds were treated with essential oils at 4% concentration for 1h and then shade dried. Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at 25± 2° C and 90 ±2 per cent relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. [14]

2.5 Root Length and Shoot Length

On fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm. [15].

Table 1. Essential oils used for testing their efficacy against *Alternaria solani*

S.No	Common name	Botanical name	Family
1	Neem	*Azadirachta indica Juss*	Meliaceae
2	Eucalyptus	*Eucalyptus globulus Labill*	Myrtaceae
3	Pongamia	*Millettia pinnata*	Fabaceae
4	Lemongrass	*Cymbopogon citratus*	Poaceae
5	Thyme	*Thymus vulgaris*	Lamiaceae
6	Clove	*Syzygium aromaticum*	Myrtaceae

Table 2. Description of disease scale [16]

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	21-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem and fruits

2.6 Vigour Index

The Vigour index was calculated and compared by adopting the following formula and expressed as whole number. [17]

3. RESULTS AND DISCUSSION

3.1 *In-vitro* Efficacy of Essential Oils on Mycelial Growth of *Alternaria solani*

All the essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass*,* Thyme and Clove showed significantly different anti-fungal activity against the pathogen. Among the various essential oils, 4% Thyme oil recorded the minimum mycelial growth of the pathogen (18.45 mm) and exhibited the maximum percent growth inhibition of pathogen (79.50%) and it was followed by Clove oil and Eucalyptus oil. Marandi et al. determined that thyme oil had the strongest antifungal activity in *in vitro* conditions and completely inhibited the fungal growth. Similar results were obtained by Palfi et al. [18]; Duduk et al. [19]; Adabayo et al. [20]; Hung et al. [15]; Lu et al. [21]; Moghaddam et al. [22] and Nguyen et al. [23]. Antifungal properties of essential oils depend on the properties of plants they are extracted from [24] and their chemical composition [25]. (Table 3)

3.2 *In-vivo* Efficacy of Essential Oils Agents on *Alternaria solani*

In-vivo efficacy of essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass*,* Thyme and Clove showed significantly different anti-fungal activity against the pathogen on seeds and seedlings of tomato cultivar PKM 1. Among the various essential oils, 4% Thyme oil recorded the maximum seed germination (84%), shoot length (6.9 cm), root length (13.9 cm), vigour index (1747) and also exhibited the lowest percent disease incidence (12.93%) and it was followed by Clove oil and Eucalyptus oil. Control treatment recorded the minimum seed germination (61%)

Table 3. *In-vitro* efficacy of Essential oils on mycelial growth of *Alternaria solani*

Essential oils	1%		2%		4%	
	Average Colony diameter (mm)	Per cent Growth Inhibition (%)	Average Colony diameter (mm)	Per cent Growth Inhibition (%)	Average Colony diameter (mm)	Per cent Growth Inhibition (%)
Neem	55.29	38.57	42.65	52.61	33.82	62.42
Eucalyptus	46.67	48.14	34.12	62.09	25.19	72.01
Pongamia	58.91	34.54	47.56	47.16	37.72	58.09
Lemongrass	49.28	45.24	38.93	56.74	29.41	67.32
Thyme	30.34	66.29	23.83	73.52	18.45	79.50
Clove	39.47	56.14	27.76	69.16	21.87	75.70
Control	90.00	-	90.00	-	90.00	-
Mean	52.85	48.16	43.55	60.21	36.64	69.17
SEd	0.91	0.84	1.09	1.19	1.01	1.78
C D (P = 0.05)	1.96	1.83	2.35	2.60	2.15	3.89

Table 4*. In-vivo* efficacy of Essential oils on *Alternaria solani* on the biometrics of tomato cultivar PKM 1

Essential oils	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index	Percent Disease Index (%)
Neem	72	6.3	13.2	1404	31.18
Eucalyptus	79	6.7	13.6	1604	22.91
Pongamia	70	6.2	13.0	1344	38.89
Lemongrass	75	6.5	13.5	1500	27.64
Thyme	84	6.9	13.9	1747	12.93
Clove	81	6.8	13.7	1661	17.21
Control	61	6.1	12.9	1159	70.23
Mean	74.57	6.50	13.40	1488	31.57
SEd	1.82	0.12	0.25	25.87	0.88
C D (P = 0.05)	3.90	0.26	0.55	55.48	1.89

and other biometrics with highest percent disease incidence (70.23%). The positive effect of essential oil of thyme in this research is in accordance with the research of Alam et al. [26]. Similar results were reported by Elshafie et al. [27]; Ćosić et al. [28]; Viuda-Martos et al. [29] and Lee et al. [30]. (Table 4).

4. CONCLUSION

The present investigation revealed that essential oils *viz.,* Neem, Eucalyptus, Pongamia, Lemongrass*,* Thyme and Clove had inhibitory effect against *Alternaria solani*. Hence these essential oils could be exploited as an alternate management strategy for chemical pesticides in the management of early blight of tomato.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Verma N, Verma S. Alternaria diseases of vegetable crops and new approaches for its control. Asian J. Exp. Biol. Sci. 2010;1(3):681-692.
2. Satish S, Mohana DC, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. J Agr Sci Tech. 2007;3(1):109-119.
3. Robin Raveau, Joël Fontaine and Anissa Lounès-Hadj Sahraoui. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A Review. Foods. 2020;9:365.
4. Nivedha M, Ebenezar EG, Kalpana K and Arun Kumar R. *In vitro* antifungal evaluation of various plant extracts against leaf blight disease of *Jasminum grandiflorum* caused by *Alternaria alternata* (Fr.) Keissler. Journal of Pharmacognosy and Phytochemistry. 2019;8(3): 2143-2147.
5. Sayago JE, Ordoñez RM, Negrillo L, Torres S, Isla MI. Antifungal activity of extracts of extremophile plants from the Argentine Puna to control citrus postharvest pathogens and Green mold. Postharvest Biol Technol. 2012;6719-24.
6. Díaz P, Cabrera A, Alem D, Larrañaga P, Ferreira F, Dalla M. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chilean JAR. 2011;71(2): 231-239.
7. Flávia Mota de Figuerêdo Alves, Kevison Romulo da Silva França and Ionaly Gomes de Araújo,. Control of *Alternaria alternata* using melaleuca essential oil (*Melaleuca alternifolia*). Journal of Experimental Agriculture International. 2019;40(3):1-10.
8. Tomasz M. Karpinski. Essential oils of lamiaceae family plants as antifungals. Biomolecules. 2020;10:103.
9. Marina Palfi, Paško Konjevoda, Karolina Vrandečić, Jasenka Ćosić. Antifungal activity of essential oils on mycelial growth of *Fusarium oxysporum* and *Bortytis cinerea.* Emirates Journal of Food and Agriculture. 2019;31(7):544-554.
10. Abada KA, Mostafa SH, Mervat R. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. Egyptian J. Appl. Sci. 2008;23:47–58.
11. Prathima P, Thiruvudainambi S, Kalpana K. Molecular characterization of *Alternaria alternata* (Fr.) Keissler Causing Leaf Blight Disease of Marigold. Int. J Pure App. Biosci. 2018;6(6):1286-1291.
12. Vincent JM. The esters of 4-hydroxybenzoic acid and related compounds. Part I. Methods for the study of their fungistatic properties. J. Soc. Chem. Ind. 194766:149-155.
13. Wheeler BEJ. An introduction to Plant disease. John Wily and Sons Limited, London. 1969:301.
14. ISTA. Proceedings of International Seed Test Association, International Rules for Seed Testing. Seed Sci Technol. 1993;21: 1152.
15. Hung VP, Chi PTL, Phi NTL. Comparison of antifungal activities of vietnamese citrus essential oils. Nat. Prod. Res. 2013;27(4-5): 506-508.
16. Datar VV, Mayee CD. Assessment of loss in tomato yield due to early blight. Indian Phytopath. 1981;3(4)191-195.
17. Abdul-Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. Crop Sci. 1973;13:630-633.
18. Palfi M, Konjevoda P, Vrandečić K, Ćosić J. Antifungalna aktivnost eteričnih ulja i njihovih glavnih komponenti na rast micelija Colletotrichum coccodes. Poljoprivreda. 2018;24(2):20-26.
19. Duduk N, Markovic T, Vasic M, Duduk B, Vico I, Obradovic A. Antifungal activity of three essential oils against *Colletotrichum acutatum*, the causal agent of strawberry anthracnose. J Essent. Oil Bearing Plants. 2015;18(3):529-537.

20. Adabayo O, Dang T, Belenger A, Khanizadeh S. Antifungal studies of selected essential oils and commercial formulation against *Botrytis cinerea*. J. Food Res. 2013;2(1):217-226.
21. Lu M, Han Z, Xu Y, Yao L. Effects of essential oils from Chinese indigenous aromatic plants on mycelial growth and morphogenesis of three phytopathogens. Flavour Fragr. J. 2013;28(2):84-92.
22. Moghaddam M, Pourbaige M, Tabar HK, Farhadi N, Hosseini SMA. Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. J. Essent. Oil Bearing Plants. 2013;16(4): 506-512.
23. Nguyen PA, Strub C, Fontana A, Schorr-Galindo S. Crop molds and mycotoxins: Alternative management using biocontrol. Biol. Control. 2017;104:10-27.
24. Piyo A, Udomsilp J, Khang-Khun P, Thobunluepop P. Antifungal activity of essential oils basil (*Ocimum basilicum* Linn.) and sweet fennel (*Ocimum gratissimum* Linn.): Alternative strategies to control pathogenic fungi in organic rice. Asian J. Food Agro-Ind. 2009;2-9.
25. Ćosić J, Vrandečić K, Jurkovic D. The effect of essential oils on the development of phytopathogenic fungi. In: Sharma, N (Ed.), Biological Controls for Preventing Food Deterioration: Strategies for Pre-and Postharvest Management, John Wiley and Sons, Ltd. 2014;273-291.
26. Alam SB, Benyelles NG, Dib MEA, N Djabou, N. Tabiti, J. Paolini, A. Muselli , J Costa. Antifungal activity of essential oils of three aromatic plants from Western Algéria against five fungal pathogens of tomato (*Lycopersicon esculentum* Mill). J. Appl. Bot. Food Qual. 2014;87:56-61.
27. Elshafie HS, Mancini E, Camele I, De Martino L, De Feo V. *In vivo* antifungal activity of two essential oils from Mediterranean plants against postharvest brown rot disease of peach fruit. Ind. Crops Prod. 2015;66:11-15.
28. Ćosić J, Vrandečić K, Poštić J, Jurković D, Ravlić M. In vitro antifungal activity of essential oils on growth of phytopathogenic fungi. Poljoprivreda. 2010;16(2):25-28.
29. Viuda-Martos, M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez J. Antifungal activity of lemon (*Citrus limon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. Food Control. 2008;19(12):1130-1138.
30. Lee SO, Choi GJ, Jang KS, Lim HK, Cho KY, Kim JC. Antifungal activity of five plant essential oils as fumigant against post-harvest and soilborne plant pathogenic fungi. Plant Pathol. J. 2007;23(2):97-102.

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