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Efficacy of different Plant essential oils against *Penicillium expansum* causing fruit rot of grapes

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Abstract

Penicillium rot (PR) caused by *Penicillium expansum* is a severe fruit rot of grapes in Pakistan, causing serious threat for the environment and responsible to market losses. The objective of this study is to find out some effective plant essential oils as an alternative to synthetic fungicides against *P. expansum* causing fruit rot of grapes. For this purpose, three selected plant essential oils (EOs) *viz.* Olive (EO), Citrus peel (EO) and Fenugreek (EO) at 400, 600 and 800 ppm concentrations were studied by using different methods under *in vitro* condition as well as application on grapes against previously isolated culture of *P. expansum* designated as (Isolate ID. APE10TL3) on grapes bunches respectively. Results showed that Citrus peel (EO) at all concentrations showed significant result to control the mycelial growth (89.3, 94 and 97.5%) in contact assay method as well as 90.1, 96.3 and 99% growth inhibition regarding fungal culture transfer (FCT) experiment while, in case of well diffusion method 41%, 46% and 52% growth inhibition was recorded at 7 day of incubation followed by olive (EO) and fenugreek (EO) as compared to control 0% growth inhibition was measured. Moreover, the Citrus peel (EO) was further evaluated for the presence of anti-fungal compounds viz. terpene, alkaloids, phenolic and saponins employing standard protocols and found positive for the presence of all compounds. During the application of citrus peel essential oil at (800 ppm) concentration on fruit bunches for the determination of decaying percentage. The result showed 12.53% decay caused by *P. expansum* on treated bunches up to six days of storage and control was 85.14% calculated. Keeping in all view, Citrus peel (EO) possessing good inhibitory action upon *Penicillium expensum* might be used as a potential candidate for preservation and extension of shelf-life of grapes commercially.

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Introduction

Grapes (*Vitis vinifera* L.) are widely cultivated, economically important and highly nutritious fruit throughout the world (Ali *et al*., 2010). Grapes are also familiar as a "Queen of fruits" due to good source of multi-vitamins such as A, C as well as it contains a lot of bioactive compounds *viz*. anthocyanins, carotenoids and some important antioxidants which has an important role to enhance the immune system. (Rathi and Rajput, 2014). In Pakistan, grapes are mostly cultivated for fresh consumptions covered over an area about 14 thousand ha with annual production of 57 thousand tons (FAO, 2017). Besides its nutritional and medicinal values grape is one of the perishable fruit, having limited shelf life up to 3 to 4 days at ambient temperature.

The maximum perishability of this fruit during handling, storage and marketing is due to susceptibility of numerous post-harvest fungal diseases like Mucor rot (MR) (Ghuffar *et al*., 2018), Botrytis bunch rot (Javed *et al*., 2017) Alternaria rot (AR) (Ghuffar *et al*., 2018) and especially Penicillium rot (PR) associated with *Penicillium expansum* is responsible for weight loss, colour changes, softening of grape berries, increased the market losses and eventually has badly impact on economy (Prusky, 2011). In addition, *Penicillium* genus produced mycotoxin such as Patulin which are carcinogenic for human health and physically deteriorate the fruit health (Neri *et al*., 2009). Penicillium expansum is an aggressive plant pathogen due to the production of cell wall-degrading enzymes and also produces a wide range of cytotoxic mycotoxins including citrinine and penicillic acid (Duduk *et al*., 2017).

For the control of penicillium rot farmers is being sprayed synthetic fungicides on small fruits. However, these fungicides have some residual effect on berries' skin which may lead to development of resistant fungi, oncogenic risk, handling hazards, and threats to the environment (Nunes, 2012). Therefore, many restrictions regarding application of chemical fungicides on small fruits are banned in many countries of the world (Dayan *et al*., 2009). Nowadays, researchers have keen interest to provide some safer alternatives which would have non-hazardous effects on environment in the replacement of chemical fungicides for farmers. They successfully found some biological methods, such as use of plant essential oils (EOs) are an exciting alternative. These Plant essential oils are volatile compounds, broad spectrum, anti-fungal activity, eco-friendly and more acceptable to the public (Isman, 2000). Keeping in all the view, present study was conducted to find out the efficacy of different plant essential oils against *Penicillium expansum* causing fruit rot of grapes under *in vitro* as well application on grapes bunches.

Materials and methods

Collection of pathogenic fungal culture

The culture of *P. expansum* previously isolated from infected grape bunches of Taiffi cv. designated as Isolate APE10TL3 obtained from mycology lab, Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi (AAUR) with Genebank submission ID. Pen 12, Accession # MF467902 (Ghuffar *et al*., 2018) respectively. Stock culture of fungal isolate was preserved at 4°C in glass vials by using silica gel technique (Nakasone *et al*., 2004). Preserved fungal culture of *Penicillium expansum* was re-cultured by transferring beads onto freshly prepared Potato Dextrose Agar (PDA) in Petri dishes and incubated for 7 days at ±25°C. After revival, culture was purified which placed in incubator for seven days at ±25°C using PDA media and finally used for further management trail under *in vitro* conditions.

In vitro screening of Penicillium expansum by using Plant essential oils (EOs)

Extraction of Plant Essential oils (EOs) through Soxhlet's appratus

Matured leaves of Olive (*Olea europaea*), Fenugreek (*Trigonella foenum-graecum*) and Citrus peel (*Citrus sinensis*) were taken from horticultural research station of PMAS-UAAR. These botanical materials were first dried under shadow, grinded well in grinder machine and subjected for extraction process through Soxhlet's apparatus followed by (Şahin *et al*., 2003). Finally, extracted Plant essential oils (EOs) put in a clean glass vials and stored in refrigrator at 4°C until further tests.

In Vitro Contact Assay

To find out the efficacy of different Plant essential oils (EOs) on mycelial growth of *P.expansum* poisoned food technique was used (Prakash *et al*., 2015). For this purpose, 50ml of prepared Potato Dextrose Agar media were kept in 100mL conical flasks, sterilized for 20min and kept under sterilized hood to cool up to 60°C then plant (EOs) were added to each flasks and shacked gently to prepare PDA media containing 400, 600 and 800 ppm of concentrations. 9cm Petri plates were poured with PDA containing known concentrations of Plant (EOs). 5mm plug of 7 days old culture of *Penicillium expansum* were kept in the center of each Petri plate whereas, in control sets PDA free of any essential oils were used. After that Petri dishes were incubated at ±25°C for 7 days and finally, mycelial growth inhibition percentage (MGI) was recorded by using the following formula $= \frac{c-t}{c} \times 100$ (D'auria *et al.,* 2005) where c means (diameter of control), t (diameter of treatment). Analysis was conducted 3 times.

Fungal culture transfer Experiment

Transfer experiment was done to check the viability of the fungal culture which utilized in contact assay. For this purpose, from seven days old contact assay fungal plates 6 mm plugs of fungal culture were shifted in a newly plates. No essential oils (EOs) were used at this stage later, plates were incubated at ±25°C for 7 days. Mycelial growth of the pathogen was measured by using formula described earlier. The data was recorded after 7th day of incubation with three replications (Feng and Zheng, 2007).

Well diffusion technique

Agar well diffusion is reliable technique used to screen out the anti-fungal activity of different plants essential oils. According to this method, two wells were made aseptically with a sterile corked borer at equidistant from each other and one well was filled by *Penicillium expansum* inoculum (10^9 spores/mL) while second with plant essential oils at defined concentrations of 400, 600 and 800 ppm respectively. Furthermore, Agar plates were incubated at ±25°C. The plant (EOs) were diffused in the agar medium which inhibited the growth of the *P. expansum*. Linear growth inhibition was measured after 7 days and mycelial growth of pathogen was calculated by using the formula as same as previously described *in vitro* contact assay experiment.

Qualitative Phytochemical analysis of Plant Essential oils

For qualitative phytochemical secreening of various metabolities such as phenolic compounds, alkaloid, Terpenoid (Evans, 1997) and Saponins (Kokate, 1999) were performed.

Application of plant essential oil on grapes against P.expansum

In order to find out the efficacy of essential oils against pathogen. Mature and healthy bunches of grapes (Taiffi cv.) used for this experiment. The fruit bunches of control as well as of treatment sets washed in running water and surface sterilized with 0.1% sodium hypochlorite solution and then washed with distilled water. Fruit bunches treated by dipping for 3 mints at most effective concentration of essential oil by poisoned food technique and kept in perforated thermo-pole box (one bunch per box). The fruits inoculated by 1ml of the standard spore suspension of decaying pathogen. For fruit inoculation spores from 3-day-old culture suspended in sterile distilled water. Each bunches inoculated by spraying with 40µl of spore suspension of Mucor fragilis. Decaying % was calculated by using the following formula at three days of interval.

Decaying % $= \frac{No.of\ fungal\ infected\ berries\ in\ bunch}{Total\ number\ of\ berries\ in\ bunch\ examined} X\ 100.$

Three replicates were kept for treatment along with the control sets.

Statistical analysis

Statistical analysis of all experiments were conducted in triplicate and data were expressed as mean ± standard deviation after analyzed via CRD two factorial design (statistix 10.0 v). The statistical significance was set at a confidence level of $p < 0.05$.

Results

In vitro Contact Assay

After the application of three selected Plant essential oils (EOs) under *in vitro* condition by using contact

assay method. Data recorded after 7 days revealed that Citrus peel oil 89.3, 94 and 97.5 percent at all applied concentrations (400, 600 and 800 ppm) followed by Olive (EO) 61.3, 68.2, 73.1% while Fenugreek (EO) showed least effective 53.6%, 59.8% and 61.4% respectively. Whereas, in control set none of mycelial inhibition % was measured shown in Fig 1.

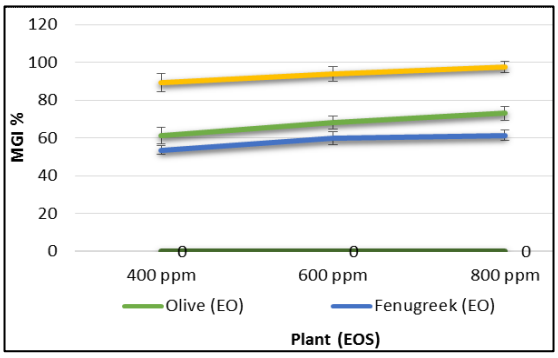

Fig. 1. Efficacy of Plant essential oils at different concentrations after 7 days of incubation against *Penicillium expansum*.

Fungal culture transfer Experiment

Similar result was found regarding in transfer experiment on 7th day demonstrated that citrus peel essential oil at concentrations 400, 600 and 800 ppm showed significant result 90.1, 96.3 and 99.2 percent followed by olive (EO) oil 66.4, 70.2 and 76.3% value while fenugreek oil showed minimum effectiveness (58.9, 62.4 and 69.1%) as compared to control where 0 % growth inhibition was recorded (Fig 2).

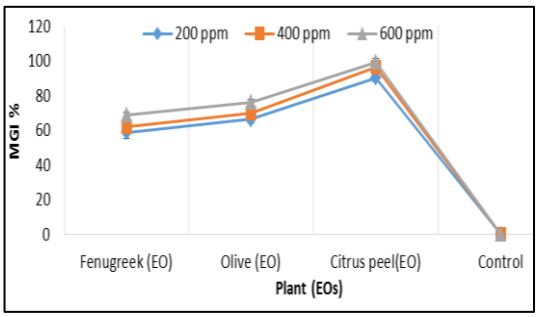

Fig. 2. Effectiveness of Plant (EOs) on growth inhibition of *Penicillium expansum* on 7th day through transfer experiment.

Well diffusion method

From three selected plant essential oils Citrus peel (EO) was applied at pre-defined three doses of 400 ppm, 600 ppm and 800 ppm per well through well diffusion technique produce 41%, 46% and 52% growth inhibition zone percent respectively at 7th day of incubation after adding extract and fungal spore suspension in their respective wells while, olive (EO) showed the inhibition zone of pathogen at 400 ppm and 600 ppm doses after Citrus peel (EO) by expressing 32% and 39% growth inhibition respectively while 800 ppm dose showed 43% zone of inhibition at 7th day of incubation. In case of Fenugreek (EO) expressed growth inhibition of 22% at dose of 400 ppm while 26% as well as 39% at doses of 600 ppm and 800 ppm per well in PDA media as compared to control which showed 0% growth inhibition (Fig 3). Fungal growth was recoded at 7 day of incubation.

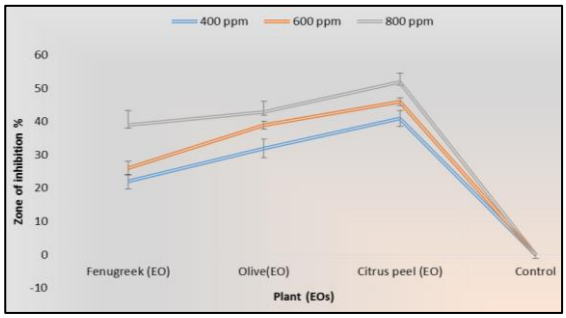

Fig. 3. Effect of plant essential oils applied at three (400 ppm, 600 ppm and 800 ppm) concentrations on growth inhibition zone of *Penicillium expansum* through well diffusion technique.

Qualitative Phytochemical analysis of Plant Essential oils

The result of qualitative analysis revealed that all the tested metabolites including Terpene, Alkaloid, Phanolic and saponins were present in Citrus peel (EO) (Table 1). Similar work was done by (Naz *et al.*, 2013) for the phytochemical screening of Tamarix indica and Tamarix passernioides by testing twelve metabilities on different organic solvent.

Application of plant essential oil on grapes against Penicillium expansum

The decaying % of treatment during 3rd storage day was calculated 5.96% as compared to control 34.16 percent, while on the 6th storage day decaying% was recorded 12.8 percent which covered the 2st disease score on treatment sets and control was 82.14% falls

on 5th disease score Table 2. The similar methodology was reported for the Chitosan treatment against *Botrytis cinerea* and *Rhizopous stolonifer* on strawberry by (Ghouth *et al.*, 1991).

Table 2. Qualitative phytochemical screening of Plant essential oils.

		Phytochemical analysis			
Sr.	Plant essential oils	Terpenes	Alkaloids	Phenolic	Saponins
		Salkowski test	Wagner reagent	Ferric chloride	Foam Test
1	Oilve oil	+	+	-	-
2	Fenugreek Oil	-	+	-	+
3	Citrus peel oil	+	+	+	+

Present (+) Absent (-).

Table 2. Effect of Citrus peel oil (800 ppm) on Incidence of Penicillium rot after three and six days of storage.

	Storage days	Decaying Percentage	Decay scoring system
	3	5.96±3.21	1
Control	6	34.14±3.85	3
Citrus peel oil	3	12.8±2.3	2
800 ppm	6	82.14±2.1	5

Disease rating scale (0) No symptoms (1) Up to 10 (2) 11-25 (3) 26-40 (4) 40-60 (5) above 60. ± Standard deviations.

Discussion

In last few years, used of synthetic fungicides are the primary means of controlling post-harvest pathogens but has some limitations due to resistance to fungicides among fungal pathogens and high development cost of synthetic chemicals. Therefore, scientists have been studied successfully some alternatives in the replacement of chemical fungicides especially plant derived products as disease control agents because of less toxic effects, eco- friendly and wide public acceptance (Seema *et al.*, 2011).

In few years, interest on plant (EO) has been increased for the control of post-harvest fungal pathogens due to environmental friendly nature (Gnanamanickam, 2002). In this study, we examined the anti-microbial activities of of some plant essential oils (Citrus peel, Olive and Fenugreek) against *Penicillium expansum* causing fungal fruit rot and demonstrated that Citrus peel (EO) oil among all tested plants at different concentrations had considerable effect on the growth rate respectively. Similar findings were also reported by Velazquez *et al.* (2013) revealed that Citrus peel essential oils (EO) showed maximum efficacy against *P. expansum* due to presence of important anti-fungal compound Limonene (96.62%) respectively. Antimicrobial effects for their phenolic compounds are identified by (Bagamboula *et al.*, 2004). According to their expalnation, concentrations of plant essential oils are directly effect on fungal growth of pathogens. Higher concentrations of essential oils can inhibit maximum fungal growth. Recently, some studies have been conducted by several scientists on determination of anti-fungal activity of plant essential oils against fungal pathogens (Satish *et al.,* 2007; Jamil *et al.,* 2007; Anwar and Rashid, 2007). Essential oils are natural, volatile, aromatic oily liquids compounds identified as secondary metabolites from various plant organs (Mar *et al.,* 2011a). Essential oil application is a very important technique for managing post-harvest diseases due to their moderately secure position, their broad acceptance by customers and for future multi-purpose functional use are gaining increasing interest (Mohamedy *et al.*, 2015). The mechanism of plant essential oils involved, inhibit the hyphal growth, interruption in nutrient uptake, disruption of mitochondrial structure and eventually disorganization of fungal pathogens discussed by (Patel and Jasrai, 2011).

Our result is in agreement with the findings of Tzortzakis and Economakis (2007) who used Lemongrass oil at 500ppm concentration for controlling the mycelial growth of *P. expansum*, the findings in this study confirmed that Citrus peel essential oil can be used as natural fungicides as well as replacement of the synthetic fungicides.

Conclusion

Plant essential oils (EOs) has an important role in the management of post-harvest diseases The results obtained in the current work illustrated that Citrus peel (EO) has anti-fungal activity against *Penicillium expansum* both *in vitro* and application on fruit bunches. So, it is interested to state that we can used Citrus peel (EO) as less harmful to enviornemnt and less expensive for controlling the *Penicillium expensum* under field conditions commercially.

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References

Ali K, Maltese F, Choi YH, Verpoorte R. 2010. Metabolic constituents of grapevine and grape-derived products. Phytochemistry Reviews **9(3),** 357-378.

Anwar F, Rashid U. 2007. Physico-chemical characteristics of Moringa Oleifera seeds and seed oil from a wild provenance of Pakistan. Pakistan Journal of Botany **39(5),** 1443-1453.

Bagamboula CF, Uyttendaele M, Debevere J. 2004. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri. Food microbiology **21(1),** 33-42.

Dauria F, Tecca M, Strippoli V, Salvatore G, Battinelli L, Mazzanti G. 2005. Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. Medical mycology Journal **43(5),** 391-396.

Dayan FE, Cantrell CL, Duke SO. 2009. Natural products in crop protection. Bioorganic & medicinal chemistry **17(12),** 4022-4034.

Duduk N, Lazarevic M, Zebeljan A, Vasic M, Vico I. 2017. Blue mould decay of stored onion bulbs caused by *Penicillium polonicum, P. áglabrum* and P. áexpansum. Journal of Phytopathology **165(10),** 662-669.

Evans WC. 1997. Trease and Evans phamacognosy. (14th ED) Harcourt Brace and Company. Asia Pvt. Ltd. Singapore 343.

Feng W, Zheng X. 2007. Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food control **18(9),** 1126-1130.

Ghaouth AE, Arul J, Grenier J, Asselin A. 1992. Antifungal activity of chitosan on two post harvest pathogens of strawberry fruits. Phytopathology **82,** 398-402.

Ghuffar S, Irshad G, Aslam MF, Naz F, Mehmood N, Hamzah AM, Mehmood A, Zeshan MA, Rehman S, Gleason ML. 2018. First report of *Mucor fragilis* causing bunch rot of grapes in Punjab, Pakistan Plant Disease **102(09),** 1858.

Ghuffar S, Irshad G, Naz F, Rosli HB, Hyder S, Mehmood N, Zeshan MA, Raza MM, Mayer CG, Gleason ML. 2018. First report of two *Penicillium* spp. causing post-harvest fruit rot of grapes in Pakistan Plant Disease **102(5),** 1037.

Ghuffar S, Irshad G, Shahid M, Naz F, Riaz A, Khan MA, Mehmood N, Sattar A, Asadullah HM, Gleason ML. 2018. First report of *Alternaria alternata* causing fruit rot of grapes in Pakistan Plant Disease **102(08),** 1659.

Isman MB. 2000. Plant essential oils for pest and disease management. Crop protection **19(8-10),** 603-608.

Jamil A, Shahid M, Khan MM, Ashraf M. 2007. Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pakistan journal of Botany **39(1),** 211-221.

Javed S, Javaid A, Anwar W, Majeed R, Akhtar R, Naqvi S. 2017. First report of Botrytis bunch rot of grapes caused by *Botrytis cinerea* in Pakistan. Plant Disease **101(6),** 1036.

Kokate CK. 1999. Practical Pharmacognasy. (4th ED) Vallabh Parkashan Publications, New Dehli, India **115.**

Mari M, Leoni O, Iori R, Cembali T. 2002. Antifungal vapour-phase activity of allyl-isothiocyanate against Penicillium expansum on pears. Plant pathology **51(2),** 231-236.

Mohamedy ERS, Gamal ENG, Bakeer ART. 2015. Application of chitosan and essential oils as alternatives fungicides to control green and blue moulds of citrus fruits. International Journal of Current Microbiology and Applied Science **4(6),** 629-643.

Naz F, Qamarunnisa S, Shinwari ZK, Azhar A, Ali SI. 2013. photochemical investigations of Tamarix indica and Tamarix passernioides Del.ex Desv. Leaves from Pakistan. Pakistan journal of Bot\any **45(5),** 1503-1507.

Neri F, Donati I, Veronesi F, Mazzoni D, Mari M. 2010. Evaluation of *Penicillium expansum* isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts. International journal of food microbiology **143(3),** 109-117.

Nunes CA. 2012. Biological control of postharvest diseases of fruit. European Journal of Plant Pathology **133(1),** 181-196.

Patel RM, Jasrai YT. 2011. Evaluation of Fungitoxic Potency of Medicinal Plants Volatile Oils (VOs) against Plant Pathogenic Fungi. Pesticide Research Journal **23(2),** 168-171.

Prusky D. 2011. Reduction of the incidence of postharvest quality losses, and future prospects. Food Security **3(4),** 463-474.

Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adıguzel A, Kotan R. 2003. Evaluation of antimicrobial activities of *Satureja hortensis* L. Journal of Ethnopharmacology **87(1),** 61-65.

Satish S, Mohana DC, Raghavendra MP, Raveesha KA. 2007. A2ntifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. The Journal of Technology **3,** 109-119.

Seema MSS, Sreenivas ND, Devaki NS. 2011. *In vitro* studies of some plant extracts against *Rhizoctonia solani* Kuhn infecting FCV tobacco in Karnataka Light Soil, Karnataka, India. Journal of Agriculture science and Technology **7(5),** 1321-1329.

Tzortzakis NG, Economakis CD. 2007. Antifungal activity of lemongrass (Cympopogon citratus L.) essential oil against key postharvest pathogens. Innovative Food Science & Emerging Technologies **8(2),** 253-258.

Velázquez NMJ, Avila SR, Palou E, Lopez MA. 2013. Antifungal activity of orange (*Citrus sinensis* var. *Valencia*) peel essential oil applied by direct addition or vapor contact. Food Control **31(1),** 1-4.