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Grapes post-harvest decaying process, associated fungal pathogens and their ecofriendly control by plant extracts and oils

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Abstract

Grapes (*Vitis vinifera* L.) are amongst widely consumed fruit in the world. In Pakistan, about 66 thousand tons of grapes are produced annually from an area of 78.30 thousand hectares. Grapes are highly perishable commodityduring transportation, storage, and marketing for general consumption and post-harvest losses are up to 16-23%. Numerous biotic and abiotic factors reduce the production and quality of grapes. In this study, samples of rotten grapes were collected from different fruit markets of Pishin (Balochistan) and Faisalabad (Punjab). The samples were subjected to isolate the fungal pathogens, responsible for the post-harvest decay of fruits. The fungal pathogen isolated were identified as *Aspergillus flavus* and *A. carbonarius* causing post-harvest decay of grape berries. The pathogenicity of the two isolated pathogens was evaluated ondifferent temperatures (5, 20, 30 & 40°C) on Thompson seedless variety of grapes. The temperature 40°C was found conducive for infection and proliferation of fungi. Five organic origin chemicals namely Neem, Castor and Clove oils, with concentrations of (500, 1000, 2000 ppm) for each oil and also Neem and Marigold Extracts with concentrations of (12.5, 25, 50%) of both extracts were used as antifungal agents. Among the tested organic compounds, Clove oil @ 2000 ppm was found better in delaying thedecay process of both fungal pathogens. The decay was reduced up to 50% as compared to control. This study indicates that post-harvest application of essential oils can potentially enhance the storage life of grapes prior to marketing.

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Introduction

Grapes (*Vitis vinifera* L.) of *Vitaceae* familyare among the most delicious fruits in world. In Pakistan the area under grapes cultivation is 78.30 thousand hectares with total production of 66 thousand tons (FAO, 2017). There are many abiotic stresses (drought, winter, cold and hot) and biotic (fungi, bacteria, viruses, MLOs and insects), which affect the quality production and cause post-harvest losses during storage and marketing. Some major grape field diseases are powdery mildew (*Uncinula necator*), black rot (*Guignardia bidwelli*), downy mildew (*Plasmopara viticola*), anthracnose (*Elsinoe ampelina*) and crown gall (*Agrobacterium vitis*) (Odile *et al*., 2006). Post-harvest losses due to fungal infections to grapes berries may be direct during ripening, packing, storage and transportation by a latent pathogen, thus becoming curious to grapes (Pezet *et al*., 2003).

In Pakistan, post-harvest losses due to diseases, during packing and transport accounts to 16-23% *(*Aujla *et al*., 2011). Nelson, 1979 reported that major post-harvest decay of grape are grey mold *(Botrytis cinerea*), sour rot (*Aspergillus carbonarius*), Rhizopus rot (*Rhizopus stolonifer*) and blue mold (*Penicillium expansum*). Genus *Aspergillus* is a serious threat for table grapes, wines and rasins as its mycotoxins are alsoharmful to human(Perrone *et al*., 2007; Somma *et al.,* 2012). The contamination of grapes and wine products are mostly due to Ochratoxin A (OTA) (Cabañes *et al.,* 2002). *A. carbonarius* and *A. niger* are the main to producer of OTA which results in inferior quality of grapes and its by proucts (Stefanaki *et al*., 2003).

Post-harvested decay is reduced by sulfur dioxide (SO_2) treatment, bunches are usually stored in the presence of SO_2, this compound is registered as a supportive in most countries, while it has been removed from the GRAS (Generally Recognized as Safe) list and classified as a pesticide in USA (Anon, 1986). SO_2 causes phytotoxicity symptoms, such as discoloration, bleaching, sulfurous taste and browning of the rachis of grapes (Zoffoli *et al*. 2008).

Keeping in view the notorious effects of syntheticfungicides on environment and human health, a study was designed to test the efficacy of natural products like neem, castor andclove oils along with neem and marigold extracts as antifungal agents at different concentration. The antifungal activities of essential oils are well documented being safe, easily decomposable and non-toxic (Gogoi *et al*., 1997). Many researchers agree the effectiveness of plant extracts as fungicides, among which include cinnamon, lemongrass oils, oregano, palmarosa and clove (Marin *et al*., 2004).

Cinamamic aldehyde present in*Cinnamon casia*oil and eugnol present in leaves ofclove and cinanamon are famous for their antifungal activities (Davidson and Naidu, 2000). Neem oil solution (2-10%) controled*A. niger, A. alternate* and *F. oxysporum* (Locke, 1995). Plant extracts having antifungal and antibacterial effects are being used to control plant diseases commercially (Lee *et al*., 2007). It has been also confirmed that different types of phenolic compounds are present in plant extracts (Al-Zoreky, 2009). *Azadirachta indica* is commonly known as 'neem, have a strong antifungal effect (Murthy and Sirsi, 1957).

The present study was aimed to isolate and identify the pathogens associated with decay of grapes and to assess their pathogenicity on harvestedgrapes. Further, it was also a mandatory to work out the optimum temperature for fungal growth and to increase the shelf lives of harvested fruit using eco-friendly plant origin fungicides.

Materials and method

Isolation and identification of pathogens associated with post-harvest decaying of grapes

The samples of rotten (diseased) grapes were collected from different vineyards, local fruit stores and different markets of districtsof Pishin and Faisalabad. Pathogens were isolated on potato dextrose agar. The growing pathogens were identified and purified on the basis of morphological characteristics.

Pathogenicity and temperature optimization

Pathogenicity test was carried out on 5, 20, 30 and 40°C tostandardize the optimum temperature. Inoculum was prepared from fresh culture, spores were maintained up to 1×10^7 spores/ml and growth behavior of fungi was observed.

Inoculation and incubation

Thompson, a seedless variety of grapes is being widely consumed in Pakistan. 100 grams of bunches were prepared. Each bunch was immersed in 70% ethanol for two minutes to sterilize the surface, bunches washed of twice in sterilized distilled water and dried in air flow chamber. Three to four grape berries in each bunch were punctured up to 2mm deep with the help of sterilized common pin and about 4µl of adjusted spores solution (1×10^7 spores/ml) injected to each puncture berry with the help of micropipette and berries were placed in sterilized plastic boxes which thereafter were incubated at 5, 20, 30 and 40°C for 8 days.

In-vitro management through oils and plant extract

Essential oils

Plant essential oils neem (*Azadirachta indica*), castor (*Ricinus communis*) and clove (*Syzygium aromaticum*) purchasedfrom the market were sterilized by heating at 45°C under 125 psi pressure. Solutions were prepared as 2000ppm (200mg/100ml), 1000 ppm (100mg/ 100ml) and 500 ppm (50mg/100ml) in acetone (Tripathi *et al.*, 2008).

Plant extracts

Leaves of marigold (*Tagetes*) andneem (*Azadirachta indica*) were collected from University of Agriculture Faisalabad main campus and were sterilized in 70% ethanol. About 40 grams of leaves were blended in 100ml of sterilized distilled water. After blending aqueous solution were passed through muslin cloth and then filtered from whatman filter paper and kept in refrigerator until use (Riaz *et al.*, 2008). The extracts were used in three different concentrations 12.5, 25 and 50%.

Evaluation of fruit decay

Infection data were collected using disease rating scale, 1-9 (0) no symptoms; (1) 1-5%, small white growth on inoculated portion; (2) 10-15%, little extent of fungal growth on inoculated portion; (3) 20-25%, spores production and little extent to adjacent berries; (4) 30-40%,the inoculated berries covered with fungus growth almost; (5) 50-60%, infection started on all berries; (6) 70-80%, severe infection; (7) >80 all berries completely covered with fungus growth (Artés-Hernández *et al.*, 2004).

Statistical analysis

Trial was laid out in CompletelyRandomized Design (CRD) and the results were subjected to analysis to differentiate treatments and concentrations turkey's (HSD) test were used for comparing different means. With three concentrationsalong with control having three replications each.

Results

Isolated pathogens

Isolated pathogens are (*R. stolonifer, A. carbonariu, A. flavus* and *A. niger*). *In-vitro* various plant essential oils and plant extract were evaluated against *A. flavus* and *A. carbonariu.*

Pathogenicity and temperature optimization

The pathogenicity of *A. carbonariu* and *A. flavus* was standardized. *A. carbonariu* showed maximum decay of 80.4% at 40°C after 8 days followed by *A. flavus* with decay values of 78.3% at the same temperature, while minimum decay growth was recorded up to 7.11% at 5°C on *A. carbonariu* after lapse of 8 days. It might be concluded that 30°C and 40°C temperature is more favorable for growth of fungi. It was learnt that grapes could be stored at 5°C to save precious fruit from decay (Fig.1.).

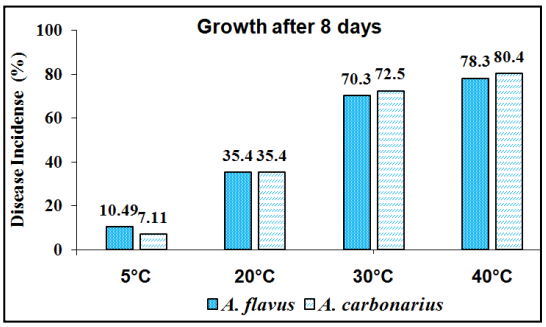

Fig. 1. Pathogenicity and temperature optimization of *A. flavus* and *A. carbonarius*.

In-vitro management through oils and plant extract

Essential oils

Data regarding impact of natural oils (neem, castor and clove oil) on fungal growth (*A. flavus* and *A. carbonarius)* on grapes after 3, 5 and 8 days after picking are depicted in (Table 1.). It is evident that with the passage of time fungal infection increased but remained under control as compared to check.

Three days data showed that minimum infection (1%) was showed by clove oil @ 2000 ppm against *A. flavus* and 1.64% decay by *A. carbonarius* while maximum damage (9.22%) was recorded in control. Similarly, after five days post-harvest decay data showed that minimum infection 3.50% was marked when applied 2000 ppm clove oil on *A. flavus* while 5.63% infection was recorded in *A. carbonarius* by antifungal effects of clove oil @ 2000 ppm. Infection data analyzed after 8 days of harvesting, showed that minimum damage 11.23% was observed by applying clove oil @ 2000 ppm against *A. flavus* and *A. carbonarius*, respectively followed by castor oil. While maximum infection was observed 75.55% in control. Overall scenario showed that among the test material, Clove oil @ 2000 ppm might be the best solution to increase the shelf life of grapes. Our finding can get support from (Sukatta *et al.*, 2008; Lopez-Malo *et al.*, 2007).

Table 1. Pathogenic effects of neem oil, castor oil and clove oil on *A. flavus* and *A. carbonarius* growth on grapes.

Fungal strains	Treatment	3 days				5 Days				8 Days			
		Control	500 ppm	1000 ppm	2000 ppm	Control	500 ppm	1000 ppm	2000 ppm	Control	500 ppm	1000 ppm	2000 ppm
A. flavus	Neem Oil	7.86	4.33	3.82	3.13	26.55	10.73	9.62	7.72	63.96	29.3	21.56	20.5
	Castor Oil	6.49	5.3	4.32	3.32	31.59	14.0	9.37	8.51	66.78	37.9	28.6	25.5
	Clove Oil	9.22	2.70	1.53	1.00	30.03	9.40	5.66	3.50	64.78	28.8	17.6	11.23
A. carbonarius	Neem Oil	5.67	5.21	4.41	3.86	27.80	13.66	12.26	8.27	76.25	42	34.8	18.97
	Castor Oil	6.23	4.36	3.33	2.99	28.98	11.11	9.65	8.60	66.46	36.5	23.5	14.44
	Clove Oil	8.16	3.32	2.87	1.64	30.77	9.55	7.75	5.63	75.55	26.7	22.1	11.28
CV			14.00				6.48				3.71		
Tukey's Value			1.65				2.85				3.41		

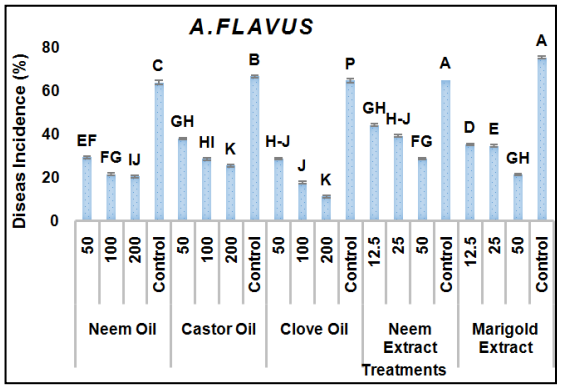

Fig. 2. Graphical representation of all treatments against *A. Flavus.*

Plant extracts

The data regarding pathogenic effects of neem and marigold extracts against *A. flavus* and *A. carbonarius* on grapes presented in (Table 2.). It is depicted that with passage of time fungal infection increase but remained under control. Marigold extract 50% after three days of harvest showed a decay of 2.20% followed by 50% neem extract with a decay value of 2.4% while control showed a decay infection 7.99% against *A. flavus*.Similarly, 50% marigold extract treatment showed decay value of 2.94% after three days of harvesting while control showed a decay infection up to 9.35% against *A. carbonarius*. With lapse of time, after 5 days of harvesting decay percentage increased to 33.88% in control while 50% Neem extract showed minimum decay of 7.33% and more decay of fruits decay were recorded under low concentration of treatment. Similarly after 8 days of harvesting, maximum decay of fruit were recorded as 75.5% against 21.33% decay in fruit flask treated with 50% marigold extract. Critical comparison showed that oils proved more effective then plant extract against decay causing organisms *i.e.A. flavus* and *A. carbonarius* thus these treatments need to be standardized to increase the shelf life of grapes. (Anjum Malik *et al.*, 2016) evaluated the efficacy of marigold and neem leaf extract against thevarious post-harvest diseases of fruits and vegetable that developed by fungal pathogen.

Table 2. Pathogenic effect plant extracts on fungal growth on harvested grapes.

Fungal strains	Extracts	3 days				5 Days				8 Days			
		Control	12.5%	25%	50%	Control	12.5%	25%	50%	Control	12.5%	25%	50%
A. flavus	Neem Extract	7.99	5.64	4.99	2.4	30.14	14.5	12.3	9.61	64.9	44.33	39.33	28.76
	Marigold Extract	6.97	4.33	3.33	2.20	31.81	13.03	13.00	8.11	75.5	35.37	31.61	21.33
A. carbonarius	Neem Extract	9.35	5.10	3.32	3.12	33.88	11.16	9.46	7.33	70.5	44.76	39.43	32.9
	Marigold Extract	5.66	3.33	3.32	2.94	33.59	19.40	14.50	10.6	72.6	58.8	39.44	32.92
CV		14.48				7.62				3.26			
Tukey's Value		1.84				3.38				3.77			

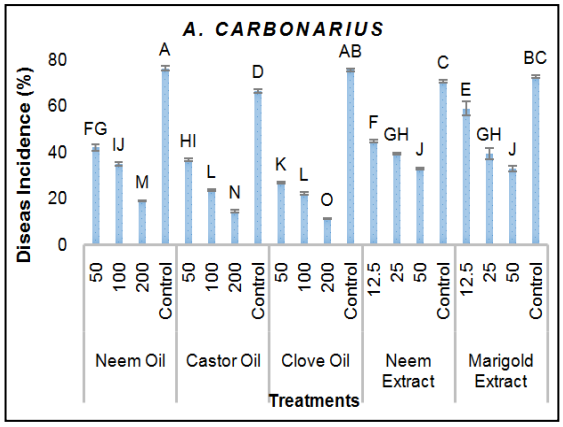

Fig. 3. Graphical representation of all treatments against *A. Carbonarius*

Discussion

Gabler *et al.* (2004) exposed spores of *A.niger* to ethanol at 25-50°C andthey revealed maximum inhibition of spore at 40°C. Esteban *et al.* (2004) reported*A. carbonarius* and *A.niger* explained that better growth at 10-40°C and produced much OTA at 20-25°C. Due to production of OTA at wide range they also declared that *A. carbonarius* and *A.niger* can mostly infect grapes.

In present study, the pathogenicity test of isolated fungi (*A. flavus* and *A. carbonarius* were done on 5, 20, 30 and 40°C with the aim to find out the optimum temperature for storage for grapes. After 8 days,*A. flavus* showed maximum decay expansion of 78.3% at 40°C and *A. carbonarius* gave maximum spread of 80.4% at 40°C. These studies made it clear that two pathogens showed minimum growth at 5°C and maximum proliferation of fungus at 30 and 40°C. So if grapes stored at 5-8°C, the decay will be very slow, whereas *A. carbonarius* was still able to produce infection even at 5°C.

Xing *et al.* (2012) stated that themain constituent of clove bud oil is eugenol which is strong antioxidant, and it controlled the *A. flavus* at different concentrations with greater success but at 3% it inhibited more than 80% infection. He suggested that clove oils can be used as good agent for control of fungal pathogens.Sukatta *et al.* (2008) argued that clove oil showed better result on grapes post-harvest pathogens (*A. niger*, *R. stolonifer* and *A. alternate*) with different concentration, at higher 400mg/ml. Amagase *et al.* (2001) stated that castor oil is a good source of monounsaturated 18-carbon fatty acid, which include ricinoleic acid. It has hydroxyl group on 12th carbon. So, it makes castor oil more polar compared to most fats, while garlic oil has allicin derivative component that's why both have more antimicrobial activity.In present study oils of neem, castor and clove were test against *A. flavus* and *A. carbonarius* at different concentration. All oils showed better result even on low concentration, but clove oil wasbetter than other oils

Conclusion

Aspergillus species were prominent in decay as compared to other fungi. There was maximum proliferation of fungi at higher temperature, thereby maximum losses can occur. Recommendations for natural storage temp is 5-8°C. All oils and extracts were effective in controlling *A. carbonarius* and

A. flavus, but clove oils proved the best as compared to other oils. Selection of solvent is an issue in the use of oils and itneeds to be addressed as the oils are soluble greatly in acetone (organic solvents). The results are based on *in-vitro* trials on fruit.

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References

Al-Zoreky, NS. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International journal of food microbiology **134,** 244-248.

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. 2001. Intake of garlic and its bioactive components. The Journal of nutrition **131,** 955S-962S.

Anjum Malik A, Ahmed N, Babita CH, Gupta P. 2016. Plant Extracts in Post-Harvest Disease Management of Fruits and Vegetables. A Review Journal of Food Process Technology **7,** 592-2.

Anon. 1986. GRAS status of sulfating agents for use on fresh and frozen foods revoked. Fed. Regist **51,** 25021.

Artés-Hernández F, Aguayo E, Artés F. 2004. Alternative atmosphere treatments for keeping quality of 'autumn seedless' table grapes during long-term cold storage. Postharvest Biology and Technology **31,** 59-67.

Aujla KM, Shah NA, Ishaq M, Fraooq A. 2011. Postharvest losses and marketing of grapes in Pakistan. Sarhad Journal **27,** 485-490.

Cabañes FJ, Accensi F, Bragulat MR, Abarca ML, Castellá G, Minguez S, Pons A. 2002. What is the source of ochratoxin A in wine? International Journal of Food Microbiology **79,** 213-215.

Esteban A, Abarca ML, Bragulat MR, Cabañes FJ. 2004. Effects of temperature and incubation time on production of ochratoxin A by black *Aspergilli*. Research in Microbiology **155,** 861-866.

FAO. 2017. Food and Agriculture Organization. http://faostat.fao.org/. (Assessed on July 07, 2017).

Gabler FM, Mansour M, Smilanick JL, Mackey BE. 2004. Survival of spores of *R. stolonifer*, *A. niger*, *B. cinerea* and *A. alternata* after exposure to ethanol solutions at various temperatures. Journal of applied microbiology **96,** 1354-1360.

Gogoi RS, Baruah P, Nath SC. 1997 Antifungal activity of the essential oil of Litsea cubeba Pers. Journal of Essential Oils Research **9,** 213-215.

Lee SH, Chang KS, Su MS, Huang YS, Jang HD. 2007. Effects of some Chinese medicinal plant extracts on five different fungi. Food control **18,** 1547-1554.

López-Malo A, Barreto-Valdivieso J, Palou E, San Martín F. 2007. *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. Food Control **18,**1358-1362.

Marin S, Velluti A, Ramos AJ, Sanchis V. 2004. Effect of essential oils on zearalenone and deoxynivalenol production by *Fusarium graminearum* in non-sterilized maize grain. Food Microbiology **21,** 313-318.

Murthy SP, Sirsi M. 1957. Pharmacological studies on *Melia azadirachta*. Part I. Antibacterial, antifungal and antitubercular activity of neem oil and its fractions. In *Symp. Utilization* Indian Med. Pl., Lucknow p.55.

Nelson KE. 1979. Harvesting and handling California table grapes for market (Vol. 1913). UCANR Publications.

Odile C, Réjean B, Jacques L, Wendy MC. 2006. Agriculture and Agriculture Food Canada. Category. No. A52-74/2006E-PDF. ISBN 0, 662-43594-X.

Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC, Samson RA. 2007. Biodiversity of *Aspergillus* species in some important agricultural products. Studies in mycology **59,** 53-66.

Pezet R, Viret O, Perret C, Tabacchi R. 2003. Latency of *B. cinerea* Pers.: Fr. and biochemical studies during growth and ripening of two grape berry cultivars, respectively susceptible and resistant to grey mould. Journal of phytopathology **151,** 208-214.

Stefanaki I, Foufa E, Tsatsou-Dritsa A, Dais P. 2003. Ochratoxin A concentrations in Greek domestic wines and dried vine fruits. Food Additives and Contaminants **20,** 74-83.

Sukatta U, Haruthaithanasan V, Chantarapanont W, Dilokkunanant U, Suppakul P. 2008. Antifungal activity of clove and cinnamon oil and their synergistic against postharvest decay fungi of grape in vitro. Kasetsart Journal. Nature Science **42,** 169-174.

Tripathi P, Dubey NK, Shukla AK. 2008. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. World Journal of Microbiology and Biotechnology **24,** 39-46.

Xing Y, Xu Q, Li X, Che Z Yun J. 2012. Antifungal activities of clove oil against *Rhizopus nigricans, A. flavus and Penicillium citrinum* in-vitro and in wounded fruit test. Journal of food safety **32,** 84-93.

Zoffoli JP, Latorre BA, Naranjom P. 2008. Hairline, a postharvest cracking disorder in table grapes induced by sulfur dioxide. Postharvest Biology and Technology **47,** 90-97.