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Management of Grey mold of Pomegranate (*Punica granatum* L.) through essential oils

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Abstract: Pomegranate (*Punica granatum* L.*)* is an important fruit growing all over the world. Different pathogens attack on pomegranate fruit and cause considerable post-harvest losses to farmers. Synthetic fungicides are used against postharvest rots in pomegranates. Essential oils are considered as best substitute of fungicide. Keeping in view the importance of essential oils, investigation was done to explore some good essential oils having the potential management of post-harvest rots of pomegranate. The samples showing typical symptoms were collected from selected markets of Faisalabad and brought into the Seed Pathology Laboratory for isolation, purification and identification of different pathogens associated with sample of pomegranate. *In-vitro* evaluation of different essential oils viz. Eucalyptus, Lemon, Sage, Fennel were tested against grey mold pathogen of pomegranate. The results showed that sage oil gives best result by giving 73% growth inhibition fungus while least control was exhibited by eucalyptus oil (40 and 41 percent) after 5 and 7 days respectively against the *Botrytis cinerea*.
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Introduction

Pomegranate (*Punica granatum* L.*)* is one of the oldest and most important fruit. It is a large shrub that is primarily deciduous in nature and belongs to family *Punicaceae* (Mercure, 2007). It is grown in tropical and sub-tropical areas in the world and it can survive in various climate and soil type. Pomegranate has ability to grow in diverse agro-climatic conditions which enabled its distribution throughout Himalayas to the Eurasia (Levin, 2006). Production of pomegranate has been increasing worldwide because of its number of health benefits (Basu and Penugond, 2009). Its annual production is 1.5 million tons around the globe. As pomegranate is among the important fruit commodities, it is widely cultivated around the globe. Pomegranate fruits are rich in carbohydrates, mineral and modest source of gelatin and contain sufficient amount of Calcium, Iron and Sulphur (Waskar, 2006). It is vulnerable to various kind of diseases and fruit production is essential issue in the whole world (Bardas *et al*., 2009). Many fungal pathogens attack on pomegranate, but the most important plant pathogenic fungi include *Botrytis cineria*, *Aspergillus niger*, *Penicillium digitatum*, *Penicillium expansum* and *Alternaria alternata* (Adaskaveg, 2012). The most important pathogen that cause grey mold disease or fruit decay problem worldwide is *Botrytis cineria* (Tedford *et al*., 2005).

Initial infection of postharvest disease due to grey mold takes place during blooming season through flower parts which leads towards dormant infection. At early stages of fruit formation, pathogen enters through mechanical damage or wounds and openings created by insects. Under suitable conditions characteristic symptoms comprising of discoloration of complete fruit initiating from crown or blossom end and covers the fruit entirely. Several types of symptoms such as rotting, blackening, softening and bruising also appear on fruit surface at lateral stages. Depending on the growing season, it can destroy 30-50% of fruits in cold storage (Teksur *et al*., 2014).

B. cinerea is most widely studied necrotrophic pathogen responsible for causing grey mold rot in almost 500 species of plants and has disastrous effect on economically important crops (Dean *et al*., 2012). Grey mold caused by *Botrytis cinerea* is most common and economically most important disease of pomegranate and during storage under favorable conditions it can cause 30-50% destruction in pomegranate fruits (Teksur *et al*., 2014). It has potential to live inside the stems, leaves, flowers, seeds, fruits and may express its symptoms during both pre and post-harvest. It is a well-known post-harvest pathogen of fresh fruits and vegetables. Because of its economic importance it is ranked as second most important and destructive pathogen (Dean *et al*., 2012).

For control of *B. cinerea* is based on application of synthetic fungicides which counts about 8 percent global fungicides market and annual expenditures of *B. cinerea* control exceeds from 1 billion in world (Dean *et al*., 2012). Chemical fungicides should be changed by natural substitutes such as essential oils like eucalyptus and cinnamon essential oils. Number of different studies depicted the antimicrobial activities of these natural oils against many plant pathogens (Antunes and Cavavo, 2010). *Thymus vulgaris* and *Carum copticum* oils are being used for the control of post-harvest decay of fruits caused by *Penicillium digitatum* and *Alternaria alternate* (Abdolahi *et al*., 2010). Thyme oil effectively managed the *B. cinerea*, *Rhizpous stolonifer*, *Alternaria alternate*, and *Colletotrichum gloeosporioides* under lab conditions (Sellamuthu *et al*., 2013).

Keeping in view all above factors present study is designed to use synthetic fungicides and essential oils against grey mold disease of pomegranate fruit. Study was carried out under following objectives

- To evaluate the different fungicides to control postharvest grey mould problem
- To evaluate essential oils against this pathogenic fungus

MATERIALS AND METHODS

Survey and Sampling

A survey was carried out in different markets and shops of district Faisalabad, Pakistan in (December- February) 2019. Pomegranate fruits having the symptoms of rots and discoloration were collected randomly. Infected fruits were separated from healthy once and were kept in polythene bags. All the samples were labelled properly and were brought into the Seed Pathology Lab, Department of Plant Pathology, University of Agriculture Faisalabad for further studies. Percentage of disease incidence (PDI) was calculated by applying the following formula (Abdulsalam *et al*., 2015).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

Sterilization of glassware and preparation of media

All the glass wares comprising of Petri plates, conical flasks, test tubes etc that were used in laboratory experiments were initially washed with detergent powder by using tap water and were air dried. After drying these were wrapped in clean paper and sterilized in autoclaved.

Potato dextrose agar medium (PDA) was used for isolation and culturing the pathogen. The ingredients of PDA medium were as follows

Potato starch	20 g
Glucose	20 g
Agar	20 g
Water	1000 ml

Isolation of pathogen

Infected fruits showing the symptoms of rotting were selected for isolation of fungal pathogen. Small infected portions of pomegranate fruits were cut into small pieces (3-5 mm) and were sterilized with the help of sodium hypochlorite (NaOCl). Samples were then rinsed by using sterilized distilled water and placed on sterilized filter papers. Sterilized diseased samples were brought into laminar flow chamber for inoculation. Samples were than inoculated into petri plates containing PDA medium with the help of sterilized forceps. Pates were wrapped and placed in incubator at 24^0C which examined regularly for growth of fungus.

Purification and identification of fungus

Samples were purified by transferring the hyphal tips to separate plates containing PDA medium. Plates were then incubated at 25^0C and growth of fungus was observed regularly. Identification of fungi was made according to cultural and morphological characteristics using related literature (Gilman, 1957; 19, 20, 21, 22). Fungal cultures were stored by inoculating on agar slants and were maintained at 4^0C for further studies.

Evaluation of Essential oils against *B. cinerea*

Different oils were collected from scientific stores Faisalabad, manufactured in Pakistan.Efficacy of different essential oils was assessed against *Botrytis cinerea* Fresh fruits was injured with the help of needle in circular shape, then fungus was inoculated with the help of needle. Then fruits were covered by coating it with essential oil was stored at room temperature, rotting symptoms was checked after 1^{st} ,2^{nd}, and 3^{rd} interval.

Statistical analysis

Data was analyzed statistically; ANOVA was used to check the significant result of data while for the mean value LSD (least significance differences) was used to contrast the difference.

Results

Application of essential oils to control Grey mold of pomegranate

Invitro antifungal potential of four essential oils such as Fennel oil, sage oil, lemon oil and Eucalyptus oil were evaluated at three concentrations (3, 6 and 10%) and growth inhibition %age was calculated after 5 and 7 days. All essential oils significantly ($p \leq 0.05$) inhibited

the growth of fungus. Growth inhibition increased by increasing the concentration of essential oils. Maximum growth inhibition (73%) was observed in fruit treated with sage oil followed by fennel oil (61.3%), lemon oil (48.6%) and eucalyptus oil (41.7%) after 7 days at 10% concentration. At 6% concentration maximum growth inhibition (64%) was observed in fruit cured with sage oil followed by fennel oil (53%), lemon oil (48.6%) and eucalyptus oil (41.7%) after 7days. Minimum growth inhibition was observed at 3% concentration as shown in Fig.2.

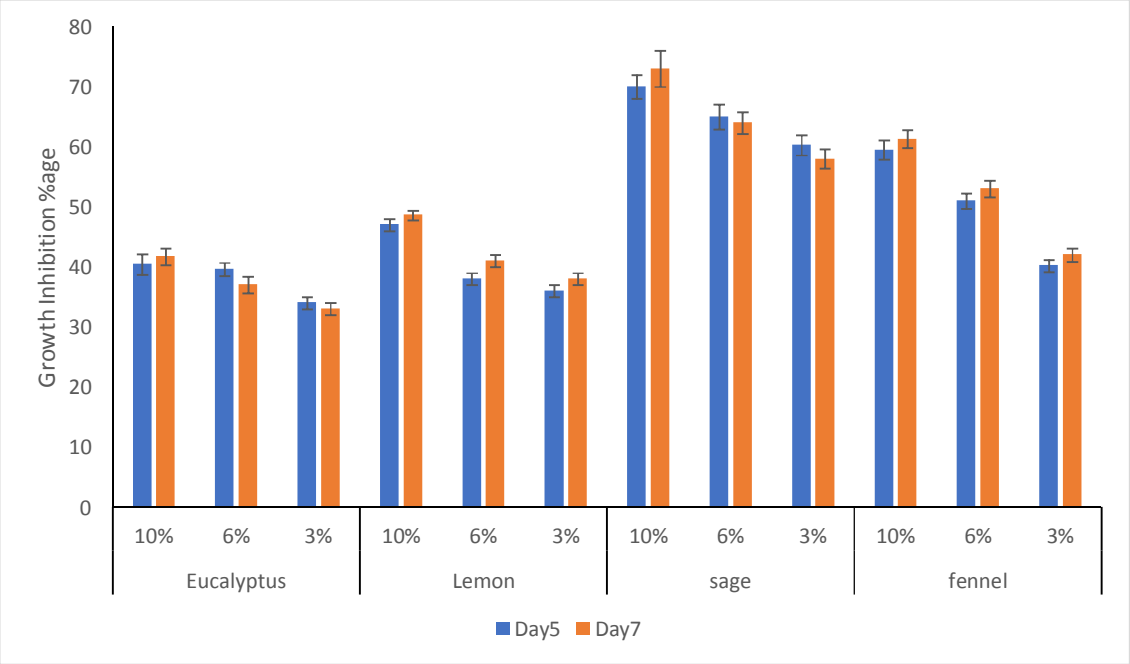

Fig.1. Effect of different concentrations of essential oils on growth inhibition (%) of *Botrytis cineria*

Table 1: ANOVA for Evaluation of different essential oils on growth inhibition % of *Botrytis cineria*

SOV	DF	SS	MS	F	P≥F
Rep	2	14.6	7.29		
Treatment (T)	3	8423.3	2807.78	4200.25	0.0000
Conc. (C)	2	1710.3	855.17	1279.27	0.0000
Days (D)	1	20.1	20.06	30	0.0000
TxC	6	279	46.5	69.56	0.0000
TxD	3	24.2	8.06	12.05	0.0000
CxD	2	6.8	3.39	5.07	0.0102
TxCXD	6	15	2.5	3.74	0.0041
Error	46	30.8	.67		
Total	71	10524			

Table. 2. *In-vitro* evaluation of different essential oils against *Botrytis cineria*

Sr#	Treatments	Growth inhibition (%)
T_1	Eucalyptus	37.5 d
T_2	Lemon	41.61 c
T_3	Sage	65.64 a
T_4	Fennel	51.27 b
	LSD	0.548

Table 3: Interaction of means of growth inhibition produced by different treatments and concentration (TxC) against *Botrytis cineria*

Sr.No.	Treatments	Growth inhibition (%)		
		3%	6%	10%
1	Eucalyptus	33.5 i	38.16 h	40.83f
2	Lemon	37.3 h	39.5 g	47.8 e
3	Sage	60.8 c	64.5 b	71.7 a
4	Fennel	41.3 f	52 d	60.5 c
5	LSD	0.9502		

Table 4: **Interaction of means of growth inhibition (%) by different treatments and days (TxD) against *Botrytis cineria***

Sr.No.	Treatments	Growth inhibition (%)	
		Day 5	Day 7
1	Eucalyptus	37.8 g	37.1 h
2	Lemon	40.4 f	42.7 e
3	Sage	65.1 b	66.2 a
4	Fennel	50.4 d	52.4 c
5	LSD	0.775	

Table 5: **Interaction of means of growth inhibition (%) by different concentration and days (CxD) against *Botrytis cineria***

Sr.No.	Concentration	Growth inhibition (%) at different days	
		Day 5	Day 7
1	3%	42.7 e	43.7 d
2	6%	48.4 c	48.7 c
3	10%	54.2 b	56 a
	LSD	0.6719	

Table 6. Interaction of means of growth inhibition (%) produced by different treatments, concentrations and days (T x C x D) against *Botrytis cineria*

Treatment	Growth inhibition (%)					
	After 5 days			After 7 days		
	3%	6%	10%	3%	6%	10%
Eucalyptus	34 p	39.3 lm	40.4 kl	33 p	37 no	41 j
Lemon	36 o	38 mn	47 i	38.6 m	41 jk	48.5 h
Sage	60 de	65 c	70 b	61 d	64 c	73 a
Fennel	40.7 jkl	51 g	59.6 e	42 j	53 f	61.4 d
LSD	1.3438					

Discussion

Many fungal pathogens attacked on pomegranate, but the most important plant pathogenic fungi include gray mold (*Botrytis cineria*) rot, black heart (*Aspergillus niger*) rot, green mold (*Penicillium digitatum*) rot and Alternaria (*A. alternata*) rot and blue mold (*P. expansum*) rot (Adaskaveg, 2012). The most important pathogen that cause fruit decay worldwide is *Botrytis cineria* Pers (Tedford *et al.,* 2005).

Natural antimicrobial agents such as essential oils can be used in food industry only if the compounds they release over time and their effects on target plant and pathogen are well-known and understood. Our study indicated that essential oils may possess antifungal activity against grey mould disease agent *B. cinerea* and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread. Suppression of mycelial growth, spore germination and germ tube elongation by essential oil treatments could make a major

contribution to limiting the spread of the pathogen by lowering the spore load in the storage atmosphere and on surfaces.

Four types of essential oils including Eucalyptus, Lemon, Sage and Fennel was used in this experiment to test their efficiency against isolated fungi on PDA media. All essential oils were tested on different doses (3, 6 and 10 %) against *Botrytis cinerea* and data was recorded after 5 and 7days intervals. Results of the experiment demonstrated that all essential oils show significant response against the isolated pathogen. On all doses Sage oil gave best result against isolated pathogen (*B. cinerea*) followed by Fennel oil which showed significant inhibition. Lemon and eucalyptus found to be least significant against *B. cinerea* as compared to Sage and Fennel oils.

My results are in line with (Sukatta *et al*., 2008) conducted an experiment to find the efficacy of essential oil from clove and cinnamon against 6 fungi causing postharvest rots pathogens. Clove oil showed the complete growth inhibition against *Aspergillus niger, Alternaria alternata, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Phomopsis viticola and Rhizopus stolonifera*.

Results indicated by Sharma and Tripathi, 2006 described the maximum growth inhibition oils due to their inhibitory effect on germ tube growth, spore germination as well as mycelia growth against 10 post-harvest disease spreading agents. Xing *et al*., 2012 evaluated the *in vitro* and *in vivo* antifungal activities of clove oil against *Aspergillus flavus*, *Penicillium citrinum* and *Rhizopus nigricans*. The minimum inhibitory concentrations of clove oil against *A. flavus*, *P. citrinum* and *R. nigricans* were 25, 25 and 50 µL/mL respectively. These results indicated that clove oil has a good potential to be a natural antifungal agent for fruit applications. Whereas in this study sage oil was most effective in controlling the pathogen.

Conclusions

Natural antimicrobial agents such as essential oils can be used in food industry only if the compounds they release over time and their effects on target plant and pathogen are well-known and understood. Our study indicated that essential oils may possess antifungal activity against grey mould disease agent *B. cinerea* and can be exploited as an ideal treatment for future plant disease management programs eliminating fungal spread. Suppression of mycelial growth, spore germination and germ tube elongation by essential oil treatments could make a major contribution to limiting the spread of the pathogen by lowering the spore load in the storage atmosphere and on surfaces.

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