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In vitro bioefficacy of essential oils against *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate

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Abstract
Pomegranate (*Punica granatum* L) is one of the important fruit crops grown in India. Fruit rot of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., has becoming a major limiting factor in cultivation of pomegranate in some regions of India. The present investigation was carried out to test the *in vitro* bioefficacy of different essential oils against *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate were carried out in the Department of Plant Pathology, College of Agriculture, Latur during the year 2017-18. Among the different essential oils However, Cinnamon oil was found most effective with least mycelial growth (43.80 mm) and numerically highest mycelial inhibition (50.58%), followed by Nutmeg oil (62.87 mm and 29.76%), Safflower oil (64.66 mm and 27.76%), Mustard oil (65.50 mm and 26.81%), Neem oil (68.55 mm and 23.22%), Castor oil (69.36 mm and 22.28%), Coconut oil (71.48 mm and 20.04%) and Groundnut oil (71.51 mm and 20.02%). The essential oils were evaluated *in vitro* against the pathogen *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate by using paper disc method.

Keywords: *Colletotrichum gloeosporioides*, essential oils, fungal, fruit rot, pomegranate

Introduction
Pomegranate (*Punica granatum* L.) is an ancient, delicious fruits consumed worldwide, gaining lot of attention of the world over, because of its high economic value and nutritional values. It is one of the important fruit crops in arid and semi-arid regions commercially important in both tropical and subtropical countries known for its drought tolerance which thrives well in dry tropical conditions with marginal soils of low fertility. Being the most adaptable subtropical fruit crop, its cultivation has increased rapidly creating its image as an important cash crop in global market. Globally India is ranked first in area and production. During 2015-16, pomegranate was cultivated over 2.09 lakh ha with an annual production of 24.42 lakh MT and productivity of 12.00 MT/ha in India (Anonymous, 2016) [3]. Maharashtra is the leading producer of pomegranate in India followed by Karnataka, Gujarat and Andhra Pradesh (Anonymous, 2013) [2]. Maharashtra considered as pomegranate basket of India contributes more than 70 per cent of the total area under pomegranate followed by Andhra Pradesh, Uttar Pradesh, Rajasthan, Gujarat and Karnataka which are the leading states; cultivating pomegranate commercially on a large scale.

However, the crop is under threat due to number of serious diseases such as bacterial blight (*Xanthomonas axonopodis* pv. *punicae*), wilt due to *Ceratocystis fimbriata*, anthracnose (*Colletotrichum gloeosporioides*) and leaf spot and severe fruit rotting due to *Alternaria alternata*, *Cercospora* sp., *Pseudocercospora* sp., *Drechslera* sp. and *Sphaceloma* sp. etc., are more or less equally important and harmful in some orchards and also take a heavy toll on the crop (Khosla and Bhardwaj, 2013) [7]. Among these; severe spotting and fruit rotting due to *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc; remains hitherto unexplored but potentially dangerous pathogen on pomegranate and considered to be an emerging disease.

In recent years, there has been a major thrust on pesticide residue free organic pomegranate production. Taking the task into consideration, efficient essential oils need to be explored to fit into the management schedule. Use of essential oils for the management of various diseases of crop plants is eco-friendly and environmentally safe. Therefore, present investigation aimed to evaluate essential oils (*in vitro*) against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc causing fruit rot in Pomegranate.

Material and Methods
Eight essential oils were evaluated *in vitro* against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc, applying paper disc method (Kishore and Pande 2007). Seven days old

cultures of the test pathogen (*Colletotrichum gloeosporioides*) grown on PDA were used for the study. The PDA medium was then poured (20 ml / plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each test essential oils the two discs of 5 mm Whatman filter paper no. 1 were dipped in 1ml test essential oils and then placed into PDA poured Petri plates. All the treatment plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week old actively growing pure culture of the test isolates of *Colletotrichum gloeosporioides* and three plates / treatment / replication were maintained. Plates containing plain PDA without any essential oils and inoculated with mycelial disc of the test pathogen isolate served as untreated control. All these plates were incubated at 28 ± 2 ^{0}C temperatures for a week or till the untreated control plates were then fully covered with mycelial growth of the test pathogens. Following essential oils reported to possess antimicrobial and therapeutic properties and available locally were collected from the Latur market and used during present studies.

The lists of essential oils used along with their botanical names are given in Table 1.

Table 1: List of essential oils with their botanical names.

Sr. No.	Local name	Botanical name
1.	Groundnut oil	*Arachis hypogea*
2.	Coconut oil	*Cocos nucifera*
3.	Castor oil	*Ricinus communis*
4.	Neem oil	*Azadirchta indica*
5.	Nutmeg oil	*Myristica fragrans*
6.	Mustard oil	*Brassica oleracea*
7.	Cinnamom oil	*Cinnamomum verum*
8.	Safflower oil	*Carthamus tinctorius*

Observations on linear mycelial growth of the test pathogen were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogens. Per cent inhibition of the test pathogens by the essential oils, over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978) [4].

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in Control plate - Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \text{X100}$$

Results and Discussion

Results (Plate 1, Fig. 1 and Table 1) revealed that all the essential oils evaluated exhibited fungistatic / antifungal activity against *Colletotrichum gloeosporioides* causingfungal fruit rot in pomegranate and significantly inhibited its growth over untreated control. Of the eight essential oils tested, Cinnamon oil was found most effective and test pathogen recorded least linear mycelial growth (43.80 mm) with highest mycelial inhibition (50.58%) of the test pathogen. The second and third best essential oils found were Nutmeg oil (mycelial growth 62.87 mm and inhibition 29.76%) and Safflower oil (mycelial growth 64.66 mm and inhibition 27.76%), respectively.

This was followed by Mustard oil (col. dia.: 65.50 mm and inhibition: 26.81%), Neem oil (col. dia.: 68.55mm and inhibition: 23.22%), Castor oil (col. dia.: 69.36 mm and inhibition: 22.28%). The essential oils Coconut oil and Groundnut oil were found least effective with 71.48 mm and 71.51 mm linear mycelial growth and 20.04 and 20.02 per cent mycelial inhibition.

These results are in conformity with the earlier findings of those workers who reported Essential oils *viz.*, Cinnamon oil, Nutmeg oil, Safflower oil, Mustard oil, Neem oil, Castor oil, Coconut oil and Groundnut oil were reported as efficient essential oils components against many *Colletotrichum* spp. including *C. gloeosporoides* by several earlier workers (Luis *et al*, 2012; Abd-Alla and Haggag, 2013; Bhuyan *et al*. 2015; Maqbool *et al*. 2010; Singh and tripathi 2015 and Bhardwaj *et al* 2014) [9, 1, 6, 10, 12, 5].

Table 2: *In vitro* bioefficacy of essential oils against *C. gloeosporioides* causing fungal fruit rot in pomegranate

Tr. No.	Treatments	Col. Dia.* of test pathogen (mm)	% Inhibition*
T_1	Groundnut oil	71.51	20.02 (26.57)
T_2	Coconut oil	71.48	20.04 (26.59)
T_3	Castor oil	69.36	22.28 (28.16)
T_4	Neem oil	68.55	23.22 (28.80)
T_5	Nutmeg oil	62.87	29.76 (33.06)
T_6	Mustard oil	65.50	26.81 (31.18)
T_7	Cinnamon oil	43.80	50.88 (45.50)
T_8	Safflower oil	64.66	27.76 (31.79)
T_9	Control (untreated)	90.00	00.00 (00)
	S.E. ±	0.39	0.66
	C.D. at 1%	1.08	1.79

*: Mean of three replications, Dia.: Diameter, Col.: Colony, Figures in parentheses are arcsine transformed values

Plate 1: *In vitro* bioefficacy of essential oils *C. gloeosporioides* causing fungal fruit rot in pomegranate

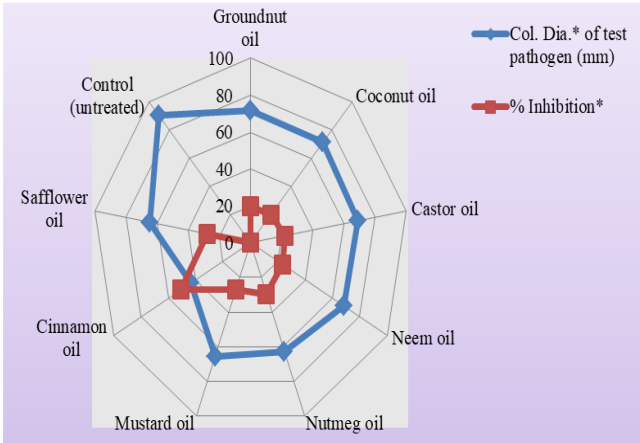

Fig 1: *In vitro* efficacy of essential oils against C. gloeosporides

T_1: Groundnut oil T_2: Coconut oil T_3: Castor oil
T_4: Neem oil T_5: Nutmeg oil T_6: Mustard oil
T_7: Cinnamon oil T_8: Safflower oil T_9: control (untreated)

In conclusion, all the, essential oils evaluated *in vitro* were found fungistatic / antifungal against *Colletotrichum gloeosporioides*. However, bioagents *viz.*, Cinnamon oil,

Nutmeg oil and Safflower oils were most efficient with significantly highest inhibition of mycelial growth of the *Colletotrichum gloeosporioides* causing fungal fruit rot in pomegranate.

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