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## *In vitro* bioefficacy of essential oils against *Alternaria solani* and *Colletotrichum capsici*, causing tomato fruit rots

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### Abstract

To develop a natural fungicide against *A. solani* and *C. capsici*, causing tomato fruit rots, a total of eight essential oils were tested *in vitro* (each @ 500 ppm & 1000 ppm) for their fumigant activity against post harvest pathogens. Among the eight essential oils *Cymbopogon nardus* resulted with highest mycelial growth inhibition (96.18%) followed by *Mentha piperita* (95.57%), *Eucalyptus globulus* (95.37%). Whereas, least inhibition of test fungus was recorded *Sesamum indicum* (44.98%). However, in *C. capsici*, *C. nardus* resulted with highest mycelial growth inhibition (96.34%), followed by *M. piperita* (95.61%), *Syzygium aromaticum* (94.82%). Whereas, least inhibition of the test fungus were recorded in *Brassica juncea* (42.67%).

**Keywords:** *Alternaria solani*, *Colletotrichum capsici*, essential oils, inhibition

### Introduction

Tomato fruits are referred as “Poor Man’s Apple”, due to their diversified nutritional values and a wide range of processed products. India is second largest producer of tomato next to china. Post harvest decays of fruits and vegetables account for significant levels of post harvest losses. It has been estimated that about 20-25% of the harvested fruits and vegetables are decayed by pathogens, during post harvest handling even in developed countries (Droby *et al.*, 2009; Abano and San-Amaoh, 2012) [3, 1]. In developing countries, post-harvest losses are often more severe (>30%) due to inadequate postharvest handling, packaging, transportation and storage (Tripathi and Dubey, 2004; Korsten, 2006; Singh and Sharma, 2007) [16, 6]. Tomato fruits, due to their low pH, high moisture content and nutrient composition are very susceptible to attack by pathogenic fungi and ultimately fruit causing rots, making them unfit for consumption, due to production of mycotoxins (Stinson *et al.*, 1981; Moss, 2002) [15, 8]. Tomato crop is prone to many fungal, bacterial, viral, phytoplasmal and nematode diseases. The major and economically important diseases of tomato are *A. solani* and *C. capsici*, causing tomato fruit rots. *A. solani* exhibited on fruits, black to brown, depressed distinct rings. The spots were extending across the fruit surface, which resulted into black rot lesions on tomato (wani, 2011) [19]. Symptoms induced by *Colletotrichum* spp. on ripe fruits/ matured tomato fruits as small, circular, sunken lesions with dark centers, containing fruiting bodies and such infected fruits possessed short shelf life and also inflicted massive fruit losses (Bankole *et al.*, 2018) [2]. The chemical fungicides are responsible to cause different diseases, such as acute and chronic neurotoxicity, lung damage, chemical burns, infant methemoglobinemia, immunologic abnormalities, adverse reproductive and varieties of cancers (Weisenburger, 1993) [3]. Biologically active natural products have the potential to replace synthetic fungicides, and exploitation of some natural products, such as flavour compounds, acetic acid, jasmonates, glucosinolates, propolis, fusapyrone and deoxyfusapyrone, chitosan, essential oils and plant extracts for the management of fungal rotting of fruits (Tripathi and Dubey 2004) [16]. Essential oils are rich source of biologically active compound with antifungal effects against both pathogen and spoilage fungi (Piccaglia *et al.*, 1993) [11]. Plants contain thousands of the constituents and are valuable sources of new and biologically anti-microbial property, such as tannin, allicin, essential oils, etc. (Gurjar *et al.*, 2012) [4]. Essential oils consist of volatile molecules, such as terpenoids, terpenes and phenol derived aliphatic and aromatic compounds, which are antiviral, bactericidal and fungicidal. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential diseases control agents (Tripathi *et al.*, 2008) [17]. Essential oils have application in folk medicine, food preservation, and as feed additive (Kurade *et al.*, 2010) [7].

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## Materials and Methods

The experiment was conducted during winter, 2020 at Department of Plant Pathology, College of Agriculture, Latur, during present investigations on *in vitro* evaluation of essential oils against *A. solani* and *C. capsici*, causing tomato fruit rots. Plant derived eight essential oils were evaluated (each @ 500 and 1000 ppm) *in vitro* against the major fungi associated with tomato fruit rots. Antifungal activity of the test essential oils were evaluated by Poisoned food technique (Nene and Thapliyal, 1993) [10] and using PDA as basal culture medium. To the cooled and molten PDA (450 C), an appropriate quantity of the test essential oil was dispensed and to this Tween 80 was added @ 0.5 per cent (V/V), so as to

facilitate dispersal of the oil with PDA medium in glass conical flasks (250 ml capacity). This PDA medium amended separately with the essential oils were dispensed (@ 20 ml/plate) into the sterile glass Petri plates (90 mm diameter) and allowed to solidify at room temperature (27 ± 20 C). Culture disc (5 mm) was cut from the periphery of actively growing 7 days aged pure culture of the test fungi, using a sterile cork borer and aseptically inoculated at the center of PDA plates. The PDA plates (without essential oil) and inoculated with culture disc (5 mm) of the test fungus were maintained as untreated control. All these plates were incubated at room temperature, for a week or till the untreated control plates fully covered with mycelial growth of test fungus.

**Table 1:** List of essential oils used

Tr. No.	Treatments (essential oils)	Tr. No.	Treatments (essential oils)
T <sub>1</sub>	<i>Eucalyptus globulus</i> (Eucalyptus)	T <sub>6</sub>	<i>Brassica juncea</i> (Mustard)
T <sub>2</sub>	<i>Ricinus communis</i> (Castor)	T <sub>7</sub>	<i>Sesamum indicum</i> (Sesame)
T <sub>3</sub>	<i>Mentha piperita</i> (Mint)	T <sub>8</sub>	<i>Cymbopogon nardus</i> (Citronella)
T <sub>4</sub>	<i>Azadirachta indica</i> (Neem)	T <sub>9</sub>	Control (untreated)
T <sub>5</sub>	<i>Syzygium aromaticum</i> (Clove)		-

Observations on radial mycelial growth / colony diameter (mm) were recorded at an interval of 24 hrs and continued upto seven days after incubation or till the untreated PDA plates were covered fully with mycelial growth of the test fungus. Based on cumulative data, per cent mycelial growth inhibition of the test fungus with the test essential oils, over untreated control was calculated by applying the following formula (Vincent, 1927%) [18].

$$\text{Growth Inhibition (\%)} = \frac{C-T}{C} \times 100$$

## Where

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

## Results and Discussion

### *In vitro* efficacy of essential oils against *A. solani*, causing tomato fruit rot

The results (Table 2, Plate 1) revealed that eight essential oils were evaluated *in vitro* (each @ 500 and 1000 ppm) against *A. solani* and the results obtained on mycelial growth and its inhibition are presented.

**Table 2:** *In vitro* efficacy of essential oils against *A. solani*, causing tomato fruit rot

Tr. No.	Treatments (essential oils)	Col. Dia.* (mm)		Av. (mm)	% Inhibition *		Av. (%)
		At ppm			At ppm		
		500	1000		500	1000	
T <sub>1</sub>	<i>Eucalyptus globulus</i>	6.00	2.33	4.16	93.33(75.03)	97.41(80.74)	95.37(77.57)
T <sub>2</sub>	<i>Ricinus communis</i>	45.67	40.67	43.17	49.26(44.58)	54.81(47.76)	52.04(46.17)
T <sub>3</sub>	<i>Mentha piperita</i>	5.40	2.57	3.98	94.00(75.82)	97.14(80.26)	95.57(77.85)
T <sub>4</sub>	<i>Azadirachta indica</i>	46.00	41.57	43.78	48.89(44.36)	53.81(47.19)	51.35(45.77)
T <sub>5</sub>	<i>Syzygium aromaticum</i>	5.77	3.23	4.50	93.59(75.33)	96.41(79.08)	95(77.08)
T <sub>6</sub>	<i>Brassica juncea</i>	50.30	42.33	46.31	44.11(41.62)	52.97(46.70)	48.54(44.16)
T <sub>7</sub>	<i>Sesamum indicum</i>	52.00	47.03	49.51	42.22(40.52)	47.74(43.70)	44.98(42.12)
T <sub>8</sub>	<i>Cymbopogon nardus</i>	5.10	1.77	3.43	94.33(76.22)	98.03(80.74)	96.18(78.73)
T <sub>9</sub>	Control (untreated)	90.00	90.00	90	0.00(0.00)	0.00(0.00)	0.00(0.00)
	S.E. ±	0.716	0.702	-	0.716	0.783	-
	C.D.(P= 0.01)	2.166	2.124	-	2.166	2.345	-

\*: Mean of three replications, Dia.: Diameter, Av.: Average, Figures in parentheses are arcsine transformed values.

### Effect on mycelial growth

At 500 ppm, radial mycelial growth of *A. solani* ranged from 5.10 to 52.00 mm. However, it was significantly least with *C. nardus* (5.10 mm), which was on par with *M. piperita* (5.40 mm), *S. aromaticum* (5.77 mm), *E. globulus* (6.00 mm), followed by *R. communis* (45.67 mm), *A. indica* (46.00 mm), *B. juncea* (50.30 mm) and *S. indicum* (52.00 mm). At 1000 ppm, radial mycelial growth of *A. solani* ranged from 1.77 to 47.03 mm.

However, it was significantly least with *C. nardus* (1.77 mm), which was on par with *E. globulus* (2.33 mm), *M. piperita* (2.57 mm), *S. aromaticum* (3.23 mm), followed by *R. communis* (40.67 mm), *A. indica* (41.57 mm), *B. juncea*

(42.33 mm) and *S. indicum* (47.03 mm).

### Effect on mycelial growth inhibition

At 500 ppm, mycelial growth inhibition of *A. solani* ranged from 42.22 to 94.33 per cent. However, it was significantly highest with *C. nardus* (94.33%), which was on par with *M. piperita* (94.00%), *E. Globules* (93.33%), *S. aromaticum* (93.59%), followed by *R. communis* (48.26%), *A. indica* (48.89%), *B. juncea* (44.11%) and *S. indicum* (42.22%). At 1000 ppm, mycelial growth inhibition of *A. solani* ranged from 47.74 to 98.03 per cent. However, it was significantly highest with *C. nardus* (98.03%), which was on par with *M. piperita* (97.14%), *E. globulus* (97.41%), *S. aromaticum*

(96.41%), followed by *R. communis* (54.81%), *A. indica* (53.81%), *B. juncea* (52.97%) and *S. indicum* (47.74%). Similarly, antimicrobial potential of the test essential oils of *C. nardus*, *M. piperita*, *E. globulus* and *S. aromaticum* against *A. solani* are in agreement with the findings of several earlier workers (Sajid *et al.*, 2020; Raghupati *et al.*, 2020) [13, 12].

### **In vitro efficacy of essential oils against *C. capsici*, causing tomato fruit rot**

The results (Table 3, Plate 2) revealed that eight essential oils were evaluated *in vitro* (each @ 500 and 1000 ppm) against *C. capsici* and the results obtained on mycelial growth and its inhibition are presented.

**Table 3:** *In vitro* efficacy of essential oils against *C. capsici*, causing tomato fruit rot

Tr. No.	Treatments (essential oils)	Col. Dia.* (mm)		Av. (mm)	% Inhibition *		Av. (%)
		At ppm			At ppm		
		500	1000		500	1000	
T <sub>1</sub>	<i>Eucalyptus globulus</i>	6.07	3.80	4.935	93.26(74.95)	95.78(78.15)	94.52(76.46)
T <sub>2</sub>	<i>Ricinus communis</i>	52.40	50.47	51.43	41.78(40.27)	43.92(41.51)	42.85(40.89)
T <sub>3</sub>	<i>Mentha piperita</i>	4.73	3.17	3.95	94.74(76.74)	96.48(79.19)	95.61(77.91)
T <sub>4</sub>	<i>Azadirachta indica</i>	52.43	46.80	49.61	41.74(40.25)	48.00(43.85)	44.87(42.06)
T <sub>5</sub>	<i>Syzygium aromaticum</i>	6.07	3.27	4.67	93.26(74.95)	96.37(79.02)	94.82(76.84)
T <sub>6</sub>	<i>Brassica juncea</i>	54.07	49.13	51.6	39.92(39.18)	45.41(42.37)	42.67(40.78)
T <sub>7</sub>	<i>Sesamum indicum</i>	51.07	48.33	49.7	43.26(41.13)	46.30(42.88)	44.78(42.00)
T <sub>8</sub>	<i>Cymbopogon nardus</i>	3.73	2.87	3.3	95.86(78.26)	96.81(79.71)	96.34(78.96)
T <sub>9</sub>	Control (untreated)	90.00	90.00	90	0.00(0.00)	0.00(0.00)	0.00(0.00)
	S.E. ±	0.719	0.911	-	0.716	0.946	-
	C.D.(P= 0.01)	2.716	2.756	-	2.166	2.833	-

\*: Mean of three replications, Dia.: Diameter, Av.: Average, Figures in parentheses are arcsine transformed values.

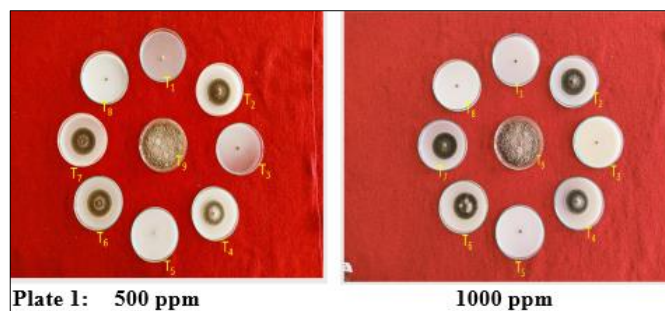
### **Effect on mycelial growth**

At 500 ppm, radial mycelial growth of *C. capsici* ranged from 3.73 to 54.07 mm. However, it was significantly least with *C. nardus* (3.73 mm), which was on par with *M. Piperita* (4.73 mm), *S. aromaticum* and *E. globulus* (6.07 mm), *S. indicum* (51.07 mm), followed by *R. communis* (52.40 mm), *A. indica* (52.43 mm), and *B. Juncea* (54.07 mm). At 1000 ppm, radial mycelial growth of *C. capsici* ranged from 2.87 to 50.47 mm. However, it was significantly least with *C. Nardus* (2.87 mm), which was on par with *M. piperita* (3.17 mm), *S. aromaticum* (3.27 mm), *E. globulus* (3.80 mm), followed by *A. indica* (46.80 mm), *S. indicum* (48.33 mm), *B. juncea* (49.13 mm) and *R. communis* (50.47 mm).

### **Effect on mycelial growth inhibition**

At 500 ppm, mycelial growth inhibition of *C. capsici* ranged from 39.92 to 95.86 per cent. However, it was significantly highest with *C. nardus* (95.86%), which was on par with *M. piperita* (94.74%), *S. aromaticum* and *E. globulus* (93.26%), followed by *S. indicum* (43.26%), *R. communis* (41.78%) *A. indica* (41.74%) and *B. juncea* (39.92%), which were on par to each other. At 1000 ppm, mycelial growth inhibition of *C. capsici* ranged from 43.92 to 96.81 per cent. However, it was significantly highest with *C. nardus* (96.81%), which was on par with *M. piperita* (96.48%), *S. aromaticum* (96.37%), *E. globulus* (95.78%), followed by *A. indica* (48.00), *S. indicum* (46.30%), *B. juncea* (45.41%) and *R. communis* (43.92%), which were on par to each other.

Thus, based on average mycelial growth inhibition, the most potential antifungal essential oils found in their order of merit were *C. nardus* > *M. piperita* > *S. aromaticum* > *E. globulus*. These results of the present study on fungicidal / fungistatic potential of the test essential oils are *C. nardus*, *M. piperita*, *S. aromaticum* and *E. globulus*, against *C. capsici* are in agreement with the findings of several earlier workers (Naik *et al.*, 2017; Jagana *et al.*, 2018) [9, 5].



**Fig 1:** *In vitro* efficacy of essential oils against *A. solani*, causing tomato fruit rot



**Fig 2:** *In vitro* efficacy of essential oils against *C. capsici*, causing tomato fruit rot

### **Conclusion**

From the results obtained on various aspects during present investigations on “*In vitro* essential oils against *A. solani* and *C. capsici*, causing tomato fruit rots” following conclusions are being drawn:

Among the essential oils tested *in vitro*, *C. nardus*, *M. piperita*, *S. aromaticum* and *E. globulus* were found efficient with significantly high mycelial growth inhibition of the fungi (*A. solani* and *C. capsici*) causing tomato fruit rots. The use of



essential oils as natural fungicides is of immense significance in view of the environmental and toxicological implications of the indiscriminate use of synthetic and reducing the problems of increasing fungi resistance.

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