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Isolation and *in vitro* evaluation of essential oils against anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc

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Abstract
Pomegranate is widely grown fruit in many regions of the world. It is regarded as "Fruit of Paradise". Anthracnose caused by the fungal pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is one of the limiting factor for low productivity of Pomegranate. Hence, *in vitro* evaluation of eight essential oils were carried out against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing anthracnose of pomegranate. Among these oils, Thyme oil (*Thymus vulgaris*) and Clove oil (*Syzygium aromaticum*) had shown the best result in controlling the fungal growth. Savory oil (*Satureja hortensis*) and Cinnamon oil (*Cinnamomum verum*) are considered as secondary best oils followed by Citronella oil (*Cymbopogon*) and Mint (*Mentha*) oils. Whereas, Tea tree oil (*Melaleuca alternifolia*) and Eucalyptus oil (*Eucalyptus globulus*) had shown poor results in fungal growth control.

Keywords: Pomegranate, Anthracnose, *Colletotrichum gloeosporioides*, mycelium growth, essential oils

Introduction
Pomegranate (*Punica granatum* L.) is an important fruit crop of tropical and subtropical regions of the world. It suffers from many economically important diseases. Among them, fruit rot, wilt, bacterial spot and are severe and causes significant losses (K.Jayalakshmi *et al*. 2013) [6]. Pomegranate belongs to the family *punicaceae*. It is symbolic of prosperity and liked for its cool and refreshing juice as well as valued for its medicinal properties. (Devanshu Dev and Somashekar Konda *et al*. 2015) [1]. It is symbol of health, fertility, eternal life and being valued for its medicinal properties to treat diabetes, heart diseases, cancer, hypertension, gastric and kidney diseases (K. Jayalakshmi *et al*, 2015) [7]. Pomegranate is considered as the" Fruit of Paradise" (K.A Burgute and S.J Magar). Pomegranate is an ancient beloved plant and fruit. Its usage is deeply embedded in human history. Anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. This disease is very harmful and spoilage through rotting that results in low market value (Ahmed Imtiaj *et al*. 2005) [5]. The symptoms on leaves observed as pinhead size of black to brown water soaked spots with circular margins. In advanced stage, these spots enlarged, coalesced and resulted in bigger patches. In severe cases, leaves dried up and dropped down. On the fruits, brown spherical depressed spots occurred in scattered form on the pericarp. In advanced stage, these spots coalesced to form necrotic patches over the surface of fruit. (Devanshu Dev and T. Narendrappa 2016) [4]. Fungal diseases are among the major conributors to global crop yield losses. The losses are often accompanied by the cost of disease control measures that are usually due to use of expensive fungicides. Frequent fungicidal use may have negative impact on the plants and improves resistance by the fungal pathogens. (Mark Angelo O. Balendres *et al.* 2010) [10]. The large scale use of pesticides in the field leads to the pesticidal residue in the soil, which affects the soil health and fertility, ultimately production of the crop. (Devanshu Dev and Somashekar Konda *et al*. 2015) [1]. Therefore, essential oils which are non hazardous to the plant life, animal life and environment as well should be recommended for controlling the disease development. Essential oils and their chemical components are known to have antifungal properties against a wide range of important plant pathogenic species. (Mark Angelo O. Balendres *et al*. 2010) [10].

Materials and Methods

Isolation of *Colletotrichum gloeosporioides*

In the present incestigation the diseases samples were collected from the field. Small tissues from infected stem or roots (5mm) along with the healthy tissue were cut with sterile scalpel. The tissues were surface sterilized with 0.1% mercury chloride for 30 seconds. The tissues were subsequently washed in three changes of sterile distilled water to eliminate mercury ions. The surface sterilized tissues were transferred on to the PDA and incubated at 25 ± 2 °C in BOD incubator and growth was observed periodically.

***In vitro* evaluation of essential oils against *Colletotrichum gloeosporioides*:** The relative efficacy of eight essential oils were evaluated under *in vitro* conditions Thyme (*Thymus vulgaris*), Clove (*Syzygium aromaticum*), Savory (*Satureja hortensis*), Cinnamon (*Cinnamomum verum*), Citronella (*Cymbopogon*), Mint (*Mentha*), Tea tree oil (*Melaleuca alternifolia*) and Eucalyptus (*Eucalyptus globulus*) at three different concentration levels i.e. 0.5, 1.0, and 2.0 (%) by using poisoned food technique (Nene and Thapliyal, 1979). From the stock double strength potato dextrose agar medium, different lots each containing 50ml double strength potato dextrose agar medium in a conical flask (150ml) were sterilized at 15 psi (1.05kg/cm²) pressure at 121.6°C for 20 minutes. Simultaneously, concentrations of different essential oils were also prepared in equal amount (50ml) of sterilized distilled water so as to get the desired concentration of essential oils after mixing the essential oil solutions in the double strength media.

Results and Discussion

In vitro efficacy of different essential oils on mycelial growth of *Colletotrichum gloeosporioides*

As fungicidal usage can pollute both soil and environment, essential oils can be used instead of the fungicides. To find out whether the essential oils can really replace the efficacy of fungicides, *in vitro* evaluation of essential oils were carried out in the plant pathology department. The essential oils used were Thyme, Clove, Savory, Cinnamon , Citronella, Mint , Tea tree oil and Eucalyptus at three different concentration levels i.e. 0.5, 1.0, and 2.0 (%) against mycelial growth of *Colletotrichum gloeosporioides.* Thyme oil and clove oil had shown the best growth inhibition in 1% and 2% concentrations. Savory and Cinnamon oils had shown better growth inhibition in 2% concentration level. Citronella and Mint oils had shown significant growth inhibition in 2% concentration level. Tea tree oil and Eucalyptus oil had shown poor growth inhibition in all concentration levels.

In vitro evaluation of different essential oils against anthracnose of pomegranate caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc

Table 1: *In vitro* evaluation of Thyme (*Thymus vulgaris*) against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. (Cm/day)

Essential oil concentration	Thyme (*Thymus vulgaris*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.715	1.325	2.850	4.675	5.850	6.725
1	0.652	0.975	1.800	3.050	4.000	5.100
2	0.600	0.700	0.875	1.925	2.625	3.050
Control	0.775	2.125	3.950	5.425	7.650	9.000
C.D	0.055	0.112	0.133	0.147	0.163	0.178
S.E(m)	0.018	0.036	0.043	0.047	0.053	0.058

Table 2: *In vitro* evaluation of Clove (*Syzygium aromaticum*) against *Colletotrichum gloeooporioides* causing anthracnose of pomegranate. (cm/day)

Essential oil concentration	Clove (*Syzygium aromaticum*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.700	1.575	2.350	4.875	6.025	7.250
1	0.629	1.075	3.250	3.850	4.650	5.350
2	0.600	0.610	0.759	0.975	1.550	2.150
Control	0.850	2.025	3.850	5.950	8.275	9.000
C.D	0.045	0.123	0.126	0.129	0.105	0.138
S.E(m)	0.014	0.034	0.040	0.041	0.043	0.045

Table 3: *In vitro* evaluation of Savory (*Satureja hortensis*) against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. (cm/day)

Essential oil concentration	Savory (*Satureja hortensis*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.725	1.600	2.125	3.925	6.325	8.325
1	0.600	1.275	1.525	3.750	5.900	6.300
2	0.600	0.825	0.950	1.950	4.575	5.425
Control	0.775	2.250	4.450	5.575	7.600	9.000
C.D	0.084	0.120	0.123	0.143	0.245	0.430
S.E(m)	0.027	0.034	0.040	0.043	0.047	0.138

Table 4: *In vitro* evaluation of Cinnamon (*Cinnamomum verum*) against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. (cm/day)

Essential oil concentration	Cinnamon (*Cinnamomum verum*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.700	1.875	2.950	5.075	7.450	8.650
1	0.650	1.325	2.300	4.200	6.350	7.200
2	0.625	1.025	1.050	2.700	4.000	4.850
Control	0.800	2.050	4.250	6.050	8.050	9.000
C.D	0.059	0.103	0.101	0.104	0.108	0.112
S.E(m)	0.019	0.032	0.034	0.035	0.037	0.041

Table 5: *In vitro* evaluation of Citronella (*Cymbopogan*) against *Colleetotrichum gloeosporioides* causing anthracnose of pomegranate. (cm/day)

Essential oils concentration	Citronella (*Cymbopogan*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.650	1.575	2.700	5.500	7.875	8.250
1	0.625	0.975	1.375	4.200	6.450	7.150
2	0.600	0.612	0.825	3.475	5.850	6.175
Control	0.850	2.025	4.550	6.400	8.350	9.000
C.D	0.087	0.095	0.133	0.156	0.230	0.241
S.E.(m)	0.028	0.031	0.043	0.056	0.068	0.075

Table 6: *In vitro* evaluation of Mint (*Mentha*) against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. (cm/day)

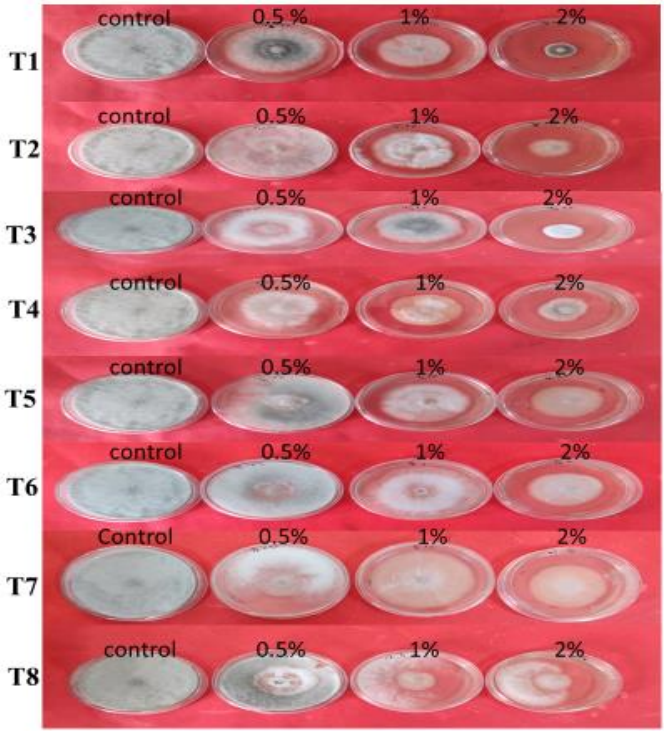
Essential oil concentrations	Mint (*Mentha*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.700	1.575	3.900	5.075	6.275	8.100
1	0.600	1.175	2.875	3.200	4.000	5.950
2	0.600	0.600	1.050	1.925	2.900	3.775
Control	0.725	2.250	4.250	6.050	7.100	9.000
C.D	0.075	0.131	0.140	0.161	0.183	0.214
SE(m)	0.024	0.042	0.052	0.059	0.062	0.069

Table 7: *In vitro* evaluation of Tea tree oil (*Melaleuca alternifolia)* against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. (cm/day)

Essential Oil Concentration	tree oil (*Melaleuca alternifolia)*					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.650	1.575	2.975	4.850	6.350	7.025
1	0.630	0.975	1.950	3.550	5.350	5.450
2	0.625	0.600	1.582	1.950	2.450	3.425
Control	0.850	2.025	3.750	5.575	8.050	9.000
C.D	0.087	0.090	0.092	0.094	0.095	0.097
S.E.(m)	0.028	0.029	0.31	0.033	0.035	0.039

Table 8: *In vitro* evaluation of Eucalyptus (*Eucalyptus globulus*) against *Colletotrichum gloeosorioides* causing anthracnose of pomegranate. (cm/day)

Essential oil concentration	Eucalyptus (*Eucalyptus globulus*)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
0.5	0.700	1.575	2.700	5.950	7.300	7.700
1	0.628	1.075	2.175	3.950	6.250	6.925
2	0.600	0.625	1.000	1.750	2.975	4.900
Control	0.850	2.025	4.275	6.450	7.675	9.000
C.D	0.045	0.123	0.129	0.136	0.149	0.157
S.E(m)	0.014	0.040	0.041	0.052	0.064	0.071



T1: Thyme oil T2: Clove oil
T3: Savory oil T4: Cinnamon
T5: Citronella T6: Mint oil
T7: Tea tree oil T8: Eucalyptus oil

Plant essential oils are ecological safer, non-hazardous and non-polluting means of plant disease management. Chandra Sekhar J, *et al*, 2020 [11] who also used same essential oils at same concentration levels against the pathogen and similar results were observed. Ali Sarkosh *et al.* reported that Thyme oil (*Thymus vulgaris* oil) had inhibited the mycelia growth of *C. gloeosporioides* in 76.11%. Dang wang, Jing zhang, and Xiaoman jia reported that clove (*Syzygium aromaticum*) oil (@ 0.5%) showed economically significant positive response in all respective parameters and was highly effective in inhibiting the growth of *Colletotricum gloeosporioides*. Thyme (*Colletotrichum gloeosporioides*) giving 100 per cent of growth inhibition.

Ali Sarkosh *et al.* also reported the efficacy of savory oil (*Satureja hortensis*) against mycelial growth of *Colletotrichum gloeosporioides.* Jeum Kyuhong *et al.* Investigated on mycelial growth of the tested fungi showed more sensitivity to high concentrations of cinnamon oil (*Cinnamomum verum*). Mark Angelo O. Balendres and Fe M. Dela Cueva found Citronella oil (*Cymbopogan)* as next most effective against *Colletotichum gloeosporioides.*

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References

1. Devanshu Dev, Somashekhar Konda ME, Puneeth N Tanuja, Prachi Singh, Narendrappa T. *In vitro* evaluation of bioagents and botanicals against *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. causing anthracnose of Pomegranate, International Journal of Ecology, Environment and Conservation. 2016; 22(3):1229-1232.
2. Dan Wang, Jing Zhang, Xiaoman Jia, Li Xin, Hao Zhai. Antifungal Effects and Potential Mechanism of Essential Oils on *Collelotrichum gloeosporioides In Vitro* and *In Vivo*. Journal of Multidisciplinary Digital Publishing Institute. 2019; 24:3386.
3. Burgute KA, Magar SJ. *In vitro* Evaluation of Systemic, Non-Systemic and Combi Fungicides against *Colletotrichum gloeosporoides* Causing Fungal Fruit Rot in Pomegranate, International Journal of Current Microbiology and Applied Sciences. 2019; 8(8):450-456.
4. Devanshu Dev, Narendrappa T0. *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. causing anthracnose of pomegranate (*Punica granatum* L.), Journal of Applied and Natural Science. 2016; 8(4):2268-2272.
5. Ahmed Imtiaj, Syed Ajijur Rahman, Shahidul Alam, Rehana Parvin, Khandaker Mursheda Farhana, Sang-Beon Kim, Tae-Soo Lee. Effect of Fungicides and Plant Extracts of *Colletotrichum gloeosporioides* Causing Mango Anthracnose, Journal of Mycobiology. 2005; 33(4):200-205.
6. Jayalakshmi K, Nargund VB, Raju J, Benagi VI. Effect of Fungicides and Plant Extracts on Growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Causing Anthracnose of Pomegranate, Bioinfolet Journal of Life Sceinces. 2013; 10(2A):502-506.
7. Jayalakshmi K, Nargund VB, Raju J, Benagi VI, Raghu S, Madhu S, *et al*. Pomegranate Anthracnose Caused by *Colletotrichum gloeosporioides*: A Menance in Quality Fruit Production, Journal of Pure and Applied Microbiology. 2015; 9(4):3093-3097.
8. Jeum Kyu Hong, Hye Ji Yang, Heesoo Jung, Dong June Yoon, Mee Kyung Sang, Yong-Chull Jeun. Application of Volatile Antifungal Plant Essential Oils for Controlling Pepper Fruit Anthracnose by *Colletotrichum gloeosporioides*, The Plant Pathology Journal. 2015; 31(3):269-277.
9. Ali Sarkhosh, Brucr Schaffer, Ana I, Vargas, Aaron J. Palmateer, Particia Lopez, Asiye Soleymani. *In Vitro* Evaluation of Eight Plant Essential Oils for Controlling *Colletotrichum, Botryosphaeria, Fusarium* and *Phytophthora* Fruit Rots of Avocado, Mango and Papaya, Journal of Plant Protection Sciences. 2017; 10:17221/49.
10. Mark Angelo O Balendresm Fe M Dela Cueva. Growth-inhibiting activity of citronella essential oil to multiple fungal plant pathogens, University of Philippines, 2010.

11. Chandra Sekhar J, Jai Prakash Mishra, Rajendra Prasad, Vedukola Pulla Reddy, Sunil Kumar, Ankita Thakur, Joginder Pal. Isolation and *In vitro* evaluation of biocontrol agents, fungicides and essential oils against stem blight of tomato caused by *Sclerotium rolfsii* (curzi) C.C Tu & Kimber, Journal of Pharmacognosy and Phytochemistry. 2020; 9(3):700-705.