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Efficacy of different essential oils, fungicides and biocontrol agents against *Aspergillus niger* the causal agent of fruit rot in Pomegranate

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Key words: Pomegranate, *Aspergillus niger*, Biocontrol, Essential oils, fungicides.

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Abstract

Different essential oils, fungicides and biocontrol agents against *Aspergillus niger* the causal agent of fruit rot in pomegranate. The importance of survey and sampling were done and the pathogenicity test against *Aspergillus niger* was performed. The antifungal potential of different essential oils like Laung, Turpentine, Castus root, Neem, Gulab and Khashkhas was carried out at different doses i.e. 5% 10% and 15% find out the effective oil for the growth inhibition of *Aspergillus niger* effect of some fungicides viz., Melodyduo, Topsin-M, Prevail, Antracol, and Cabriotop against the causal pathogen by food poisoning method at 3 different concentration (100, 200, 300 ppm). Disease incidence was recorded in Killi Oryagi (40%) followed by Killi Murtath (20%), Killi Pattankot (18%) and Killi Zangiwal (14%). Minimum disease incidence was recorded in Killi Lashti (8%). Injection method of inoculation showed a higher percentage of rotting (7.0%) as compared to the cut method of inoculation (4.05%). Minimum colony growth of *Aspergillus niger* (0.10, 0.20 and 0.30%) examined Laung at the dosage Turpentine (57.33, 45.52 and 25.13%), Gulab (41.50, 35.50 and 29.50%), Castus root (65.57, 44.45 and 32.96%), Neem oil (45.00, 42.00 and 37.00%) Maximum colony growth of *Aspergillus niger* (49.00, 45.00 and 41.00%) was observed Minimum linear colony growth of *Aspergillus niger* as observed for Prevail (13.20, 4.72 and 0.25%) at various concentrations respectively followed by Topsin-M (28.50, 20.50 and 11.50%), Cabriotop (33.00, 26.50 and 13.50%), Antracol (49.84, 33.54 and 21.96%), Alliete (44.09, 32.65 and 22.83%) and maximum growth of fungus were determined under Melodyduo (57.16, 45.31 and 37.42%). The fungus growth was observed up to 90% under control.

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Introduction

Pomegranate (*Punica granatum* L.) is an important fruit of tropical, sub-tropical and arid regions. It belongs to the family Punicaceae the name pomegranate comes from a Latin word meaning apple with many seeds and is believed to the native of the middle East (Iran, Pakistan and adjoining countries) and spread to most tropical and subtropical countries of the world. The yearly production of pomegranate in Pakistan is 50109 tons. Balochistan being the major producer counts for 65% of the entire production (Gross, 2007, da Silva *et al.*, 2013). It believed to originate from the Middle East (Iran and adjoining countries) and spread to most tropical and subtropical countries of the world. It is widely cultivated in Iran, Egypt, Pakistan, Spain, Afghanistan, and India and in some place of California, and Bulgaria. Approximately 7990 hactares land under pomegranate are cultivated in Balochistan during 2014-15, in Balochistan the main districts where pomegranate is cultivated are Loralai, Zhobe, Khuzdar, Kalat etc. (Aly *et al.*, 2011). Pomegranate has a high medicinal value. Pomegranate seeds are used to make pomegranate seed oil, which has many positive health effects both internally and externally. It is a good source of vitamins B and C, antioxidant polyphenols, pantothenic acid and potassium and also reduces systolic blood pressure by inhibiting serum of angiotensin-converting enzyme. Pomegranate fights against many diseases like cancer, heart diseases, fertility problems and improves immunity, cholesterol level, bone health, arteries and also improves the dryness of skin and hair (Tomás-Barberán *et al.*, 2013)Pomegranate has been used for ages in many civilizations for the prevention and treatment of a varied number of health maladies such as cancer, diabetes, inflammation, dental plaque, dysentery, and to fight malaria parasites and intestinal infections. It is an important source of bioactive compounds such as Ellagitannins and the Punicalagin (Bharani & Namasivayam, 2016). Pomegranate has been described by the Holy Quran as the fruit of heaven and has been mentioned twice. Local varieties of pomegranate grown in Balochistan are Red Kandhari, Zalari, Bedana, Metha Anar, Sofaid Anar and Khata Anar. Pomegranate is being attacked by several insect pests and diseases. The diseases included *Alternaria* fruit rot, *Aspergillus* fruit rot, *Botrytris* fruit rot, are the major limiting factors in terms of yield losses both qualitatively and quantitatively. Among the above-mentioned diseases, fruit rot of Pomegranate caused by *Aspergillus niger* is one of the major post-harvest infections in which it may cause considerable losses in some cases up to 94% to the pomegranate growers. In Pakistan, this disease invariably appears every year in the pomegranate orchards causing significant yield and quality losses. The disease is more severe in the rainy season and fruit symptoms appeared in two forms; spherical depressed spots occurred in the scattered form on the pericarp only and black rot restricted to internal fruit tissues. Worldwide fruit rot of pomegranate caused by fungi *A. niger*, *Aspergillus* spp., *B. cinerea*, *C. gloeosporioides*, *P. versicolor*, *Penicillium* spp., *Nematospora* spp., *Coniella* spp., *S. racemosum*, *P. granati* and *Rhizopus* spp. (Bardas *et al.*, 2009, Jamadar *et al.*, 2011, Mirabolfathy *et al.*, 2012, Sharma & Jain, 1978, Snowdon, 1990, Thomidis & Exadaktylou, 2011, Hebert & Clayton, 1963) essential oils viz. Terpentine Cstos root oil khashkhasoil neem oil *and (Mint)*were tested on *A. niger* in vitro condition. All the essential oils significantly inhibited the radial mycelia growth of the test pathogen *(A. niger)*. (Munhuweyi *et al.*, 2016) Five fungicides viz., *carbendazim* (0.05%), *mancozeb* (0.25%), companion (0.25%), *copper oxychloride* (0.3%) and *captan* (0.3%) against fruit spot and rot diseases of pomegranate were conducted. Bio-control agents like *Trichoderma viride* and other biocontrol agents were evaluated against *aspergillus niger* causing fruit rot of pomegranate. For this purpose, dual culture technique was used (Jain & Desai, 2018).

Materials and methods

Survey and sampling of pomegranate infected fruits

To record the disease incidence of pomegranate fruit rot, six different locations were selected (Killi Pathankot, Killi Zangiwal, Killi Oryagi, Killi Lashti, Killi Murtath and Killi Pathankot) in district Loralai,

Balochistan. The collected specimens were brought to a mycological laboratory at Faculty of Crop Protection, Department of Plant Pathology, Sindh Agriculture University Tandojam for isolation and identification of the causal agent. The diseases prevalence was obtained by applying the following formula.

$$\text{Disease incidence \%} = \frac{\text{Total number of fruits}}{\text{Diseased fruits}} \times 100$$

Isolation and identification of the causal fungus

Diseased specimens showing the symptoms of fruit rot was brought to mycology laboratory at Faculty of Crop Protection Department of Plant Pathology, Sindh Agriculture University Tandojam. The infected portion including fruits was cut into small pieces of (3 to 4 mm) length and was surface sterilized with 5% commercial bleach (sodium hypochlorite) for 2 minutes. The sterilized pieces were washed two times with sterilized water and shifted sterilized filter paper for drying and then sterilized portions were kept on Petri plates containing fresh potato Dextrose Agar (PDA) medium. Usually, five pieces of infected samples were kept in every plate and another isolation method i-e carrot discs method as described by (Moller & DeVay, 1968). All Petri plates were kept in an incubator at 25°C±2 temperature for 7 days to observe sporulation of the fungi. Meanwhile, diverse fungal colony appeared which was purified using the single spore isolation technique and hyphal tip method. The colony growth of the fungus was recognized on the basis of their morphological characteristic as reported (Pitt & Hocking, 1997)The information on the frequency of isolated fungi from different plant parts was calculating this formula:

$$\text{Frequency \%} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total no of pieces cultured}} \times 100$$

To perform the pathogenicity test

The pure cultures of the fungi isolated were maintained on PDA in culture tubes which were stored in the refrigerator at 24°C and used frequently. These were multiplied on 2% PDA for two-three weeks. The inoculum potential of each isolate was prepared by taking 1gm culture in 20 ml distilled water. Pathogenicity tests were carried out. The suspension was inoculated in 10 healthy fruits to see the severity of the disease.

Pure culture of the causal fungus

After identification, the pure culture of fungus was made and maintained for future use and the colony growth response of fungus was also be observed against media on potato dextrose ager (PDA).

To evaluate the efficacy of various essential oils on linear colony growth of fungus

The efficacy of different essential oils was examined under *in-vitro* conditions against the causal agents of fruit rot of pomegranate.

The essential oils were used, with two different doses with enough replications. The pathogen was cut from 8-10 days old culture plate by using sterile cork borer (5mm) and placed in the centre of the PDA plate and incubated at 28°C. Petri dishes without oil was treated as control. The radial colony growth of test pathogen (*Aspergillus niger*) was recorded by drawing two mutually perpendicular lines on the back of the Petri plates crossed each other in the center of the plate.

The data on colony growth was recorded along with these lines in millimeter after every 24 hours until the plates were completely fill in any treatment. Similarly, the efficacy of essential oils was also be evaluated through food poisoned method. In this method, the essential oils were added aseptically to the PDA medium with a drop of Tween 20.

The resulting media were immediately dispensed (20 ml/ Petri dish) into sterilized Petri plates. After.

To test the effect of different biocontrol agents against Aspergillus niger under in-vitro conditions

Bio-control agents like *Trichoderma viride* and other biocontrol agents were evaluated against *Aspergillus niger* causing fruit rot of pomegranate. For this purpose, dual culture technique was used (Rajendiran *et al.*, 2010).

To evaluate the efficacy of different fungicides

Furthermore, in-vitro studies of some selective chemical fungicides viz., Melodyduo, Topsin-M, Prevail, Antracol, and Cabriotop fungicides were evaluated against the causal pathogen by food poisoning method. For this purpose, 3 different concentration (100, 200, 300 ppm) was incorporated in the PDA medium before the poring. Medium without fungicide served as control. After solidifying of the medium, 5 mm diameter agar disk of test fungus was cut from 8-10 days old culture plate by using sterile cork borer and placed it in the centre of the PDA plate.

The inoculated plates were incubated at 25 °C. The radial colony growth of test fungus was recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the centre of the plate. The data on colony growth was recorded along with these lines in millimetre after every 24 hours until the plates were filled in any treatment.

Results

Isolation and identification

The diseased specimens were treated at the laboratory for the isolation and identification of the disease-causing organisms. For this purpose, the specimens were cultured on the artificial nutrient media (PDA) and kept under observation for seven days. The isolated fungi were then sub-cultured for purification of the actual cause of the disease.

The isolation and identification process reveal the association of different fungi from the infected parts of the pomegranate i.e. *Aspergillus niger*, *Botrytis cincerea*, *Penicillium* spp. and *Rhizopus* spp. Among all the isolated fungi, *A.niger* remains the most frequent and pre-dominant fungus and identified on the basis of their morphological characteristics with the help of electronic microscope (Fig.1).

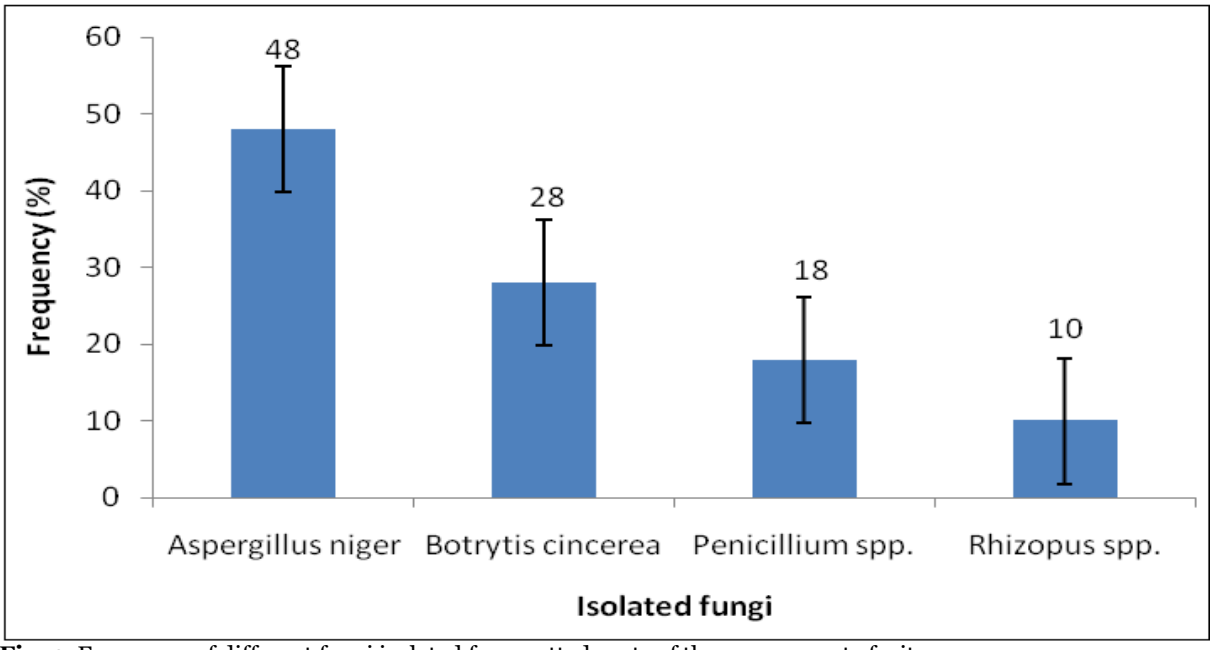

Fig. 1. Frequency of different fungi isolated from rotted parts of the pomegranate fruit.

Disease incidence (%) in different locations of district Loralai, Balochistan

The results (Fig. 2) showed that the disease incidence from different locations of Loralai was observed as 18, 14, 40, 8 and 20% in Killi Pathankot, Killi Zangiwal, Killi Oryagi, Killi Lashti and Killi Murtath, respectively. Maximum disease incidence was recorded in Killi Oryagi followed by Killi Murtath, Killi Pattankot and Killi Zangiwal. Minimum disease incidence was recorded in Killi Lashti.

Rotting (%) through cut and injection inoculation methods

Results regarding the rotting (%) through cut and

injection inoculation methods are presented in Fig.3. The data shows that 4.05%, 7.0% and 2.6% rotting of *A. niger* in pomegranate was observed by cut, injection and dip method of inoculation. On the basis of percentage, it was observed that the injection method of inoculation showed a higher percentage of rotting as compared to cut and dip method of inoculation.

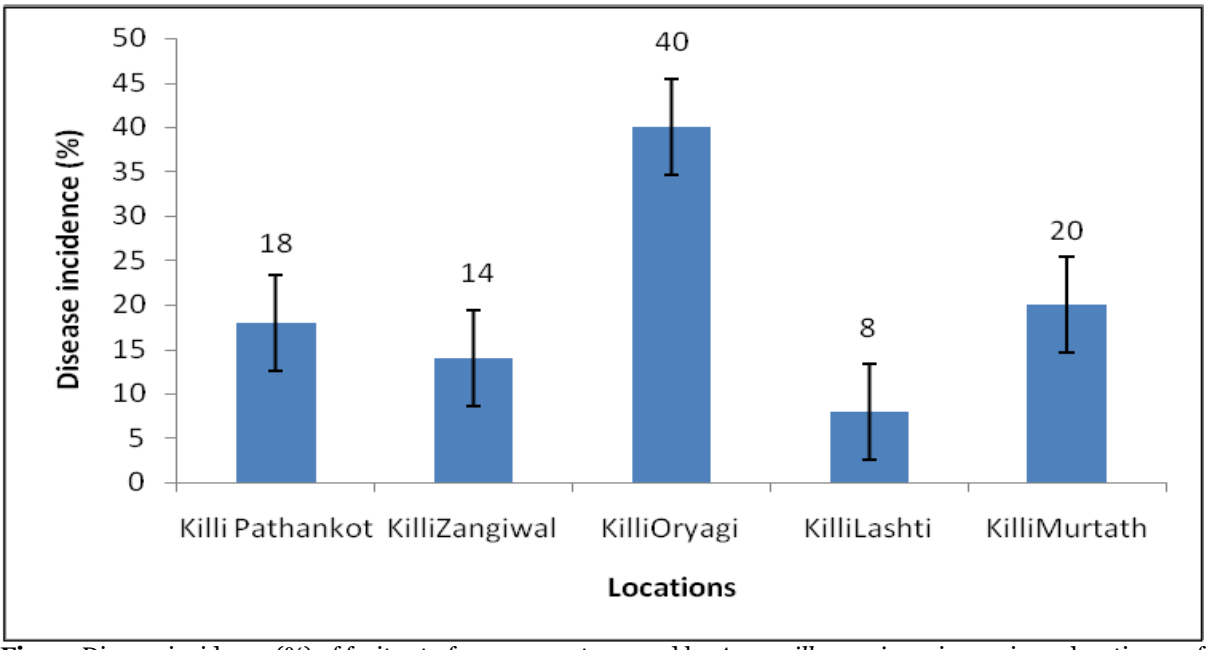

Fig. 2. Disease incidence (%) of fruit rot of pomegranate caused by *Aspergillus niger* in various locations of district Loralai, Balochistan.

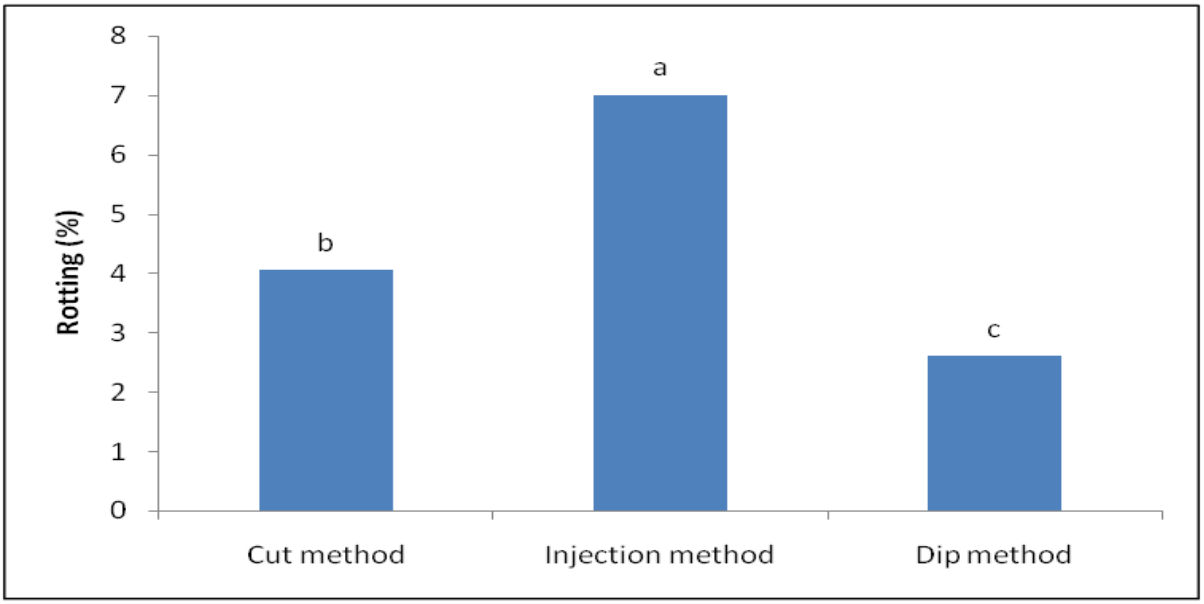

Fig. 3. Rotting (%) through cut, injection and dip inoculation methods.

Linear colony growth of under different essential oils

The results regarding linear colony growth of *Aspergillus niger* under different essential oils is presented in Fig. 5. The data clarified that minimum colony growth of *Aspergillus niger* (0.10, 0.20 and 0.30%) was examined under Laung oil at the dosage of 5%, 10% and 15% followed by Turpentine oil (57.33, 45.52 and 25.13%), Gulab oil (41.50, 35.50 and 29.50%), Castus root oil (65.57, 44.45 and 32.96%), Neem oil (45.00, 42.00 and 37.00%) at the

dosage of 5%, 10% and 15%, respectively. Maximum colony growth of *Aspergillus niger* (49.00, 45.00 and 41.00%) was observed under Khashkhas at the dosage of 5%, 10% and 15%. Under control, the *Aspergillus niger* showed (90 mm) colony growth. On the basis of means, Laung oil ranked 1st, Turpentine oil ranked 2nd, Gulab oil ranked 3rd, Castus root oil ranked 4th, Neem oil ranked 5th, Khashkhas oil ranked 6th for controlling colony growth of *Aspergillus niger* under *in-vitro* conditions. Statistical analysis of the obtained data reveals that there was a significant difference in linear colony growth of *Aspergillus niger* among the essential oils at different dosages.

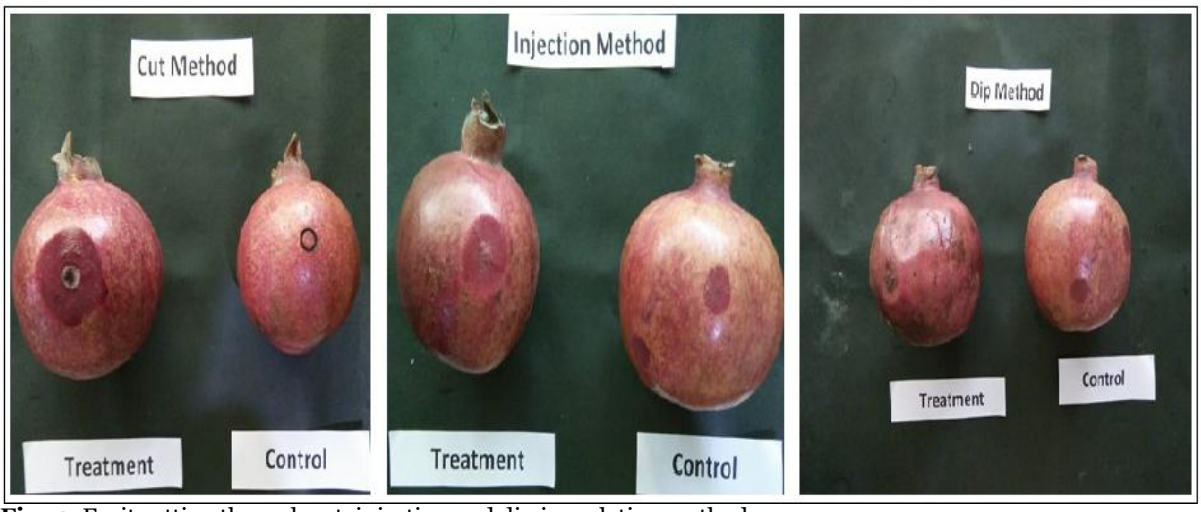

Fig. 4. Fruit rotting through cut, injection and dip inoculation methods.

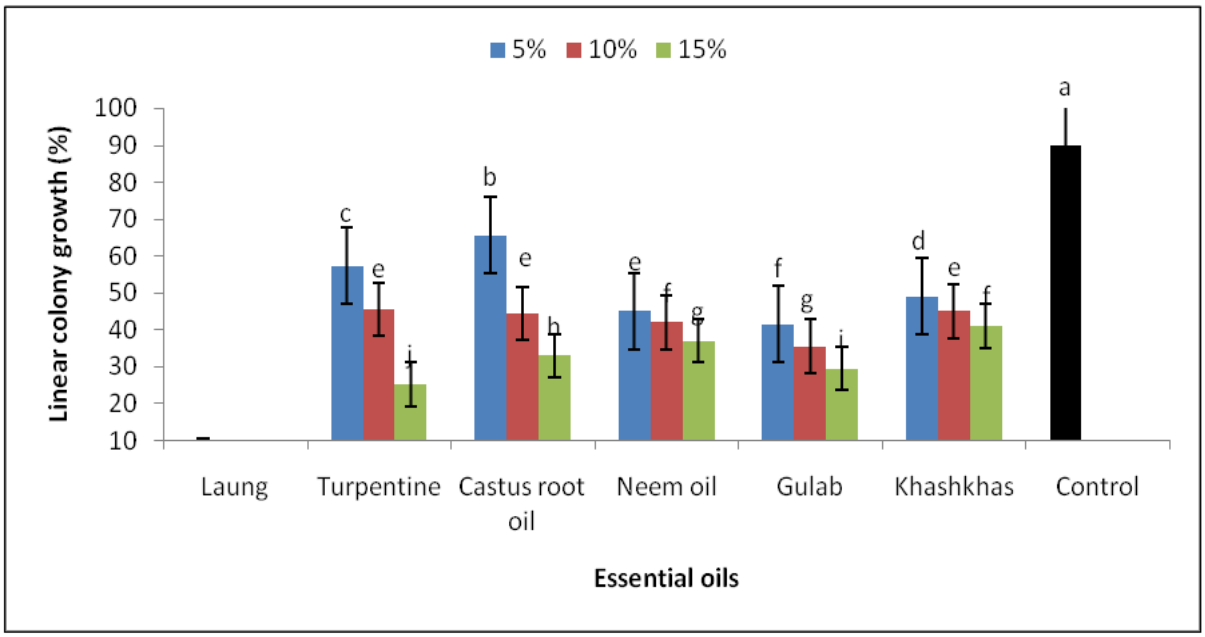

Fig. 5. Linear colony growth (%) of *Aspergillus niger* at different concentrations of essential oils in comparison with control.

Linear colony growth of Aspergillus niger under different fungicides

Results (Fig. 11) showed that minimum linear colony growth of *Aspergillus niger* was observed for Prevail (13.20, 4.72 and 0.25%) at various concentrations i.e. 100, 200 and 300ppm, respectively followed by Topsin-M (28.50, 20.50 and 11.50%), Cabriotop (33.00, 26.50 and 13.50%), Antracol (49.84, 33.54 and 21.96%), Alliete (44.09, 32.65 and 22.83%) and maximum growth of fungus were determined under

Melodyduo (57.16, 45.31 and 37.42%). The fungus growth was observed up to 90% under control. Minimum growth of the fungus was observed under Prevail followed by Topsin-M, Cabriotop, Antracol and Alliete and maximum growth of the fungus was determined under Melodyduo. Minimum linear colony growth of *A. niger* was observed at 300% ppm and maximum linear colony growth of *A. niger* was observed at 100 ppm for all fungicides. Statistical analysis of the data revealed that there was a significant difference between the fungicides at a different level of concentration for linear colony growth of fungus.

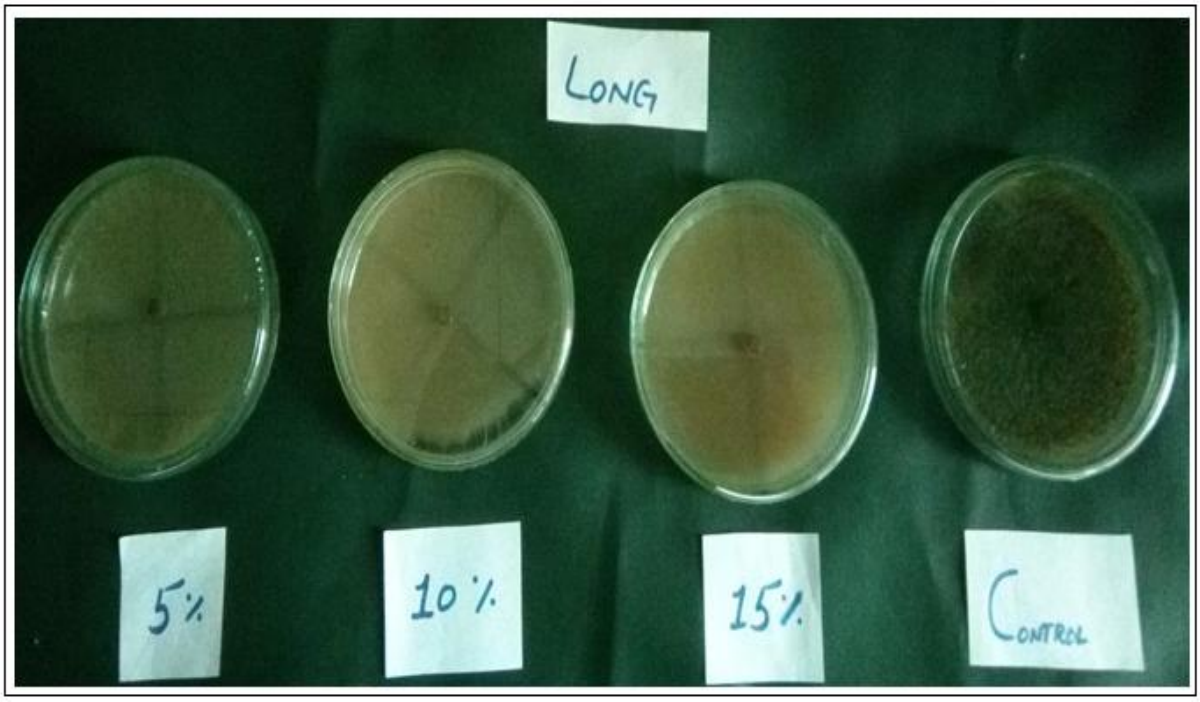

Fig. 6. Colony growth (mm) of *Aspergillus niger* at different concentrations of Long oil in comparison with control.

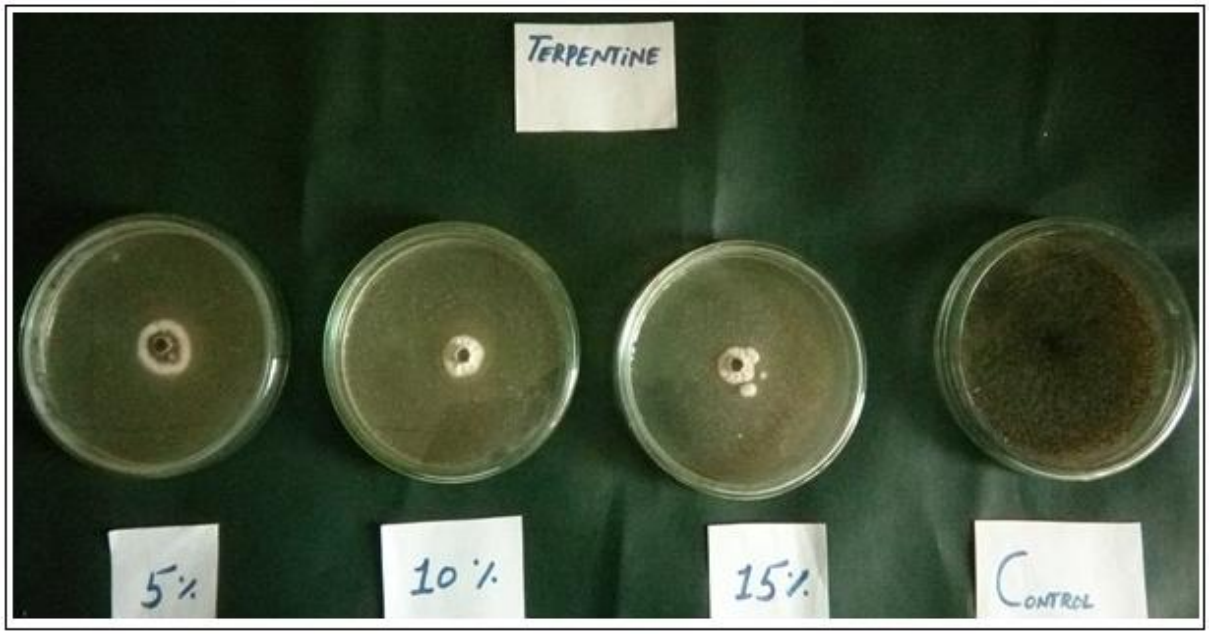

Fig. 7. Colony growth (mm) of *Aspergillus niger* at different concentrations of Terpentine oil in comparison with control.

Linear colony growth of Aspergillus niger under different biocontrol agents

The results (Fig.16) indicates that minimum linear colony growth of *A. alternata* was observed for *Fusarium* sp. (40.33%), followed by *Neurospora* spp. (41.75%), *Chactomium subaffine* (46.82%), *Lasiodiplodia theobromae* (65.1%) and *Hypoxylon* Sp1 (67.67%). Maximum mycelial colony growth (90%) was recorded in control. Statistically, there was significant ($p<0.05$) difference in mycelial colony growth between the biocontrol agents.

Fig. 8. Colony growth (mm) of *Aspergillus niger* at different concentrations of Castus root oil in comparison with control.

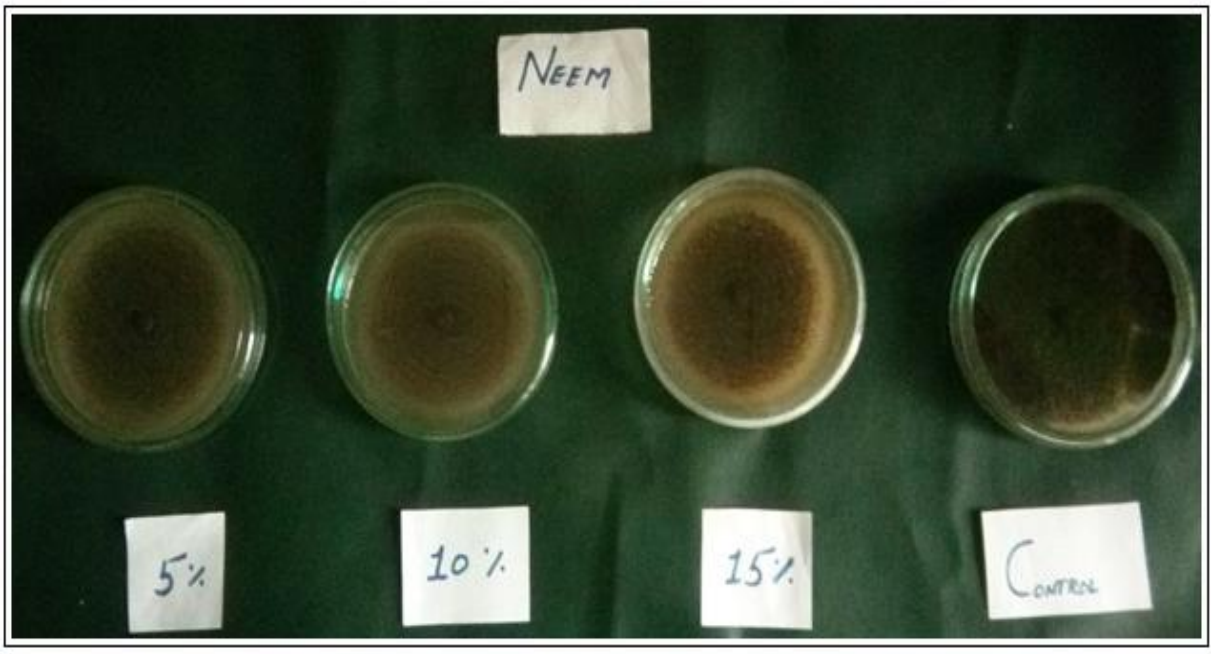

Fig. 9. Colony growth (mm) of *Aspergillus niger* at different concentrations of Neem oil in comparison with control.

Pomegranate is being attacked by several insect pests and diseases. The diseases included Alternaria fruit rot, Aspergillus fruit rot, *Botrytris* fruit rot, are the major limiting factors in terms of yield losses both qualitatively and quantitatively. Among the above-mentioned diseases, fruit rot of Pomegranate caused by *Aspergillus niger* is one of the major post-harvest infections in which it may cause considerable losses in some cases up to 94% to the pomegranate growers. In Pakistan, this disease invariably appears every year in the pomegranate orchards causing significant yield and quality losses. The disease is more severe in the rainy season and fruit symptoms appeared in two forms; spherical depressed spots occurred in the scattered form on the pericarp only and black rot restricted to internal fruit tissues.

Fig. 10. Colony growth (mm) of *Aspergillus niger* at different concentrations of Gulab oil in comparison with control.

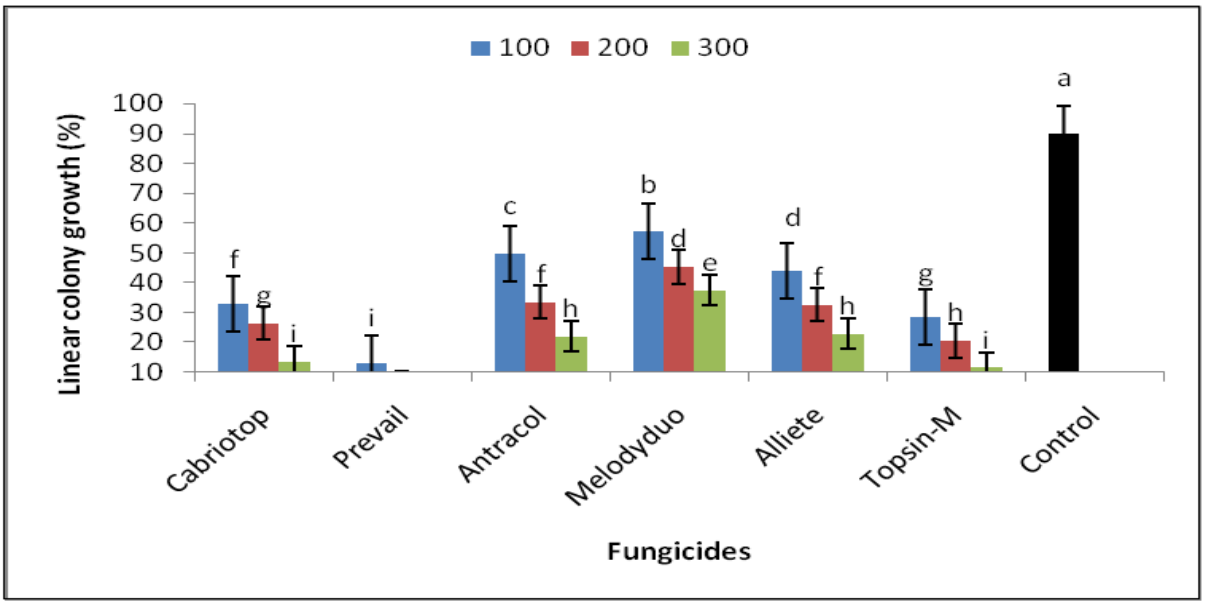

Fig. 11. Linear colony growth (%) of *Aspergillus niger* at different concentrations of fungicides in comparison with control.

Discussion

Findings of the current study revealed that maximum disease incidence was recorded in Killi Oryagi and minimum disease incidence was recorded in Killi Lashti. The results are in agreement with (Li *et al*., 2009) reported that marigold *Tagetes erecta* is an important commercial crop. A leaf spot disease of *T. erecta* was observed during 2012 and 2013 in the Beijing district. The disease was widespread, with 60 to 75% of the fields affected.

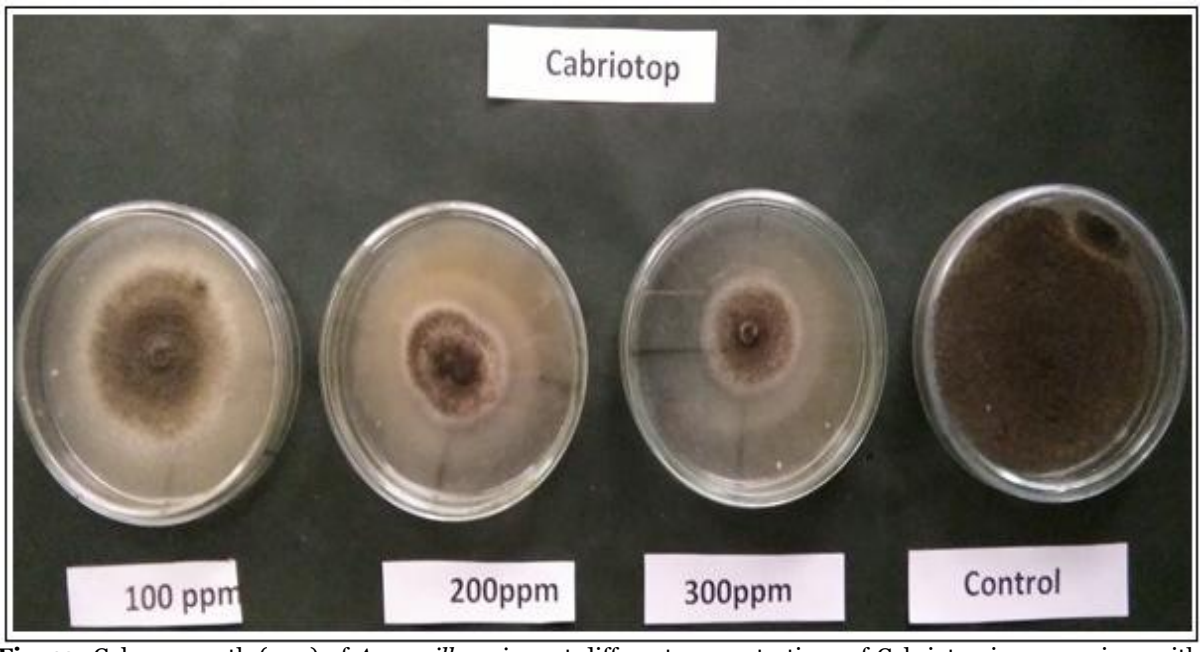

Fig. 12. Colony growth (mm) of *Aspergillus niger* at different concentrations of Cabriotop in comparison with control.

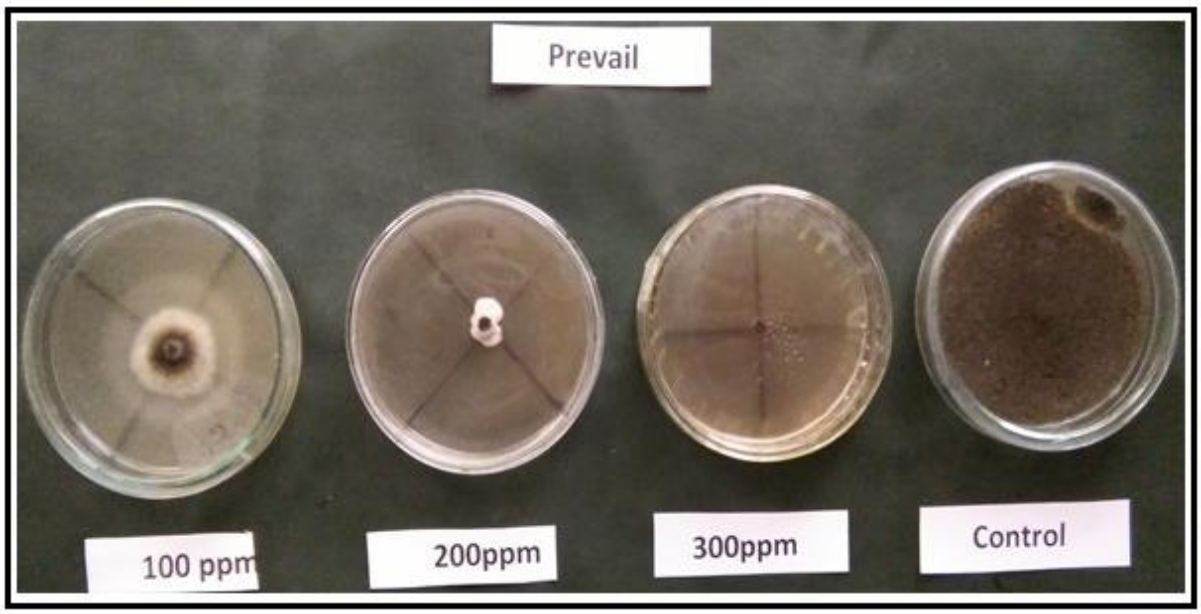

Fig. 13. Colony growth (mm) of *Aspergillus niger* at different concentrations of Prevail in comparison with control.

The crop suffers from many diseases, out of which leaf spots, leaf blight, powdery mildew, flower bud rot and damping-off are important diseases of marigold. Leaf spot caused by *A. alternate* is one of the major diseases of marigold. The leaf spot disease was widespread, with 60 to 75% of the fields affected.

Leaves of the affected plants had small, brown, necrotic spots on most of the foliage. Yield losses of flowers of up to 20 to 30% were reported (*Li et al., 2009*). In our study Laung oil ranked 1st, Turpentine oil ranked 2nd, Gulab oil ranked 3rd, Castus root oil ranked 4th, Neem oil ranked 5th, Khashkhas oil ranked 6th for controlling colony growth of *Aspergillus niger* under *in-vitro* conditions These results are in accordance with the findings of Pawar and Thakar, 2006 they stated that most of the other essential oils were found challenging to combat *A. niger*, suggesting their use as strong aromatherapeutic agents. Minimum rotting (12.53%) was recorded for Laung oil followed by Neem oil (13.30%), Tarpin oil (13.42%), Roghan e Gul (Rose oil) (14.27%), Castor oil (15.16%) and Chamomile oil (17.25%), respectively.

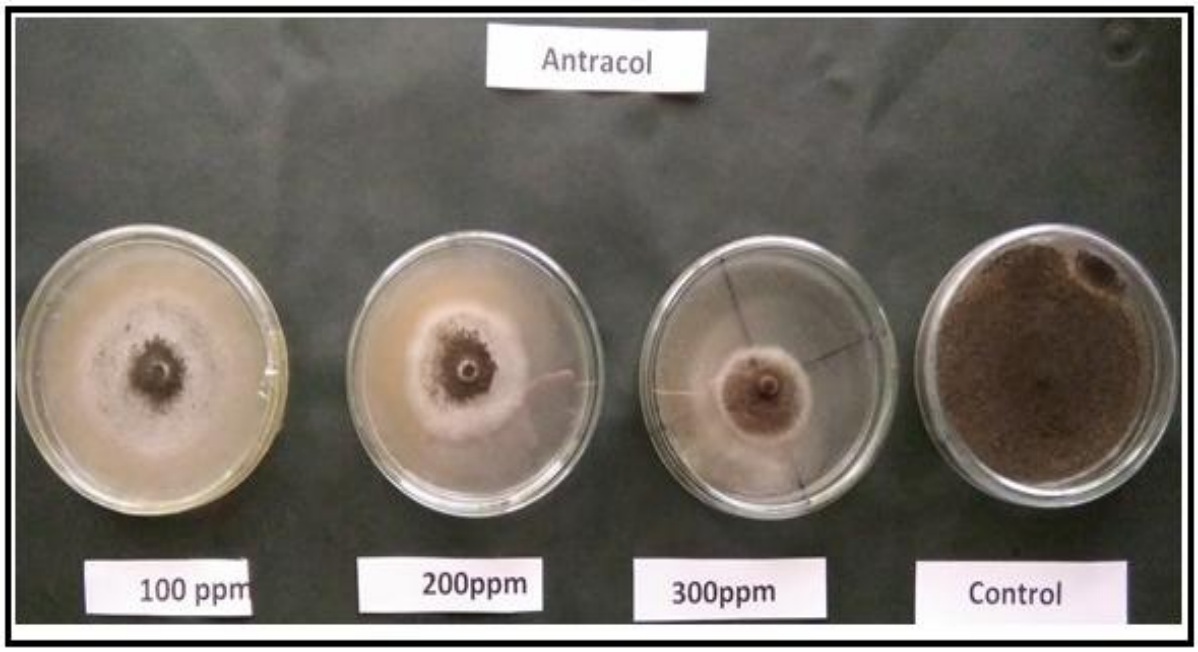

Fig. 14. Colony growth (mm) of *Aspergillus niger* at different concentrations of Antracol in comparison with control.

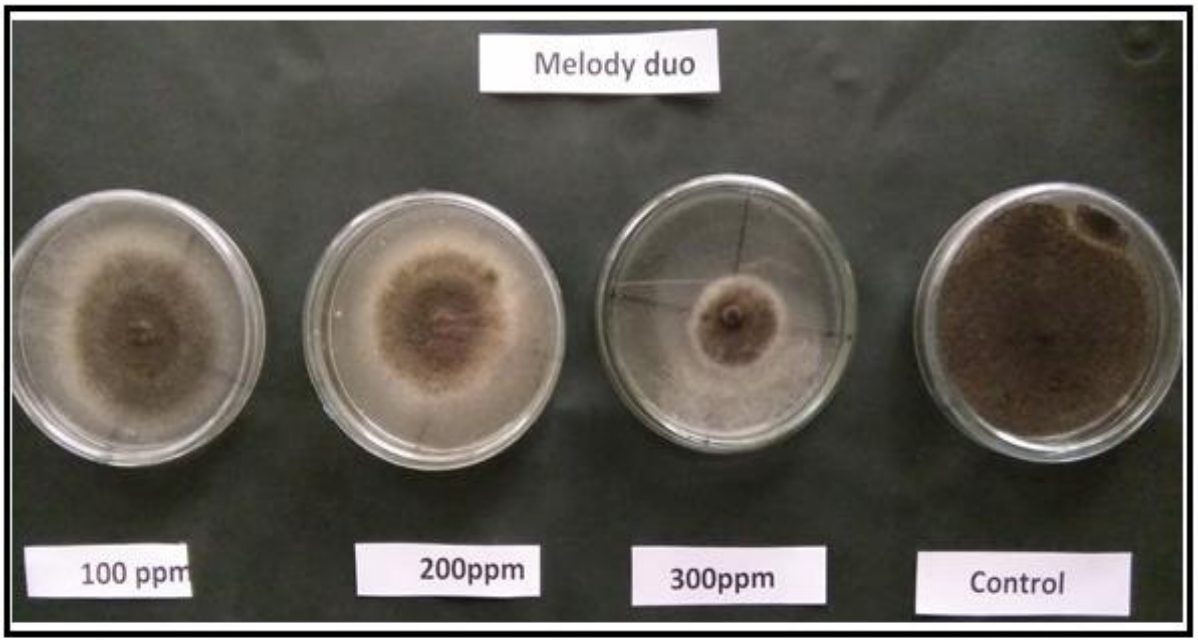

Fig. 15. Colony growth (mm) of *Aspergillus niger* at different concentrations of Melodyduo in comparison with control.

These findings agree with other studies reported by (Guynot *et al.*, 2003; Suhr & Nielsen, 2003). Several investigations showed that the antifungal activity of the volatile compounds by vapour contact is better than that obtained by broth dilution or by agar diffusion (Suhr and Nielsen,2003; Inouye *et al.*,2000). The results obtained in our experimental conditions showed that the antifungal effect of the essential oils and their major components limited the growth of fungus to a higher concentration. Even if this method is easy to use and widely utilized, it is principally a qualitative test which gives no more than an idea about the volatile fraction of the essential oils.

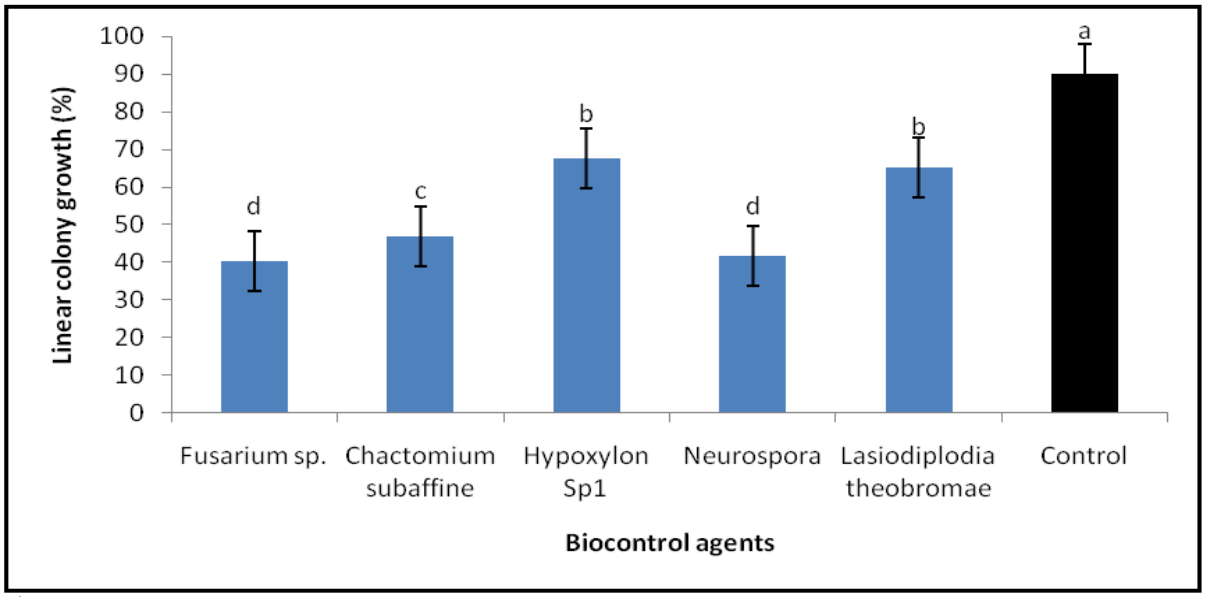

Fig. 16. Linear colony growth (%) of *Aspergillus niger* under different biocontrol agents in comparison with control.

It has been reported that the large inhibition of colonial growth of pathogen (*Aspergillus niger*) observed with some essential oils is not only due to the effect of their diffusion, but it is also due to the vapor effect of these oils in the agar medium (Inouye *et al.,* 2006). In addition, the scientists, who have evaluated the antimicrobial activity of the essential oils by the agar diffusion test, have frequently dispersed essential oils in detergents and solvents such as dimethyl sulfoxide (DMSO) (Sabulal *et al.*, 2006), ethanol and Tween 80 (Inouye *et al.*, 2000). These dispersing agents increase the diffusion of the essential oils in the agar medium. In the present study, essential oils and their major components were dispersed in an agar viscous solution (0.2%) without using any detergent or solvent. It has been previously demonstrated (Remmal *et al.*, 1993) that the dispersing agents habitually used (Tween 80, TritonX-100 and ethanol) have an inhibitory effect on the antimicrobial activity. This inhibition was confirmed later by other authors (Cavanagh & Wilkinson, 2002; Inouye *et al.*, 2000). Therefore, this widely used test does not allow accurately measure of the antifungal activity of the essential oils and their major components; it is a qualitative method which can be used as a preliminary test to select efficient essential oils. Many publications have reported the antifungal activity of the essential oils (Tantaoui-Elaraki & Beraoud, 1994; Chami *et al.*, 2005). It has been demonstrated that this property is essentially is due to the presence of some major phenolic components such as thymol, eugenol and carvacrol (Inouye *et al.*, 2000). This is in agreement which has been previously published using the same oils (Suhr & Nielsen, 2003). Rosemary oil showed a weaker activity on *Aspergillus niger* growth compared to oregano, thyme and clove oils. These findings parallel those already reported by (Inouye *et al.*, 2006). The results we have obtained with the phenolic major components using the broth dilution method revealed

that thymol and carvacrol have a more potent anti-Aspergillus effect than eugenol, mentioning that in our experimental conditions, thymol had a better fungicidal effect than carvacrol. The discrepancy between the last results and those concerning the fungicidal effect of essential oils would be explained by the fact that the oregano oil used in this work contains both carvacrol and thymol, while the thym oil contains only thymol. Several publications have previously reported that the antimicrobial activity of essential oils is due to the presence of these major components (Jeff-Agboola *et al.*, 2012; Dorman & Deans, 2000; Pina-Vaz *et al.*, 2004).The antifungal activity of these agents in the micro atmosphere method and agar diffusion method is limited to high concentrations, while the broth dilution method is more sensitive, and the anti-Aspergillus effect is observed at very low concentrations. So, in our experimental conditions, the anti-Aspergillus efficiency of these agents was better appreciated when they were applied directly into liquid medium than when they were applied as volatiles or diffused in a solid medium. These results are in agreement with those obtained by (Suhr & Nielsen, 2003).

Conclusion

On the basis of present investigation, it was concluded that Lung oil, Prevail and *Fussarium* spp. produced high efficacy in controlling the target pathogen. Laung oil, prevail fungicides and *Fussarium* spp. should be used for controlling *Aspergillus niger* in pomegranate fruit. Further studies may be conducted on the efficacy of essential oils, fungicides and biocontrol agents against other fungal species on various fruits and vegetables.

References

Aly K, Shaukat SS, Mian S. 2011. Management of plant nematodes associated with pomegranate (Punica granatum L.) using oil-cakes in Balochistan, Pakistan. Indian Journal of Nematology **41,** 1-3.

Bardas G, Tzelepis G, Lotos L, Karaoglanidis G. 2009. First report of Botrytis cinerea causing gray mold of pomegranate (Punica granatum) in Greece. Plant disease **93,** 1346.

Bharani RA, Namasivayam SKR. 2016. Pomegranate (Punica granatum L.) Peel Extract-A Study On Potential Source Of Pharmacological Activities. International Journal of Pharma and Bio Sciences **7,** 282-90.

Cavanagh H, Wilkinson J. 2002. Biological activities of lavender essential oil. Phytotherapy research **16,** 301-8.

Chami F, Chami N, Bennis S, Bouchikhi T, Remmal A. 2005. Oregano and clove essential oils induce surface alteration of Saccharomyces cerevisiae. *Phytotherapy Research: An* International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives **19**, 405-8.

Da Silva JaT, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA. 2013. Pomegranate biology and biotechnology: a review. Scientia Horticulturae **160,** 85-107.

Dorman H, Deans SG. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology **88,** 308-16.

Gross PM. 2007. Superfruits take center state. NPI Center. Retrieved October **17,** 2007.

Guynot M, Ramos A, Seto L, Purroy P, Sanchis V, Marin S. 2003. Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. Journal of Applied Microbiology **94**, 893-9.

Hebert T, Clayton C. 1963. Pomegranate fruit rot caused by Coniella granati. *Plant Disease Reporter* **47**, 222-3.

Inouye S, Tsuruoka T, Watanabe M. 2000. Inhibitory effect of essential oils on apical growth of Aspergillus fumigatus by vapour contact. *Mycoses* **43**,

17-23.

Inouye S, Uchida K, Abe S. 2006. Vapor activity of 72 essential oils against a Trichophyton mentagrophytes. Journal of Infection and Chemotherapy **12,** 210-6.

Jain K, Desai N. 2018. Pomegranate the cash crop of India: a comprehensive review on agricultural practices and diseases. International J Health Science Res **8,** 315-36.

Jamadar M, Jawadagi R, Sataraddi A, Patil D, Patil R. 2011. Status of pomegranate diseases of northern Karnataka in India. Acta horticulturae **890,** 501-7.

Jeff-Agboola Y, Onifade A, Akinyele B, Osho I. 2012. In vitro antifungal activities of essential oil from Nigerian Medicinal Plants against toxigenic Aspergillus flavus. Journal of Medicinal Plants Research **6,** 4048-56.

Li Y, Gao F, Gao F, Shan F, Bian J, Zhao C, 2009. Study on the interaction between 3 flavonoid compounds and α-amylase by fluorescence spectroscopy and enzymatic kinetics. Journal of Food Science **74,** C199-C203.

Mirabolfathy M, Groenewald J, Crous P, 2012. First report of Pilidiella granati causing dieback and fruit rot of pomegranate (Punica granatum) in Iran. Plant Disease **96, 461**.

Moller W, Devay J. 1968. Carrot as a species-selective isolation medium for Ceratocystis fimbriata. Phytopathology **58**, 123-4.

Munhuweyi K, Lennox CL, Meitz-Hopkins JC, Caleb OJ, Opara UL. 2016. Major diseases of pomegranate (Punica granatum L.), their causes and management—A review. Scientia Horticulturae **211,** 126-39.

Pawar V, Thaker V. 2006. In vitro efficacy of 75 essential oils against Aspergillus niger. *Mycoses* **49,** 316-23.

Pina-Vaz C, Gonçalves Rodrigues A, Pinto E. 2004. Antifungal activity of Thymus oils and their major compounds. Journal of the European Academy of Dermatology and Venereology **18,** 73-8.

Pitt JI, Hocking AD. 1997. Aspergillus and related teleomorphs. In. Fungi and food spoilage. Springer, 339-416.

Rajendiran R, Jegadeeshkumar D, Sureshkumar B, Nisha T. 2010. In vitro assessment of antagonistic activity of Trichoderma viride against post harvest pathogens. Journal of Agricultural Technology **6**, 31-5.

Remmal A, Bouchikhi T, Rhayour K, Ettayebi M, Tantaoui-Elaraki A. 1993**.** Improved method for the determination of antimicrobial activity of essential oils in agar medium. Journal of Essential Oil Research **5**, 179-84.

Sabulal B, Dan M, Kurup R, Pradeep NS, Valsamma RK, George V. 2006. Caryophyllene-rich rhizome oil of Zingiber nimmonii from South India: Chemical characterization and antimicrobial activity. *Phytochemistry* **67**, 2469-73.

Sharma N, Jain A. 1978. Two new fruit rot diseases of pomegranate (Punica granatum L.) caused by Coniella spp.[India]. Current Science (India*)*.

Snowdon AL. 1990. A colour atlas of post-harvest diseases and disorders of fruits and vegetables.

Suhr KI, Nielsen PV. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. Journal of Applied Microbiology **94,** 665-74.

Tantaoui-Elaraki A, Beraoud L. 1994. Inhibition of growth and aflatoxin production in Aspergillus parasiticus by essential oils of selected plant

materials. Journal of environmental pathology, toxicology and oncology: official organ of the International Society for Environmental Toxicology and Cancer **13,** 67-72.

Thomidis T, Exadaktylou E. 2011. First report of Pilidiella granati on pomegranate with symptoms of crown rot in the prefecture of Xanthi, Greece. Plant disease **95,** 79.

Tomás-Barberán FA, Ruiz D, Valero D. 2013. Health benefits from pomegranates and stone fruit, including plums, peaches, apricots and cherries. Bioactives in Fruit: Health Benefits and Functional Foods, 125-67.