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Research Article

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Eco-Friendly Management of *Botrytis cinerea* Causing Gray Mold in Pomegranate

Atifa Akhtar[1], Syed Zulfiqar Ali[1], Muhammad Waris[1]*, Kamran Hashim Jamali[2], Qasid Hussain[3], Zobia Jabeen[1], Abdul Jabbar[4], Muhammad Sharif[5], Gulshan Irshad[6], Muhammad Nawaz Sukhera[7]

[1]Department of Plant Pathology, Balochistan Agriculture College, Quetta, 87100, Pakistan.
[2]Department of Plant Breeding & Genetics, Balochistan Agriculture College, Quetta, 87100, Pakistan.
[3]PARC-Agricultural Research Institute, Jaffarabad, 80500, Pakistan
[4]Department of Plant Breeding & Genetics, Sindh Agriculture University, Tando Jam, 70050, Pakistan.
[5]Department of Soil Science, Balochistan Agriculture College, Quetta, 87100, Pakistan.
[6]Department of Plant Pathology, PMAS-Arid Agriculture University, Rawalpindi, 41600, Pakistan.
[7]Department of Plant Pathology, University of Agriculture Faisalabad, 38000, Pakistan.
*Corresponding Author: Muhammad Waris, waris.faqir@gmail.com

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ABSTRACT

Pomegranate (*Punica granatum*) is an important fruit being grown in Pakistan and throughout world. Its wonderful cultivars are naturally flavored with sweet taste juice. Pathogens invade the fruit under favored conditions and cause economic losses. The most important postharvest disease in pomegranate fruits is gray mold caused by *Botrytis cinerea*. This study aims utilization of eco-friendly essential oils against gray mold disease in fruit. According to *In-vitro* test, of coconut (*Cocos nucifera*), rose (*Rosa sp*.), sesame (*Sesamum indicum*), clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) on Pomegranate exhibited strong effect on *B. cinerea*. Oils exhibited antifungal activity on the causal pathogen and tested through contact as well as volatile phase with different concentrations inhibiting the fungal growth of *Botrytis cinerea* as compare to control. *In vitro* experiment results showed that essential oils like clove followed by cinnamon oils with 9% concentration completely suppressed the growth of mycelial fungus causing gray mold disease in pomegranate. While *In vivo* experiment showed that the essential oil cinnamon followed by clove oil at 9% concentration suppressed the fungus causing gray mold in pomegranate.

Keywords: Pomegranate, Gray mold, Management, Essential oils, eco-friendly

INTRODUCTION

Pomegranate (*Punica granatum* L.) is among the oldest edible fruits of the ancient times. Since the ancient Egyptians, the very old tree was cultivated in Egypt (Mars, 2000). It is spherical fruit having thick, reddish skin containing hundreds of juice-trappings in its inner chamber. It is one of the most known edible fruits grown in regions ranging from tropical to temperate (Jalikop, 2007). Pomegranate show medicinal importance due to anti-mutagenic and antimicrobial characteristics (Sestili *et al.*, 2007). The cultivars of pomegranate are a wonderful fruit recognized for sweet taste, with great amount of juice contents and unique aroma of the fruit. The fruit contains natural iron, potassium and folic acid (Thomidis, 2014). Gray mold spread from irrigation and rainwater that assist pre- overwintered pycnidio spores in pycnidia on tree bark and young plants causing latent infection upon storage at 5°C or with relative humidity (RH) of 90% or greater avoiding loss of weight like brown discoloration (Artes *et al*., 2000). These trees offer more than sweet fruit and elements within the bark and roots can be sever medicinally (Ahmad, *et al*., 1998). However, storage life rapidly increases with low occurrence of latent crown infection (Hess-Pierce and Kader, 2003). New technologies like development of industrial methods based on experimental findings may save 66% water comparing with the irrigation at surface (Chopade *et al*., 2001). Fungal pathogen is responsible for post-harvest losses of fruit (Korsten, 2006). Gray mold is one of the serious pathogens of pomegranate during storage, which may be controlled synthetically by fungicides efficiently (Eckert and Ogawa, 1988). In Pakistan, the total cultivated area of pomegranate is 10866.24 hectare with total production of 89035 tons (El-Qurashi *et al*., 2017). Pomegranate diseases reach at their maximum levels in rainy seasons. (Archana and Jamadar, 2014; Nel *et al*., 2007). Decay can start, especially the stem become fully removed or die. The use of essential oils has proven hazard free environment and adds towards prevention of the postharvest pathogenic activities as well as increase profitability to the growers through healthy production of the fruits (Erkan and Kader, 2011). Pomegranate prefers semi-arid mild temperature to

subtropical climate and is naturally adapts to cool winter and hot summer regions, with delicious taste in tropics and subtropics (Ozugan, 1997). Propagating form cutting procedures a plant of the same characteristics after rooting the plants starts fruiting after about three years (Kahlon, 2002). Variations exist in wild and cultivated genotype for most fruit characters like its color, size, weight, juice, acidity, sweetness etc. (Ercisli *et al*., 2007). However, asexual propagation through cuttings is an economical and easiest way for propagation and we may receive its production after 3-4 years of planting (Yazici, K. *et al*., 2005). Phytochemicals like terpenoids, sterols in seeds, leaves and bark, alkaloids in leaves and bark, fatty acids and triglycerides in seed oil (Newman *et al*., 2007). Gallyol derivatives from leaves, organic acids in juice (Ender *et al*., 2002) are used for treatment of heart disease like cancer, diabetes, aging, AIDS, brain disorders etc (Seeram *et al*., 2006). Fruit adds into dietary health through supplements and nutraceuticals (Faria and Calhau, 2011). Pomegranate are attacked by numerous pathogens at both preharvest and postharvest stages of fruits. The most important fungal diseases of pomegranate fruit include *Botrytis cinerea*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Penicillium* spp. and *Trichoderma* spp. and the affected fruits are non-marketable. The most important bacterial diseases of pomegranate are bacterial blight caused by *Xanthomonas axanopodis* pv. *Punicae granatum* in the field for which bactericide application is necessarily recommended for pathogenic inhibitions. *X. axanopodis* pv. *punicae* cause the disease in the fruit during fruit development stage (Teksur *et al*., 2014). Some pathogens like gray mold infect stored pomegranates and blowout from infected to healthy fruits through mycelium (Tedford *et al*., 2005; Palou *et al*., 2007). The pathogens causing post-harvest disease in pomegranate assess post-harvest decay during cold storage (Palou *et al.,* 2007). *Botrytis* gray molds decay is a serious issue that continues worldwide in flowers, fruits, and vegetable species as mentioned by Masih and paul, (2002). Severe postharvest losses may be caused by the gray molds. Many essential oils of plant origin and their vapor phase bioactivity makes them more emphasizing for the postharvest management of this fungi (Tripathi *et al*., 2008). There are many commercial herbal formulations, which are being utilized in pomegranate extract in a number of the world countries (Du *et al*., 1975). The five medicinal plants like rose (Rosa *sp*.), coconut (*Cocos nucifera*), clove (*Syzygium aromaticum*), sesame (*Sesamum indicum*) and cinnamon (*Cinnamomum zeylanicum*) etc under a modified environment *in vitro* and *in vivo* are being applied against gray mold for its management (D'Aquino *et al*., 2009). Tripathi *et al*., (2008) has reported that the effective control of fruit mold disease derived from five (05) essential oils; Rose oil, Sesame oil, Coconut oil, Cinnamon oil and Clove oil were evaluated for the efficacy against the pathogen, *B. cinerea in vitro* and *vivo*. (Poole and McLeod 1994). Although a major part has been carried out to examine essential oils under *in vivo* and *vitro* conditions as described by Rasooli and Owlia, (2005); Yahyazadeh *et al*., (2008). Sprays of various chemicals have been only successful to some extent, especially in damp weather and under cool condition (katane *et al*., 1989). The essential oils on post-harvest fungi have been examined to a large scale (Feng and Zheng, 2007; Regnier *et al.,* 2010). So, the study was conducted to minimize the disease.

MATERIALS AND METHODS

Survey and sampling: Pomegranate fruits were collected from market of Quetta. The experiment was conducted in Plant Pathology laboratory of Baluchistan Agriculture College, Quetta to evaluate different fungicides through essential oils against gray molds under storage. Survey was performed from local markets of different localities of different districts of Balochistan viz. Quetta, Pishin and Qila Saifullah. During survey, affected fruits were examined for presence of *Botrytis cinerea* in fruits. Samples of gray molds were collected in polythene bags to the Plant Pathology laboratory, Balochistan Agriculture College, Quetta. Storage temperature was maintained at about 4°C.

Isolation of *Botrytis cinerea:* Plant samples with diseased symptoms of gray mold caused by *Botrytis cinerea* were examined for fungal growth and structures under microscope. Samples without any fungal disease were incubated on blotter paper in petri plates for 12h in light and dark condition. During isolation the diseased and healthy portion was surface sterilized with 1% NaOCl for 2-3 minutes then placed into petri plate. Then the pieces were given two washings of distilled water in petri plate, dried into filter paper and then introduced to the PDA media. When the hyphal tip growth was observed in the medium then these fungal structures were transferred for further growth process after the duration of 7 days. Fungi colonies after purification were maintained and preserved on agar slants at 4 degrees Celsius temperature (Mirdegahan, S. H *et al.,* 2007).

Pathogenicity test: Pathogenicity test was performed by using infected and healthy fruits. Fruit was first disinfected with 1% NaOCl solution for 3 minutes and dried. Then the portion of diseased parts were transferred into blotter paper in petri dishes. Then PDA was prepared and each sample was cut for 3mm diameter using the cork borer from the growing points of the culture. The cultures were then incubated at 12h in light and 12h in dark condition. Similarly, those cultures were inoculated and kept under incubation for one week. Re-isolation from the fruit samples were showing prominent symptoms (Or – Mizrahi *et al*., 1986) the frequency of achieved fungus from unalike plant portions was calculated as under:

$$\textbf{Frequency } \% = \frac{\textbf{Number of colonized fungal pieces}}{\textbf{Total No.of culture pieces}} \times \mathbf{100}$$

Preparation of essential oils for their antifungal activity: Essential Oils of Cloves, Cinnamon, Sesame, Rose and Coconut oil were purchased from scientific company (aroma pharmacy). These oils were used in experimentation and handled in refrigeration at 5°C for future use. In clove oil, the plant part that was used for extraction in flower buds. In Rose oil from petals. In cinnamon oil from inner bark. In Sesame oil through seeds and in Coconut oil plant part used for extraction from the kernals and its usage is Oil-Extract. For the preparation of solution Potato Dextrose Agar (PDA) medium was prepared by using PDA 35.1 g and Distilled Water 1000 ml. The efficacy of variety of essential oils e.g. coconut, rose, sesame, clove and cinnamon were assessed at different concentrations at contact and volatile phases to test the antifungal activities against *Botrytis cinerea* (Table. 1). The similar study on different essential oils has been performed by Soylu *et al.,* (2006). For determining the effects through contact phase, the essential oils were dispersed through adding these oils in water (H_2O) using Ethyl Alcohol (C_2H_5OH) and Tween 20 (0.1% v/v), then the prepared solution was quickly added to PDA after it was poured into petri plate containers (90 × 20 mm wide) at a temperature of 26 ° C. Three concentrations at the rate of 3%, 6%, 9% were tested at levels from 0.2 to 24.4 µg/ml. The controls were handled with the similar amount of ethanol and Tween 20 was mixed with the PDA medium. *Botrytis cinerea* were picked and then quickly inoculated by placing in each plate's the center. Approximately 7 mm in diameter the fungus disc was marked and cut with sterile cork borer from the edges of the culture growing vigorously on the PDA petri plates. Petri dishes were then kept in the dark at temperatures rising from 22-25 °C. The growth of the experimental mold fungus was recorded radially by drawing straight lines behind petri plates crossing from the center of the incubated plate. Data taken on the growth of colony of the growing fungus was timely noted along with sketched lines in millimeters. The measurement was carried out after each 24 hours till the plates were observed as filled with fungal mass (Soylu *et al*., 2006). The observation of growth of mycelium pertaining test fungus was noted at the interval of four days during fungus incubation by measuring average diameter.

Table 1. Essential oils with different Concentrations used against *Botrytis cinerea*

S.No.	Essential Oils	Botanical Name	Concentration		
1.	Rose oil	*Rosa spp.*	3%	6%	9%
2.	Clove oil	*Syzygium aromaticum*	3%	6%	9%
3.	Coconut oil	*Cocos nucifera*	3%	6%	9%
4.	Sesame oil	*Sesame indicum*	3%	6%	9%
5.	Cinnamon oil	*Cinnamon indicum*	3%	6%	9%
6.	Control	---	---	---	---

In *Vivo* Assessment of Different Essential Oils: Fruits were arranged into groups and then were placed in polythene packages placing three layered moistened blotters at the inner end. Conidia of *B. cinerea* were recovered from 2-week old cultures by mixing 10 ml of deionized distilled water to every plate. Then the conidial suspensions were filtered by using three-layered sterile cheesecloth. The conidial concentrations of suspension were modified through serial dilution of conidia in ten test tubes and a single drop of Tween 20 was fixed to the suspension prepared. The test fruits were inoculated and then stored at 22°C for period of two weeks. Thereafter, different concentrations of essential oils were prepared and were placed in small conical glass containers i.e. 100 ml flasks in the bottom of the packages. The test fruits were placed at 22°C temperature. This experiment was repeated twice. The results of these two experiments represented the average for experimentation as prescribed by Soylu *et al*., (2006).

Determining the effects through the volatile phase, about 90 x 20 mm of glass petri dishes, were spaced up to 80 ml after adding 20 ml of agar medium. The incubated petri dishes containing altered oil concentrations were then affixed to sterile filter papers having 10 mm diameter using Whatman Schleicher and Schuell No. 41. These filter papers were then put on inner surface of the lid of glass plates to obtain final concentrations of 0.05 to 1.5 µm/ml of space. The glass dishes were then sealed with the parafilm soon after the addition of the required concentrations of oils preventing losses of essential oil vapors from the applied plates and preventing from the contaminants. Then the oil added plates were kept under a controlled environment at 22 °C. The mycelial growth of the pathogen was completed by measuring the width of the colony of control petri plates in two paths at right angles for determining the mean radial after seven (7) days of inoculation when the plate surface of these plates was covered by fungus. As the essential oils have a fungicidal nature, their effects were checked by noticing the development of restrained mycelium forming discs that have been revived, Transferring to non-treated potato dextrose agar. The fungicidal effects shown on plates have no detection of growth, whereas a fungistatic effect was observed when microbial growth was temporarily inhibited. Failure of growth of agar discs of *B. cinerea* occurred through two phases i.e. either the *B. cinerea* was transferred to agar media in absence of oils during the contact phase or effect of oils during volatile phase in which lids of

the plate containing chemicals such as ethanol and Tween 20 (0.1% v/v) with oil was added with fungus. Petri plates were kept for five (5) days in an incubator. Pathogen remains without growth, the activity of variety of concentrations of the test oils were considered as fungicidal or otherwise showing no effects. For each concentration, five plates were prepared for five further replicates. The growth values for mean were received and then inhibition percent of mycelia was converted in relation to the treatment of control values (Soylu *et al*., 2006).

Disease Severity formula was used: The effect of essential oils was checked on stored pomegranates. The severity of essential oils was recorded by fallowing formula:

$$\textbf{Disease Severity} = \frac{\textbf{Sum of all numerical rotting}}{\textbf{No.of fruit examined x Maximum Grade Value}} \times \mathbf{100}$$

Statistical Analysis: The data was applied along with replications by using CRD (Complete Randomized Design). For the statistical tests LSD (Least ignificant Difference) was used (Steel *et al*., 1997).

RESULTS AND DISCUSSION

After analysis Data showed that all concentrations of essential oils controlled the colony growth of *Botrytis cinerea* as compared with the control. Maximum reduction in linear colony growth having 4.35 cm diameter and antifungal effect was 80.26% against *Botrytis cinerea* recorded at 6% concentration of Clove Oil (*Syzygium aromaticum*) followed by reduction in linear colony growth having 3.46 cm diameter and antifungal effect was 63.84% in Cinnamon Oil. Statistically Coconut and Rose had non-significant results i.e. 2.80 cm reduction in colony growth with 51.59% efficacy percentage in Rose Oil and the reduction in colony growth (2.62 cm) was recorded in Coconut Oil with antifungal effect of 48.41%. The least reduction in colony growth occurred in colony growth of Sesame Oil i.e. 1.59 cm with 29.33% of efficacy percentage after the duration of 12 days of incubation. Statistically the difference in reduction of colony growth of pathogen and antifungal effect under different treatments were significant i.e. P<0.05 (Table 2). The Clove Oil at 6% concentration was effective in controlling the mycelial growth of *Botrytis cinerea* as compared with control having zero inhibition. There was linear trend in efficacy of Clove oil among various concentrations. The growth of the fungus gradually decreased with increase in different essential oil concentrations. Similar effects have been reported by Charo *et al*., (2003) who studied inhibition methods against three different concentrations (6%, 9%, 15%). As in 9 % concentration maximum reduction in linear colony growth was 1.7 cm diameter and antifungal effect was 81.11% was recorded in clove oil. In cinnamon oil the linear colony growth 2.3 cm diameter and antifungal effect was 74.45% was recorded. In thyme oil maximum reduction in linear colony growth was 2.8cm diameter and antifungal effect was 68.89%. In neem oil the linear colony growth was 1.7cm diameter and antifungal effect was 81.11%.

Table. 2. Efficacy of different essential oils at 3% concentration against linear colony growth of *Botrytis cinerea*

Essential oils	**Linear colony growth (cm) Days**						**Total Colony Growth (TCG)**	**Reduction in Colony Growth (RCG=C-TCG)**	**Antifungal Efficacy (%)=RCG(100)/C**
	2nd	**4th**	**6th**	**8th**	**10th**	**12th**			
Rose Oil	0.1	1.7	2.15	2.45	2.75	2.85	2.85d	3.37c	54.12c
Clove Oil	0.01	0.06	1.05	1.35	1.45	1.85	1.85f	4.37a	70.23a
Cinnamon Oil	0.06	0.08	0.28	0.56	1.45	2.37	2.37e	3.85b	61.96b
Coconut Oil	0.9	1.35	2.55	2.65	2.80	3.38	3.38c	2.84d	45.59d
Sesame Oil	0.8	2.0	2.35	2.65	2.75	3.46	3.46b	2.76e	44.31e
Control	1.35	1.72	3.15	4.7	4.9	6.22	6.22a	0.00f	0.00f
CV							0.56	0.65	0.65

Antifungal efficacy of different essential oils at 6% concentration against linear colony growth of botrytis cinerea: After analysiy data showed that all concentrations of essential oils controlled the colony growth of *Botrytis cinerea* as compared with the control. Maximum reduction in linear colony growth having 4.35 cm diameter and antifungal effect was 80.26% against *Botrytis cinerea* recorded at 6% concentration of Clove Oil (*Syzygium aromaticum*) followed by reduction in linear colony growth having 3.46 cm diameter and antifungal effect was 63.84% in Cinnamon Oil. Statistically Coconut and Rose had non-significant results i.e. 2.80 cm reduction in colony growth with 51.59% efficacy percentage in Rose Oil and the reduction in colony growth (2.62 cm) was recorded in Coconut Oil with antifungal effect of 48.41%. The least reduction in colony growth occurred in colony growth of Sesame Oil i.e. 1.59 cm with 29.33% of efficacy percentage after the duration of 12 days of incubation. Statistically the difference in reduction of colony growth of pathogen and antifungal effect under different treatments were significant i.e. P<0.05 (Table 3). The Clove Oil at 6% concentration was effective in controlling the mycelial growth of *Botrytis cinerea* as compared with control having zero inhibition. There was linear trend in efficacy of Clove oil among various concentrations. The growth of the fungus gradually decreased with increase in different essential oil concentrations.

Similar effects have been reported by Charo *et al*., (2003) who studied inhibition methods against three different concentrations (6%, 9%, 15%). As in 9 % concentration maximum reduction in linear colony growth was 1.7 cm diameter and antifungal effect was 81.11% was recorded in clove oil. In cinnamon oil the linear colony growth 2.3 cm diameter and antifungal effect was 74.45% was recorded. In thyme oil maximum reduction in linear colony growth was 2.8cm diameter and antifungal effect was 68.89%. In neem oil the linear colony growth was 1.7cm diameter and antifungal effect was 81.11%.

Table. 3 Efficacy of different essential oils at 6% concentration against linear colony growth of *Botrytis cinerea*

Essential oils	**Linear colony growth (cm) Days**						**Total Colony Growth (TCG)**	**Reduction in Colony Growth (RCG=C-TCG)**	**Antifungal Efficacy (%) RCG(100)/C**
	2nd	**4th**	**6th**	**8th**	**10th**	**12th**			
Rose Oil	0.08	0.7	2.15	2.4	2.5	2.62	2.62c	2.80sc	51.59c
Clove Oil	0.01	0.04	1.0	1.02	1.05	1.07	1.07e	4.35a	80.26a
Cinnamon Oil	0.04	0.06	0.25	0.36	1.65	1.96	1.96d	3.46b	63.84b
Coconut Oil	0.8	1.25	2.35	2.45	2.70	2.80	2.80c	2.62c	48.41c
Sesame Oil	0.7	2.95	3.25	3.55	3.75	3.83	3.83b	1.59d	29.33d
Control	1.35	1.72	3.15	4.7	4.9	5.42	5.42a	0.00e	0.00e
CV							8.04	9.60	9.60

Antifungal efficacy of different essential oils at 9% concentration against linear colony growth of *botrytis cinerea:* After analysis its was observed that concentrations of these oils checked the colony development of *Botrytis cinerea* as compared with the control. Maximum reduction in linear colony growth having 5.13 cm diameter and antifungal effect was 86.92% against *Botrytis cinerea* recorded at 9% concentration of Clove oil (*Syzygium aromaticum*) followed by reduction in linear colony growth having 4.39 cm diameter and antifungal effect was 74.37% in Cinnamon oil. Statistically Coconut and Rose had non-significant results i.e. 3.06 cm reduction in colony growth with 51.86% efficacy percentage in Rose oil and the reduction in colony growth (2.77 cm) was recorded in Coconut oil with antifungal effect of 46.95%. The least reduction in colony growth occurred in colony growth of Sesame oil i.e. 0.90 cm with 15.25% of efficacy percentage after the duration of 12 days of incubation. Statistically the difference in reduction of colony growth of pathogen and antifungal effect under different treatments were significant i.e. $P<0.05$ (Table 4). The Clove Oil at 9% concentration was very effective in prohibiting the mycelial incubation of *Botrytis cinerea* as compared with control having zero inhibition. There was linear trend in efficacy of Clove oil among various concentrations. The growth of the fungus gradually decreased with increase in the concentration of different essential oils. Similarly, Charo *et al*., (2003) described the effects of inhibition against three different concentrations as in 6%, 9% and 15% concentrations. He assessed the results that in 15% concentration, maximum reduction in linear colony growth was 1 cm diameter and antifungal effect was 88.88% was recorded in clove oil. In cinnamon oil the maximum reduction in linear colony growth was 1 cm diameter and antifungal effect was 88.89%. In thyme oil the linear colony growth was 1.8 cm diameter and antifungal effect was 80%. In neem oil the maximum reduction colony growth was 0.9 cm diameter and antifungal effect 90% was recorded.

Table.4. Efficacy of different essential oils at 9% concentration against linear colony growth of *Botrytis cinerea*

Essential oils	**Linear colony growth (cm) Days**						**Total Colony Growth (TCG)**	**Reduction in Colony Growth (RCG=C-TCG)**	**Antifungal Efficacy (%) RCG(100)/C**
	2nd	**4th**	**6th**	**8th**	**10th**	**12th**			
Rose Oil	0.06	0.5	2.1	2.3	2.6	2.84	2.84c	3.06c	51.86c
Clove Oil	0.01	0.04	0.09	0.55	0.68	0.77	0.77e	5.13a	86.92a
Cinnamon Oil	0.02	0.06	0.15	0.19	1.3	1.51	1.51d	4.39b	74.37b
Coconut Oil	0.6	1.15	2.15	2.25	2.63	3.13	3.13c	2.77c	46.95c
Sesame Oil	0.6	1.85	2.54	3.45	4.65	5.00	5.00b	0.90d	15.25d
Control	1.35	1.72	3.15	4.7	4.9	6.5	6.5a	0.00e	0.00e
CV							7.24	8.53	8.53

In *vivo* assessment of efficacy at 3% concentration of different essential oils against severity rotting: The results in figure. 1 stated that in-vivo efficacy of optimum concentration at 3% (3g/100 ml) of five essential oils out of medicinal plants against postharvest fruit deterioration losses caused by *Botrytis cinerea.* The maximum concentration doses of Rose oil (*Rosa* spp.), CLove oil (*Syzygium*

aromaticum), Cinnamon oil (*Cinnamon zeylanicum*), Coconut oil (*Cocos nucifera*) and Sesame oil (*Sesamum indicum*) were almost checked against fruit infection caused by *Botrytis cinerea* by comparing with the control. After twenty days of treatment, maximum infected fruits were 24% and the efficacy percentage against fruit infection was 76% in cinnamon oil. Clove oil treated fruits showed 50.67% of severity rotting while the efficacy percentage remained up to 49.33%. The pathogenic reduction against infection was 48.33% in Rose oil with 51.67% infected fruits. Severity rotting ratio and efficacy percentage showed non-significant results among Rose oil and Clove oil as these showed similar homogenous groups in the data. Sesame oil showed 69.33% severity rotting percentage along with 30.67% of the efficacy percentage in fruits. Maximum number of infected fruit was 80% with 20% of minimum efficacy percentage in coconut comparing with the control in which 90.67% of the fruits were infected showing only 9.33% efficacy against infection. Statistically the difference in reduction percentage and number of infected fruit percentage against *Botrytis cinerea* among different treatment applied were significant (P<0.05). The essential oils viz Cinnamon at 3% was effective for the control of pathogenic infection followed by Clove oil and Rose oil showing minor effectiveness on pomegranate fruits

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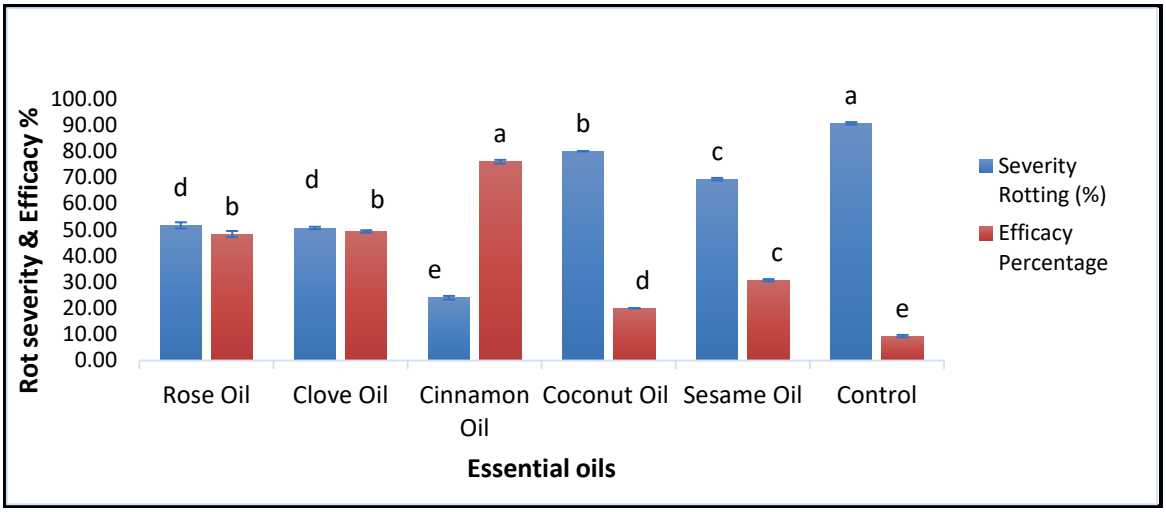

Figure 1. In *vivo* Assessment of efficacy at 3% concentration of different essential oils

In *vivo* assessment of efficacy at 6% concentration of different essential oils: The results in figure. 2 showed that in-vivo efficacy of optimum concentration at 6% (6g/100 ml) of five essential oils out of medicinal plants against postharvest fruit losses for severity were caused by *Botrytis cinerea*. The maximum concentration doses of Rose oil (*Rosa* spp.), CLove oil (*Syzygium aromaticum*), Cinnamon oil (*Cinnamon zeylanicum*), Coconut oil (*Cocos nucifera*) and Sesame oil (*Sesamum indicum*) were practiced against fruit infection caused by *Botrytis cinerea* by comparing with the control. After twenty days of treatment, maximum infected fruits were 19.33% and the efficacy percentage against fruit infection was 80.67% in cinnamon oil. Clove oil treated fruits showed 24.67% of severity rotting while the efficacy percentage remained up to 75.33%. The pathogenic reduction against infection was 73.33% in Rose oil with 26.67% infected fruits. Severity rotting ratio and efficacy percentage showed non-significant results among Rose oil and Clove oil as these showed similar homogenous groups in the data. Sesame oil showed 33.33% severity rotting percentage along with 66.67% of the efficacy percentage in fruits. Maximum number of infected fruits was 53.33% with 46.67% of minimum efficacy percentage in coconut comparing with the control in which 91.25% of the fruits were infected showing only 8.75% of minimum efficacy against infection. Statistically the difference in reduction percentage and number of infected fruit percentage against *Botrytis cinerea* among different treatment applied were significant (P<0.05). The essential oils viz Cinnamon oil at 6% considerably showed higher ratio of affectivity followed by Clove oil and Rose oil which showed moderate effectiveness for the control of pathogenic infection on pomegranate

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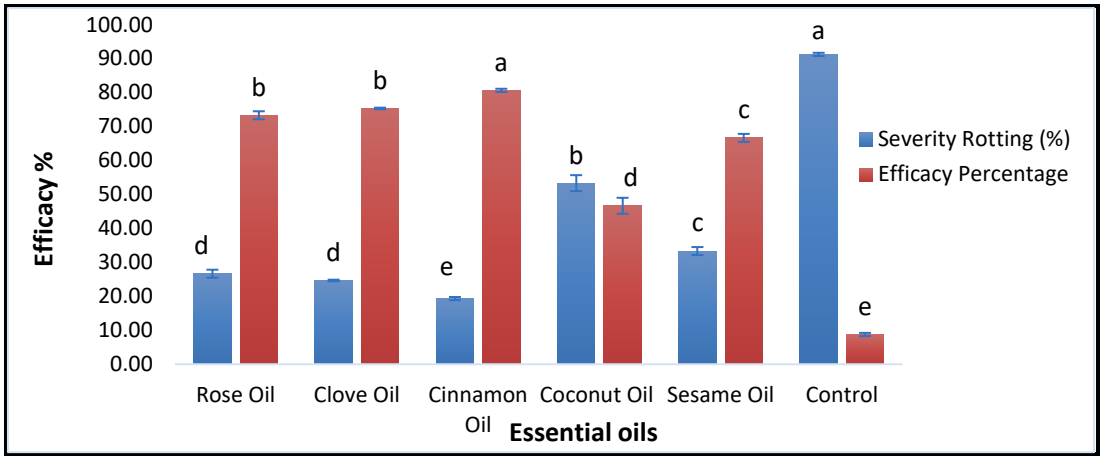

Figure. 2 In *vivo* Assessment of efficacy at 6% concentration of different essential oils

***In-Vivo* assessment of efficacy at 9% concentration of different essential oils:** Effectiveness of essential oils like Cinnamon oil, Clove oil, Sesame oil, Rose oil, Coconut oil at 9% concentration possessed strong *in vivo* antifungal action against the *Botrytis* gray mold disease. The results in figure 3 showed that in-vivo efficacy of optimum concentration at 9% (9g/100 ml) of five essential oils out of medicinal plants against postharvest fruit losses for severity were caused by *Botrytis cinerea.* The maximum concentration doses of Rose oil (*Rosa* spp.), CLove oil (*Syzygium aromaticum*), Cinnamon oil (*Cinnamon zeylanicum*), Coconut oil (*Cocos nucifera*) and Sesame oil (*Sesamum indicum*) were practiced against fruit infection caused by *Botrytis cinerea* in comparison with the control. After twenty days of treatment, maximum infected fruits were 9.33% and the efficacy percentage against fruit infection was 90.67% in Cinnamon oil. Clove oil treated fruits showed 10.67% of severity rotting while the efficacy percentage remained up to 89.33%. Severity rotting ratio and efficacy percentage showed non-significant results among Clove oil and Cinnamon oil as these showed similar homogenous groups in the data. The pathogenic reduction against infection was 84.00% in Rose oil with 16.00% infected fruits followed by 27.33% severity rotting percentage in Sesame oil along with 72.67% of the efficacy percentage in fruits. Maximum number of infected fruits was 33.33% with 66.67% of efficacy percentage in coconut comparing with the control in which 90% of the fruits were infected showing only 10% with least efficacy against infection. Statistically the difference in reduction percentage and number of infected fruit percentage against *Botrytis cinerea* among different treatment applied were significant ($P<0.05$). The essential oils viz Cinnamon and clove at 9% were highly effective for the control of pathogenic infection on pomegranate.

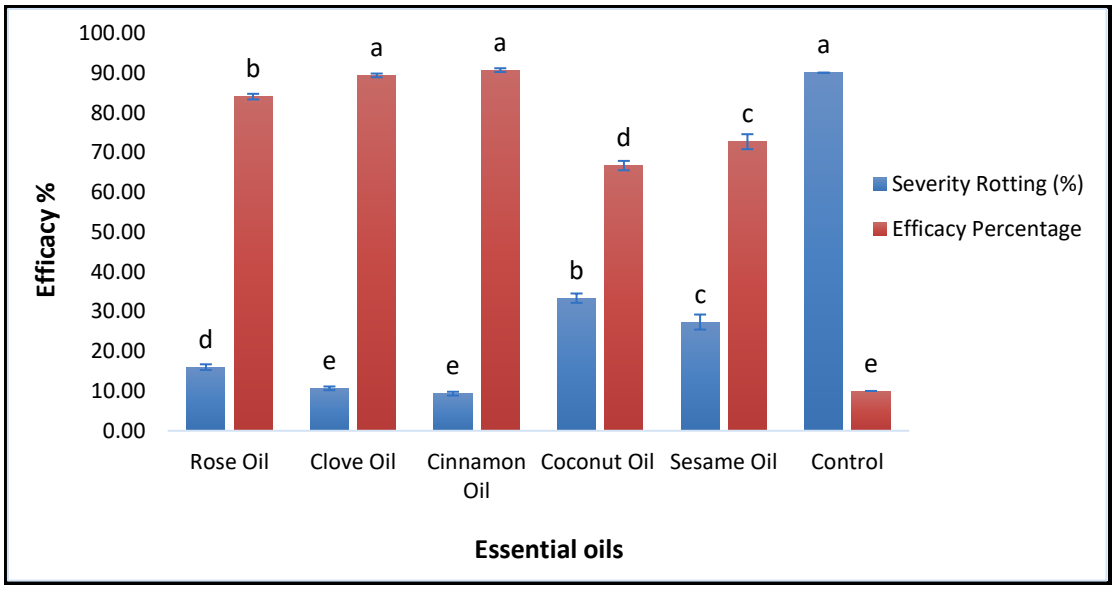

Figure. 3. *In vivo* Assessment of efficacy at 9% concentration of different essential oils

CONCLUSION

It was concluded that *In vitro* experiment showed that essential oils showed that clove followed by cinnamon oils with 9% concentration completely suppressed the growth of mycelial fungus against gray mold of pomegranate. While In *vivo*, essential oils showed cinnamon followed by clove oil at 9% concentration suppressed the mycelial growth of fungus against gray mold of pomegranate. It is recommended that clove and cinnamon oils being environment friendly as compared to fungicides may be used against the gray mold disease of pomegranate caused by the fungal pathogen *Botrytis cinerea*.

AUTHORS' CONTRIBUTIONS

The study, led by Atifa Akhtar a postgraduate scholar, benefited from the scholarly skill of Syed Zulfiqar Ali, known for his significant contributions. The experiments were executed and the data was analyzed by Muhammad Waris, Dr. Zobia, Qasid Hussain and Kamran Hashim Jamali. The manuscript underwent a rigorous review process by esteemed scholar Muhammad Sharif, Abdul Jabbar and Gulshan Irshad whose intellectual contributions and meticulous evaluations enhanced its overall quality and proof read was done by Muhammad Nawaz.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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