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Antimicrobial activity of essential oils against early blight of tomato under *in vitro* conditions

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Abstract

Fungal diseases limit tomato production, particularly the early blight caused by *Alternaria solani*. Disease control is usually done with synthetic fungicides, which have long-term residual effects and also contribute to environmental pollution. In this study, the effectiveness of essential oils from lemongrass (*Andropogon citratus*), citronella oil (*Cymbopogon nardus*), clove oil (*Syzygium aromaticum*), peppermint oil (*Mentha piperita*) and patchouli oil (*Pogostemon cablin*) were tested *in vitro* in suppressing the radial growth of *A. solani*. The suppression of fungal growth was determined by measuring the radial growth of the examined pathogen. The oils have shown a certain effectiveness against the pathogen in question. Lemongrass oil was found to be the best oil for disease suppression at concentrations of 0.02%, 0.06%, 0.1% (59.37%, 73.12% and 84.32%, respectively). Essential oils were equally effective as synthetic fungicides in limiting pathogen growth. However, for these products to be used effectively in agricultural fields, an industrial formulation may be required to increase their effectiveness.

Keywords: Early blight, essential oils, *Alternaria solani*, lemon grass oil

1. Introduction

Tomato (*Solanum lycopersicum* L.) is the most important vegetable in India. It is a widely used vegetable for its high nutritional value. However, India faced a serious disease problem that significantly reduced tomato yields. Early blight caused by *Alternaria solani* is a major disease among tomato leaf diseases. A common cooking ingredient in the Indian diet, tomatoes are used as raw fruits and processed foods such as soup, tomato sauce, gravy, marinade, pasta, and powder. Tomato paste and juice are very easy to digest, increase gastric secretion and purify the blood. They also contain nutrients and metabolites (folic acid, potassium, and vitamins A and C) that are important for human health. In India, the area, production and yield of tomatoes are 45.82 ha, from 20.7 million to 20-25 t / ha. With an 11% share of world tomato production, India is the world's largest tomato producer (United Nations FAOSTAT - 2019) [3].

2. Materials and methods

2.1 Collection and isolation of fungal pathogens

Leaf samples with typical symptoms from a farm, BHU, were taken, collected in plastic bags and sealed. They were taken to laboratory to isolate the pathogen. To obtain a culture of *A. solani*, the method described by Naik *et al.* (2010) [4] used the described standard tissue isolation method. The leaves were examined under a microscope to confirm the presence of the fungus. After confirming the presence of fungal spores, isolation was performed using a standard tissue extraction procedure as described below.

The infected leaves were brought to the laboratory and sterilized with ethyl alcohol using non-absorbent cotton. They were then cut into small 3 mm² pieces with a sterilized blade. The surface of the samples was sterilized with 0.1% mercury chloride (HgCl₂) solution for 30 seconds and washed three times for 60 seconds with sterile distilled water. Allow to air dry by placing on sterile filter paper. The samples were then transferred onto fresh Potato Dextrose Agar (PDA) medium in a Petri dish. These Petri dishes were incubated in B.O.D. at 25 ± 2°C. A pure culture of *A. solani* was obtained by cultivating hyphal tips. This mycelial growth was sub-cultured on the sloping surfaces of the PDA. This inoculated culture tube was stored in B.O.D. Incubator at 25 °C ± 2 °C for future research.

2.2 Extraction of essential oils from test plants.

The extraction of essential oils was carried out using a modified technique described by Adams, 2007. A vertical steam distillation unit consisting of a hot oven, a boiling flask, a biomass flask, a distillation head, a condenser and a collecting flask (funnel) was used for dry steam distillation of plant material. Fresh materials from each plant were placed separately in a biomass flask and the distillation unit was turned on. Steam was generated in a boiling flask by heating the distilled water with a hot oven. Steam rose to the biomass flask, where essential oils and other water-soluble plant compounds were passed as steam through the still head, condensed in a water-cooled condenser, and collected in a collecting flask (separatory funnel). The collecting flask separates oils that are heavier than water from oils that are

lighter than water and allows excess water-soluble compounds to be drained and collected.

The poison food technique was used to evaluate the efficacy of five essential oils against *A. solani* in three concentrations of 0.02%, 0.06%, 0.1%. The oils were added directly to the Potato Dextrose Agar medium after sterilization at 121 °C, 15 lbs. For 20 minutes immediately before pouring into Petri dishes according to the processing instructions. To obtain a concentration of 0.02%, it is necessary to use 10 µl. 1 of oil in the same way 30 µl and 50 µl of oil can be added to obtain concentrations of 0.06% and 0.1% in 50 ml of PDA medium. A 5 mm *A. solani* disc was removed from a 7-day culture and placed in the center of the treated Petri dishes. Plates were placed in B.O.D. at 25 ± 2 °C. Each treatment was repeated three times. Record the reading against the control data.

Table 1: Quantity of Essential oils required to make concentrations

S. No.	Essential Oils	Quantity of essential oils taken for 50ml in µl			Amount of PDA taken (ml)
		0.02%	0.06%	0.1%	
1	Lemon grass Oil	10	30	50	50
2	Patchouli Oil	10	30	50	50
3	Citronella Oil	10	30	50	50
4	Clove Oil	10	30	50	50
5	Peppermint Oil	10	30	50	50

Per cent inhibition of mycelium growth of the fungus in case of Essential oils, under *in vitro* conditions was calculated by using the formula described by Vincent (1927) [6].

$$I = \frac{C - T}{C} \times 100$$

Were,

- I = Per cent reduction in growth of *A. solani*
 C = Radial growth of fungus (mm) in control
 T = Radial growth of fungus (mm) in treatment.

3. Results and Discussion

a. Antimicrobial Activity of the Essential Oils against the Test Pathogens:

The rate of inhibition of pathogens was tested using various essential oils available in the local market. The essential oils selected were citronella oil, patchouli oil, peppermint oil, clove oil, lemongrass oil. The calculations were recorded using a PDA as a control. The effects of essential oils were tested at three different concentrations, namely 0.02%, 0.06% and 0.1%. The reported degree of suppression is listed in a table. 2.

Of the five essential oils tested, lemongrass oil was the most effective essential oil that had minimal mycelial growth (1.27 cm). This was significantly lower than for the other essential oils. However, the maximum mycelial growth (4.12 cm) was observed in patchouli oil. At higher concentrations (0.1%),

minimal mycelial growth was observed in all essential oils. In the case of an interaction between essential oils and concentration, minimal mycelial growth (1.27 cm) was found in 0.1% lemongrass oil, which was equivalent to 0.1% citronella oil (2.23 cm) with 0.1% peppermint oil (2.56 cm), 0.1% clove oil (3.92 cm), 0.1% patchouli oil (4.12 cm).

The results clearly show that lemongrass oil was the best oil for pathogen suppression at concentrations of 0.02%, 0.06%, 0.1% (59.37%, 73.12% and 84.32%, respectively). Citronella oil also showed an effect after lemongrass oil with 40.87, 50% and 72.12% at 0.02%, 0.06% and 0.1%, respectively. The lowest percent inhibition was reported with patchouli oil with less than 50% inhibition. Moderate suppression of pathogens was observed with clove oil and peppermint oil with suppression of 51% and 68%, respectively, at a concentration of 0.1%. The suppression of the growth of pathogens could be due to the action of an antifungal effect that helped reduce the growth of fungi.

These results are compared to those reported by Ashour *et al.*, (2016) [2] who showed that a complete inhibition of fungal growth with mint, lemongrass, thyme and sweet basil oil at a concentration of 2% was more effective. at 100% suppression of fungal growth compared to the control. Rahmatzai *et al.*, (2016) [5] also recorded the concentration of 1% lemon oil as the maximum 27% inhibition of pathogen growth in their results. With cinnamon oil (21.6%) a moderate suppression of fungal growth was observed.

Table 2: *In vitro* evaluation of different Essential oils on mycelial growth of *Alternaria solani*

S. No.	Essential Oils	Radial growth (cm)			Percent Inhibition (%)		
		0.02%	0.06%	0.1%	0.02%	0.06%	0.1%
1	Lemon Grass Oil	3.25	2.15	1.27	59.37	73.12	84.32
2	Patchouli Oil	6.20	5.03	4.12	22.5	37.12	48.50
3	Peppermint Oil	5.31	3.58	2.56	33.62	55.25	68.00
4	Clove Oil	4.91	4.05	3.92	38.62	49.37	51.00
5	Citronella Oil	4.73	4.00	2.23	40.87	50.00	72.12
	Control	8.00	8.00	8.00	0.00	0.00	0.00
		Essential Oils	Concentration	Essential oils X Concentration			
	SEm ±	0.109	0.085	0.19			
	CD at 5%	0.318	0.246	0.55			

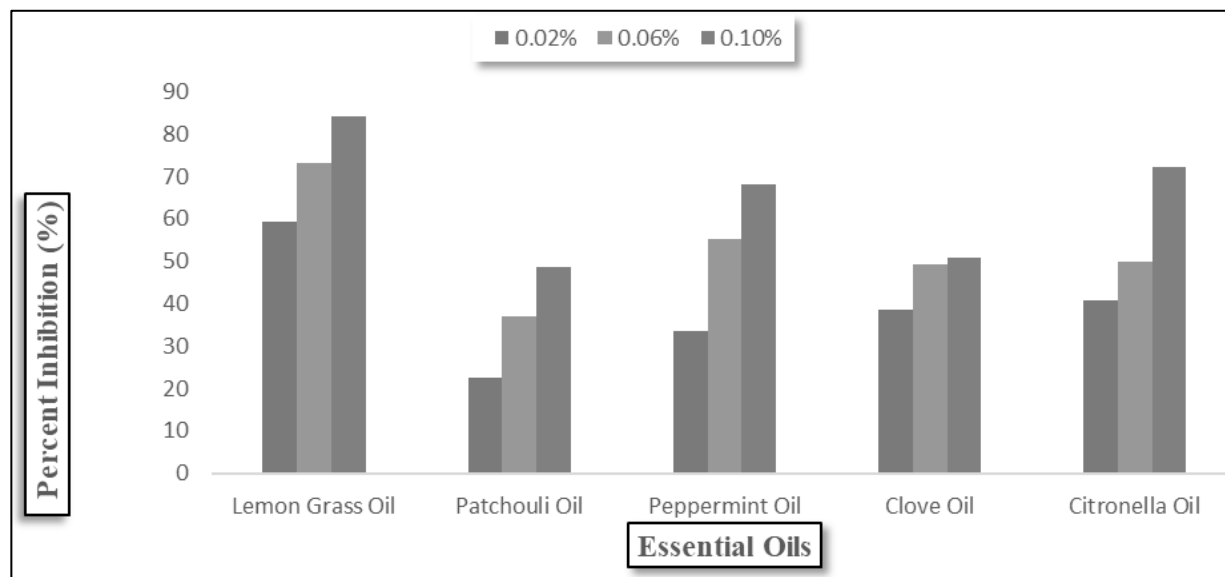


Fig 1: *In vitro* evaluation of different Essential oils on mycelial growth of *Alternaria solani*.

4. Conclusion

Therefore, these essential oils are recommended for industrial use to make less bulky fungicidal products that are effective for use in the field. The scope of these antimicrobial agent testing systems can also be expanded by testing their efficacy on a large number of pathogens. Furthermore, as this study was conducted *in vitro*, a confirmatory *in vivo* experiment in a greenhouse is required.

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