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Research Article

Bioprotective effects of different plant extracts and essential oils against *Rhizopus stolonifer* and *Aspergillus niger* causing post-harvest decay in grapes

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ABSTRACT

The grapes (*Vitis vinifera*) are one of the largely consumed fruits in the world. Grape production in Pakistan has increased in the recent years. These are mainly consumed fresh or as dried raisins. Grapes are highly perishable, which makes them liable to rot during transport, storage, and marketing. The perishable nature of grapes leads to a considerable amount of post-harvest loss. The production and quality of grapes are adversely affected by several abiotic and biotic factors. Two different cities Pishin (Balochistan) and Faisalabad (Punjab) fruit market and stores were selected to collect rotten grapes. The fungal pathogens isolated were identified as *A. niger* and *R. stolonifers*. The pathogenicity of isolated pathogens was assessed at different temperatures (5, 20, and 30 °C) on the Thompson seedless grape variety. Infection and proliferation of fungi were found conducive at 30°C. As antifungal agents, three organic origin oils, neem, cinnamon, and clove oils with levels i.e., 500, 1000, 2000 ppm as well as marigold and neem extracts with diverse concentrations of like 12.5, 25, and 50% were employed. Among these organic compounds, cinnamon and clove oils at 2000 ppm exhibited 6.54% and 15.3% decay against *A. niger*, respectively. Meanwhile, against *R. stolonifer*, cinnamon and clove oils at 2000 ppm exhibited 12.22% and 6.74% decay, respectively. This study's findings focus on the effectiveness of both essential oils and plant extract in significantly delaying the decay process caused by fungal pathogens. The present study underscores the protective ability of the plant extracts and natural oils which could be a way forward for eco-safe management of post-harvest diseases of grapes before they reach the market.

Keywords: *Aspergillus*; *Rhizophus*; temperature; oils and plant extracts; post-harvest diseases; grapes.

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INTRODUCTION

Grapes (*Vitis vinifera*) of the family (Vitaceae) are among the most widely consumed fruits globally. It is cultivated on an area of 7.12 million hectares, with about 74 million tons of production worldwide, with Europe contributing 34.9%, Asia 31% and America 20%. (FAOSTAT, 2021). In Pakistan, grapes are cultivated on an area of 16160 hectares with a production of 87584.01 thousand tons (FAO, 2021). Fungal pathogens are the primary causal agent of postharvest rotting of fresh fruits and vegetables (Gatto *et al.*, 2011). In postharvest decay, at least 100 species of fungi are responsible (Tripathi and Dubey, 2004).

In favorable conditions, postharvest diseases can cause up to 55% of production losses (Sanzani *et al.*, 2016). Grapes are susceptible to many fungal diseases in the field and during storage. Due to various postharvest pathogens, storing grapes in humid and warmer climates is difficult (Barkai-Golan, 2001). Postharvest losses in Pakistan are 16-23% due to diseases, packing and transport (Aujla *et al.*, 2011). Fungal pathogens responsible for grapes' diseases include *A. niger*, *A. flavus*, *A. terreus*, *A. ochraceus*, *Alternaria alternata*, *Botrytis cinerea*, *C. gloeosporioides*, sp, Mucor sp, *Penicillium* spp. *Cladosporium* and *R. stolonifer* (Barkai-Golan, 2001; Lichter *et al.*, 2002; Walker *et al.*, 2011; Rousseaux *et al.*, 2014). The most harmful postharvest disease of grapes is gray mold, caused by *Botrytis* spp. Some diseases, such as blue mold, can emerge during storage and are attributed to *Penicillium* spp. (Sanzani *et al.*, 2013). Other illnesses, such as rots brought on by *Aspergillus* spp. are more severe in warm weather conditions (during marketing and transit) (Droby and Lichter, 2007).

Some of the most prevalent black molds found on grapes comprise species such as *A. tubingensis*, *A. carbonarius*, *A. niger* and *A. welwitschiae* (Perrone *et al.*, 2007). *Aspergillus* spp. is the main cause of mycotoxin (ochratoxin A (OTA)), produced on grapes and mostly produced by *A. carbonarius* and *A. niger* (de Souza Ferranti *et al.*, 2018).

Rhizophus stolinifer attacks bunches of grapes in the vineyard before harvest and continues growth during storage and transportation, causing severe economic damage. Growth and development of *R. stolonifer* are reduced at 0°C and above 37.8°C (Nelson, 1979; Lisker *et al.*, 1996).

Considering the hazardous effects of synthetic fungicides, which cause serious environmental and health problems, new methods are adopted to check grapes' decay, including biological control agents like bacteria and yeast (Janisiewicz and Korsten, 2002). Pesticides of organic nature such as essential oils, plant extracts and other natural compounds (Ippolito *et al.*, 1997), oil of tea tree (Burgiel and Smaglowski, 2008), cinnamon leaf, common thyme, and linseed oils (Cosic *et al.*, 2010), neem, eucalyptus, datura, sweet basil, oleander oils and extract of garlic (Nashwa and Abo-Elyousr, 2012) have been used against many pathogens. Such plants have high levels of antifungal compounds such as cinamamic aldehyde present in cinnamon cassia oils and cinnamon bark oils and eugenol in clove (Davidson and Naidu, 2000). Plant extracts of many plants have been reported to possess antibacterial and antifungal properties (Satish *et al.*, 1999; Bouamama *et al.*, 2006; Okigbo and Ogbonnaya, 2006; Shariff *et al.*, 2006).

This study aimed to isolate, characterize, and evaluate the pathogens linked to grape rot and their pathogenicity on harvested grapes. Furthermore, assessing the ideal temperature for fungal growth and increasing the shelf life of harvested fruit through the application of environmentally friendly bioprotectants derived from plant sources.

MATERIALS AND METHODS

Isolation and identification of pathogens.

Frequent surveys were conducted in grape vineyards and markets in districts Faisalabad (Punjab) and Pishin (Balochistan). The grapes samples were brought to the laboratory of the Department of Plant Pathology at the University of Agriculture Faisalabad for analysis. The rotten (diseased) grape berries samples were kept in the refrigerator at 4°C for preservation and to prevent cross-contamination. Infected berries were surface sterilized in 70% ethanol; some were not sterilized because there was a chance of damage to the surface spores of fungi. Pathogens were isolated on potato dextrose agar and yeast peptone dextrose agar and kept in an incubator at 27 ± 1 °C. The pathogens were purified and identified based on morphological characteristics such as mycelium growth, Hyphae (shape, septation, width and color) and Conidia (size, shape, and type of fruiting body) (Diba *et al.*, 2007).

Pathogenicity and temperature optimization

The isolated pathogens were confirmed through pathogenicity test at 5, 20 and 30°C to find out the optimum temperature for the growth of pathogens. The fresh culture was used to prepare the Inoculum, sterilized water with a small amount of tween-80 with a volume of (0.06%) was added to a Petri plate, and spores were gently mixed with a glass rod. The spore suspension was filtered through by 4 layers of muslin cloth to remove mycelium. spores were counted by using a hemocytometer, and the final count was maintained up to 1×106 spores/ml (Li *et al.*, 2021).

Inoculation and incubation

bunches of Thompson Seedless grapes, each weighing 100 grams, underwent thorough surface sterilization process by immersion in 70% ethanol for 2 minutes. Subsequently, the grapes were washed in distilled sterilized water and dried in a laminar flow chamber. To create standardized points of entry for the pathogens, 2-4 berries within each bunch were punctured up to a depth of 2 mm using a sterilized common pin. 5 µl of an adjusted spore solution, calibrated to a concentration of 1×106 spores/ml, into each punctured berry using a micropipette. The treated grapes were carefully placed under sterilized disposable plastic boxes, and these boxes were then stored at three different

temperatures (5, 20, and 30°C) over an 8-day period. Importantly, each temperature-pathogen combination was replicated three times to ensure the robustness and trustworthiness of the results.

Evaluation of *in-vitro* efficacy of plant extract and natural oils in different pathogens

Plant based oils

Plant based oils of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*) and neem (*Azadirachta indica*) were purchased from the shop and then sterilized through a sterile membrane filter with the size of (0.2- 0.4 um) (Enrico *et al.*, 2017). Solutions were prepared as 2000 ppm (200 mg/100 ml), 1000 ppm (100 mg/100 ml) and 500 ppm (50 mg/100 ml) in acetone organic compound (Tripathi *et al.*, 2008; Shamsullah *et al.*, 2020).

Plant extracts

Fresh leaves of marigold (Tagetes) and neem were collected from the university premises and sterilized using 70% ethanol. 40 grams of leaves were grinded in 100 ml of distilled sterilized water in a pestle and mortar. After the grinding process, the solution was passed from muslin cloth and then Whatman filter paper. Further contaminations were removed by passing the solution through a membrane filter with a size ranging between 0.2 to 0.4 micrometers. The resulting extracts were then stored in a refrigerator until their utilization (Riaz *et al.*, 2008). Notably, these extracts were employed in the study at three separate concentrations: 12.5%, 25%, and 50%.

Table 1. Disease rating scale for evaluation of fruit decay.

Disease rating scale	Infection percentage	Decay explanation
0	(0)	no infection
1	1-5%	inoculated portion shows only small white growth
2	10-15%	fungal growth is little extended on inoculated portion
3	15-25%	spore production observed and infection spread to neighboring berries
4	25-40%	inoculated berries almost surrounded with fungus
5	40-55%	little infection started on all berries
6	55-70%	all berries are covering with infection
7	70-80%	severe infection
8	90-100%	almost all berries totally infected

Statistical analysis

The trial was conducted in a Completely Randomized Design (CRD) and turkey's (HSD), with a probability value of 0.05, to compare different means.

RESULTS

Isolated pathogens and their characterization

From decaying grapes, four isolated pathogens were *Aspergillus. flavus Rhizopus stolonifer, Aspergillus carbonarius,* and *Aspergillus niger*. However, characterization and management were done against *A. niger* and *R. stolonifer.* The morphological characterization on basis of colony color and shape, fruiting bodies and spores showed that these are *A. niger* (Figure 1A and B) and *R. stolonifer (*Figure 1C and D).

Pathogenicity test and temperature standardization

When the pathogens were subjected to different temperatures, the *A. niger* at 30°C showed maximum decay of 92% after 8 days, followed by *R. stolonifer* with 76% decay, while low decay was noted up to 2.3% at 5°C on *R. stolonifera* and 6.5% on *A. niger* after lapse of 8 days. (Figure 2).

Evaluation of *in-vitro* efficacy of plant extract and natural oils against different pathogens

Essential oils

Less fungal growth of *A. niger* and *R. stolonifera* on grape berries was observed after using natural oils (neem, clove and cinnamon oil) after 3, 5 and 8 days (Table. 1) as compared to control treatments. After 3 days of treatment, cinnamon oil at a concentration of 2000 ppm, minimum infection (1.1%) was observed against *A. niger*. In comparison, *R. stolonifer* exhibited a decay of only 1.64%. The control, on the other hand, experienced a significantly higher maximum decay of 9.11%.

Similarly, after 5 days, the antifungal effects of cinnamon oil at 2000 ppm continued to demonstrate impressive results, with a minimal infection rate of 3.40% against *A. niger*. Simultaneously, the application of clove oil at 2000 ppm resulted

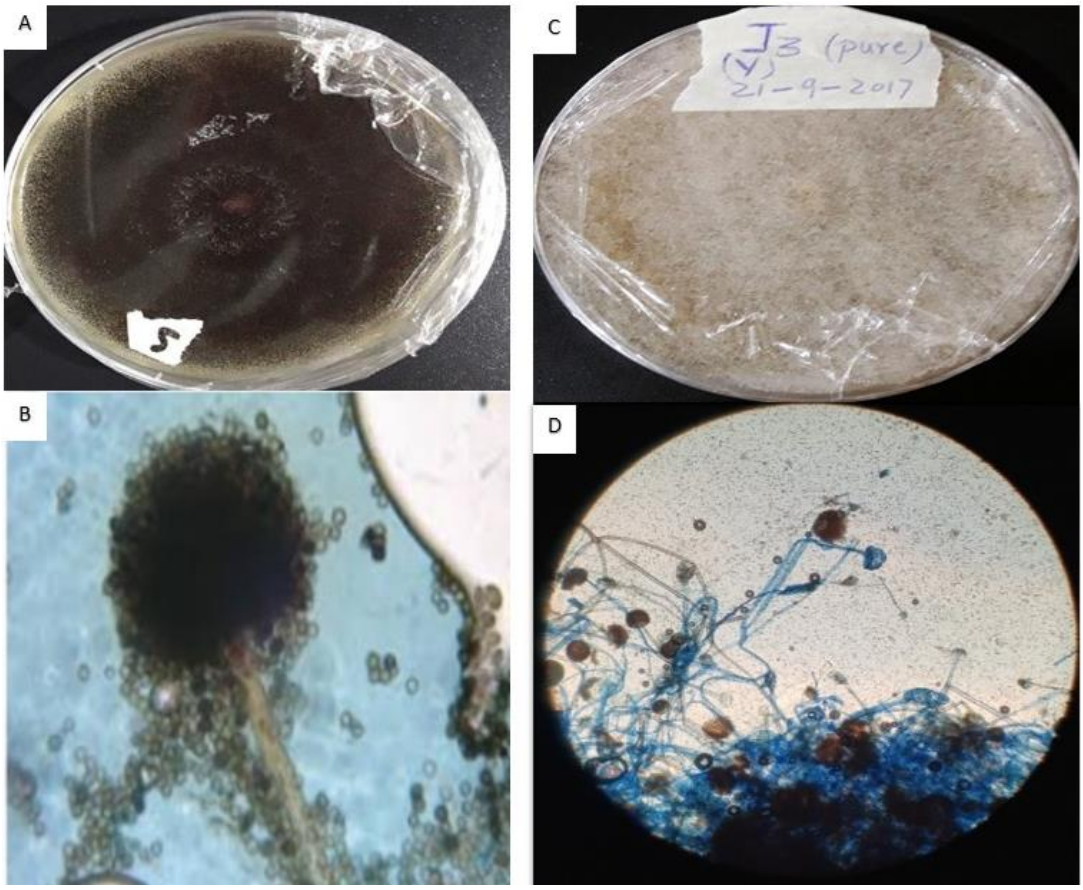

Figure 1. A is a colony of *A. niger*. B is a spore of *A. niger* under a microscope. C is a colony of *R. stolonifera.* D is spores of *R. stolonifera* under a microscope.

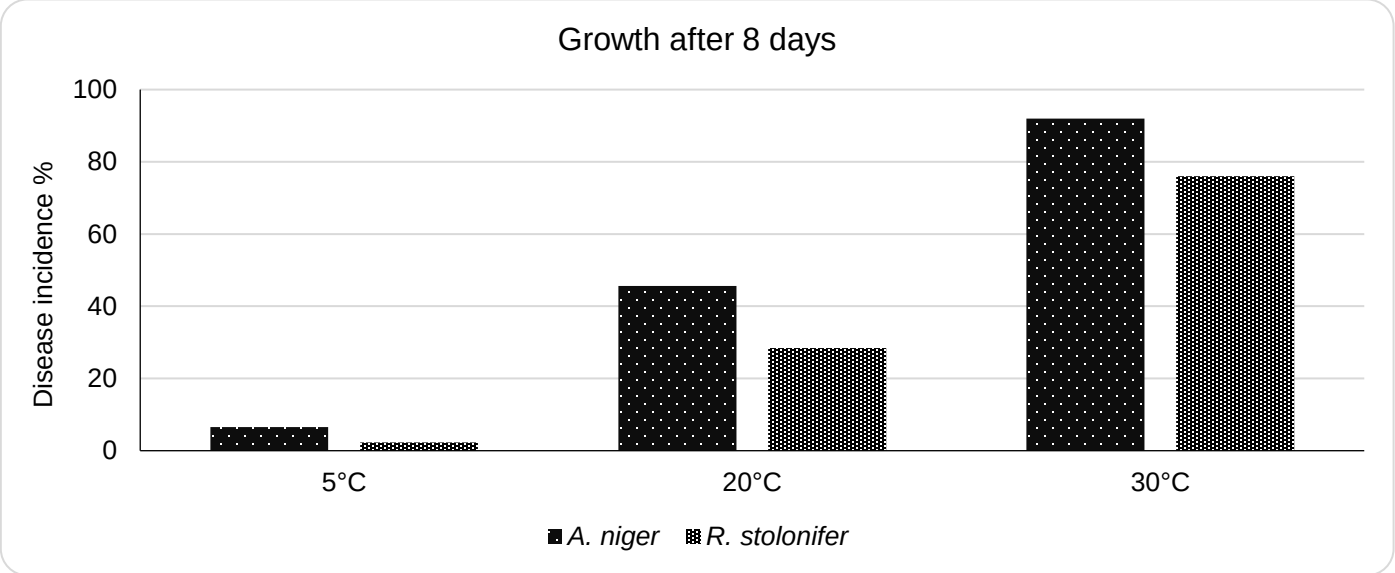

Figure 2. Pathogenicity test and temperature optimization.

in a 4.59% infection rate in *R. stolonifer*. These findings reinforce the sustained effectiveness of both cinnamon and clove oils in controlling fungal growth, contributing to the overall health and quality of the grapes. After 8 days of treatment, the oils showed incredible results, cinnamon oil at 2000 ppm exhibiting minimal damage at 6.54% against *A. niger* (Figure 3). On *R. stolonifer*, clove oil exhibited impressive performance with a low infection rate of 6.74%

(Figure 4), followed by neem oil, which showed 19.7% infection; the control grapes experienced a significantly higher maximum infection at a rate of 80.1%.

Table 1. *In-vitro* management through essential oils.

Fungal Species	Treatment (Oils)	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. niger	Neem	8.21	4.66	3.43	2.63	26.55	14.27	11.17	11.50	74.37	31.3	19.4	19.6
	Clove	7.21	4.74	2.27	1.60	30.3	10.1	6.5	5.20	80.1	28.4	22.2	15.3
	Cinnamon	9.21	1.7	0.53	1.1	31.5	8.53	4.94	3.40	75.7	19.7	14.3	6.54
R. stolonifer	Neem	7.1	4.13	3.25	3.32	31.4	19.1	17.3	9.59	72.55	40.3	31.42	19.7
	Clove	6.50	3.32	2.87	1.64	29.6	12.33	8.27	4.59	70.67	25.51	17.77	6.74
	Cinnamon	6.50	4.36	3.33	2.9	27.5	14.46	11.34	7.55	68.33	30.85	21.33	12.22
CV		13.03				6.65				5.47			
Tukey's Value		1.65				3.791				2.507			

Whereas CV is the abbreviation of coefficient of variation

Table. 2. *In-vitro* management through plant extracts

Fungal Species	Treatment (Extracts)	3 days				5 Days				8 Days			
		Control	12.5%	25%	50%	Control	12.5%	25%	50%	Control	12.5%	25%	50%
A. niger	Neem	7.210	6.51	4.9	3.16	32.70	19.58	18.3	11.8	72.48	44.48	35.3	29.3
	Marigold	8.20	5.21	3.8	2.11	32.5	15.23	13.6	7.57	75.75	35.59	28.2	20.5
R. stolonifer	Neem	7.70	4. 71	3.3	2.32	33.48	11.16	9.46	8.33	72.55	41.33	33.6	28.6
	Marigold	8. 42	5.36	4.33	3.12	28. 50	19.40	14.5	10.6	71.2	52.33	39.4	32.9
CV		14.54				6.35				4.05			
Tukey Value		1.66				4.047				3.791			

Whereas CV is the abbreviation of coefficient of variation.

Plant extracts

The data highlights the inhibitory impacts of neem and marigold extracts against *A. niger* and *R. stolonifer* on grapes over time (Table. 2). As observed, fungal infection increased gradually but stayed under control. Specifically, after three days of treatment, the 50% marigold extract exhibited a decay of 2.11% against *A. niger*, while the 50% neem extract showed a slightly higher decay value of 3.16%, both significantly lower than the control with a decay infection of 8.20%. Similarly, in the case of *R. stolonifer*, the 50% neem extract displayed a decay value of 2.32%, contrasting with the control's decay infection of 8.42%.

After 5 days of treatment, the control group showed a significant increase in decay percentage, reaching 33.48%. In a 50% marigold extract exhibited a minimal decay of 7.57%, showcasing its effectiveness even at a lower concentration (Table. 2). However, it is noteworthy that under lower concentrations of treatment, a higher decay of fruits was recorded. After 8 days of treatment, the decay of fruit was recorded in the control group at 75.75%, while the 50% marigold extract demonstrated a significantly lower decay of 20.47% against *A. niger* (Figure 3). On the other hand, neem extract against *R. stolonifer* showed a decay percentage of 32.92% (Figure 4).

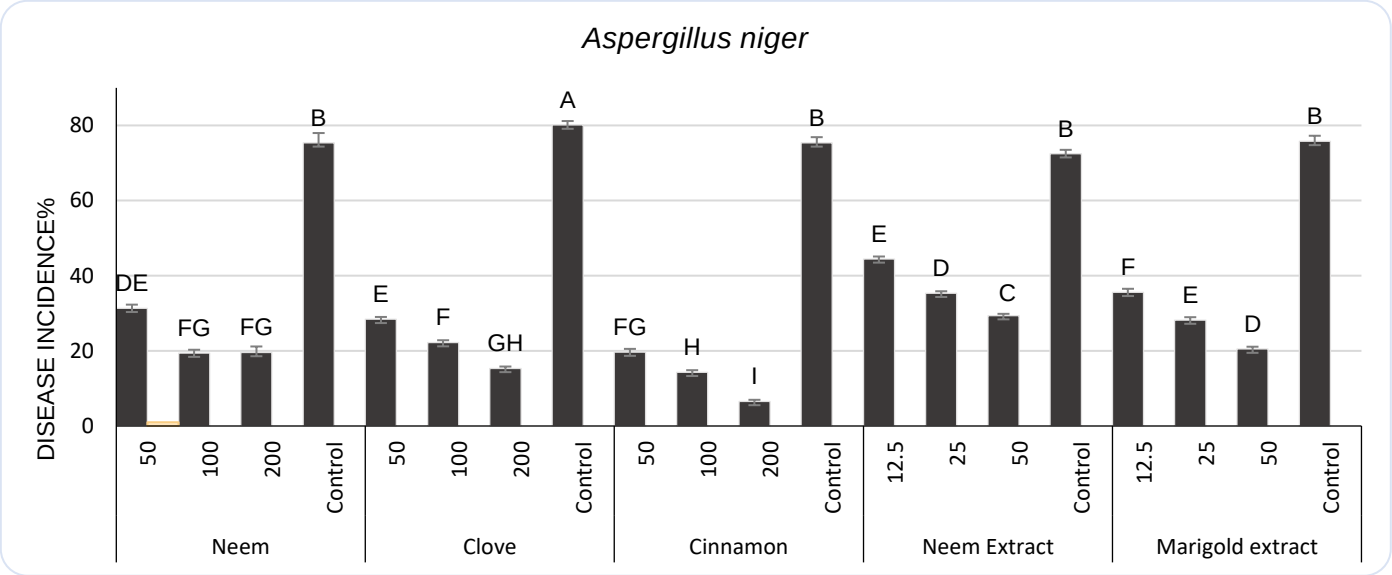

Figure 3. *In-vitro* management through plant oils and Extract against *A. niger*.

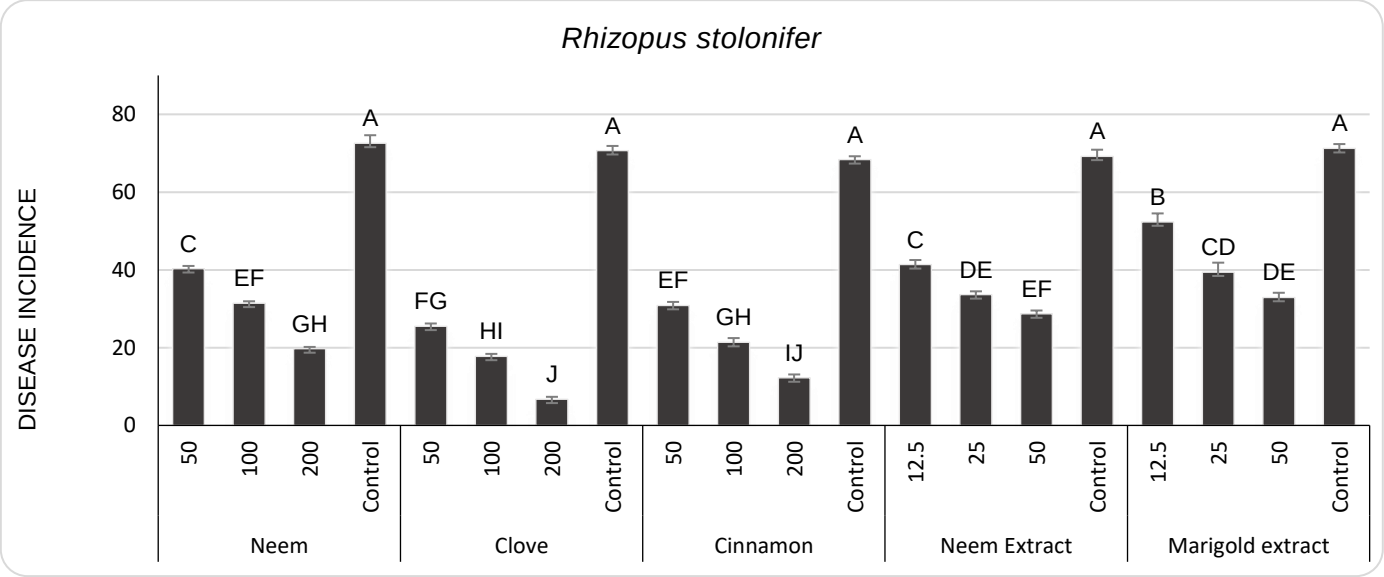

Figure 4. *In-vitro* management through plant oils and Extract against *R. stolonifera.*

DISCUSSION

A study conducted by Gabler *et al.*, (2004) in which *A. niger* and *R. stolonifer* spores were exposed to ethanol at temperatures ranging from 25 to 50°C. The results showed that there was the highest inhibition of spore growth at 40°C. Furthermore, Esteban *et al.*, (2004) investigated that *A. carbonarius* and *A. niger* produces more ochratoxin A (OTA) at 20 to 25°C.

In the present study, the pathogenicity of isolated fungi *A. niger and R. stolonifer* at different temperatures 5, 20, and 30°C was done. Within an 8-day observation period, the findings revealed that *A. niger* exhibited a maximum decay expansion of 92% at 30°C, while *R. stolonifer* showed the highest spread of 76% at the same temperature (Figure 2). While at 5°C, both pathogens exhibited minimum decay and maximum decay at 30°C. Notably, grapes stored at temperatures between 5-8°C will exhibit slower decay. It is concluded that a temperature of 30°C is more favorable for the reproduction and growth of fungi. It is knowledgeable that storing grapes at 5°C could effectively preserve the fruit and mitigate decay.

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Figure 5. A and B are Cinnamon oils against *A. niger at different* concentrations C is clove oil, and D is cinnamon oil against *R. stolonifera* at 2000ppm.

In the present study, the pathogenicity of isolated fungi *A. niger and R. stolonifer* at different temperatures 5, 20, and 30°C was done. Within an 8-day observation period, the findings revealed that *A. niger* exhibited a maximum decay expansion of 92% at 30°C, while *R. stolonifer* showed the highest spread of 76% at the same temperature (Figure. 2). While at 5°C, both pathogens exhibited minimum decay and maximum decay at 30°C. Notably, grapes stored at temperatures between 5-8°C will exhibit slower decay. It is concluded that a temperature of 30°C is more favorable for the reproduction and growth of fungi. It is knowledgeable that storing grapes at 5°C could effectively preserve the fruit and mitigate decay.

Leaf extracts of marigold and neem were evaluated against the various post-harvest fungal diseases of fruits and vegetables (Anjum Malik *et al.*, 2016; Satish *et al.*, 2007). In our study, marigold extract at 50% against *A. niger* showed 20.47 decay, while on *R. stolonifera*, 32.92 decay was observed. However, both extracts at low concentrations were not very effective.

Perdones *et al*., (2014) stated that the main components of cinnamon oil are cinnamaldehyde, eugenol, and cinnamic acid. Besides these components, it contains cinnamic acid and cinnamyl alcohol, which possess antifungal, antibacterial and antioxidant activity against pathogenic microorganisms. Tzortzakis, (2009) investigated the antifungal activity of cinnamon oil 25 and 500 ppm against *Botrytis cinerea*, *Cladosporium herbarum*, *Colletotrichum coccodes*, *Rhizopus stolonifera* and *A. niger* reduces spores' germination in vitro and in vivo. In research against 6 fungi, the minimum inhibitory concentrations (MICs) of clove and cinnamon oil and their combinations were tested. The cinnamon oil showed lower MICs values than clove oil against all pathogens except *R. stolonifera*, therefore demonstrating that clove oil was more effective than cinnamon oil (Sukatta *et al.*, 2004).

Xing *et al.*, (2012) reported that the prime component of clove oil is eugenol, which is a powerful antioxidant. They studied the effect of clove oil against *Aspergillus*, *Rhizopus*, and *Penicillium*, with different concentrations, and the infection was inhibited at 3% more than 80%.

In research, table grapes were fumigated with thyme, cinnamon, and oregano essential oils at 2% or 4% and sprayed at concentrations of 0.5% and 1%. The grapes were stored for 4 weeks at a temperature of 2°C ± 1°C. All treatments exhibited a more noticeable effect on maintaining the quality of the grapes. Furthermore, these treatments effectively reduced the growth of *B. cinerea (*Elsayed *et al.*, 2022). Another research underscores the potency of clove and

cinnamon oils against grape post-harvest pathogens (*A. niger*, *R. stolonifera* and *A. alternata*). The study revealed that the optimal concentration for achieving enhanced results was notably higher, specifically at 400 mg/ml (Arik and Arik, 2017).

In the current investigation, the antifungal activity of cinnamon, clove, and neem oils was assessed against *A. niger* and *R. stolonifera* at various concentrations. Notably, all three oils demonstrated promising results even at lower concentrations. However, in our findings against *R. stolonifera* after 8 days at 2000 ppm, clove, cinnamon, and neem oils exhibited superior effectiveness and showed 6.74, 12.22 and 19.7 decay percentages, while at 500 ppm, the decay was 25.51, 30.85 and 40.3, respectively (Figure. 5. C and D).

The clove, cinnamon, and neem oils at 2000 ppm showed 15.3, 6.54 and 19.6% decay in response to *A. niger*, while at 500 ppm, the decay was 28.4, 19.7 and 31.3 respectively Figure 5. A and B). At different concentrations, even at low concentrations, all oils showed better results, but clove oil was better on *Rhizophus* and cinnamon oil was better on *A. niger* than other oils. Our results are in line with the previous literature (Velluti *et al.*, 2003; Lopez-Malo *et al.*, 2007 Singh *et al.*, 2007; Shamsullah *et al.*, 2020). A critical analysis revealed that oils were more efficient against*. A. niger* and *R. stolonifer* compared to plant extracts. As a result, there is a compelling need to standardize these treatments, recognizing their capability to enhance the shelf life of grapes.

CONCLUSION

Compared with other fungi *Aspergillus* species were prominent in decay. In *in-vitro* trials, maximum proliferation of fungi is recorded at higher temperatures. Storage temperature 5-8°C considerably reduced disease incidence. All oils were effective in controlling *A. niger* and *R. stolonifer,* but clove oils were the best on *R. stolonifera*, while cinnamon oil proved to be best against *A. niger*. The study provides a valuable foundation for developing standardized protocols to extend the freshness and quality of grapes by effectively combatting decay-causing organisms.

AUTHOR CONTRIBUTIONS

SS mainly performed the experiments, MI, KN and SUK helped in data analysis, NA helped in interpretation, MJ and NA revision of the manuscript, AA supervised the study.

COMPETING OF INTEREST

The authors declare no competing interests.

REFERENCES

Anjum, M. A., Ahmed, N., Babita, C. H., & Gupta, P. (2016). Plant Extracts in Post-Harvest Disease Management of Fruits and Vegetables. A Review. Journal of Food Process Technology, 7, 592–2.

Arik, U. O., & Arik, C. (2017). Use of some essential oils in grape storage. Full text proceedings book.

Artes-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, K. M., Shah, N. A., Ishaq, M., & Fraooq, A. (2011). Postharvest losses and marketing of grapes in Pakistan. Sarhad Journal of Agriculture, 27, 485–490.

Barkai-Golan, R. (2001). Postharvest diseases of fruits and vegetables development and control. Amsterdam: Elsevier.

Bouamama, H., Noel, T., Villard, J., Benharref, A., & Jana, M. (2006). Antimicrobial activities of the leaf extracts of two Moroccan Cistus L. species. Journal of Ethnopharmacology, 104, 104–107.

Burgiel, Z. J., & Smaglowski, M. (2008). Fungistatic properties of the tea tree oil. Zeszyty Problemowe Postepow Nauk Rolniczych, 13–18.

Cosic, J., Vrandečić, K., Postic, J., Jurković, D., & Ravlić, M. (2010). In-vitro antifungal activity of essential oils on growth of phytopathogenic fungi. Poljoprivreda, 16, 25–28.

Davidson, P. M., & Naidu, A. S. (2000). Phyto-Phenols. In A. S. Naidu (Ed.), Natural Food Antimicrobial Systems (pp. 265–294). CRC Press.

De Souza Ferranti, L., Fungaro, M. H. P., Massi, F. P., da Silva, J. J., Penha, R. E. S., Frisvad, J. C., Iamanaka, B. T. (2018). Diversity of *Aspergillus* section *Nigri* on the surface of *Vitis labrusca* and its hybrid grapes. International Journal of Food Microbiology, 268, 53–60.

Diba, K., Kordbacheh, P., Mirhendi, S. H., Rezaie, S., & Mahmoudi, M. (2007). Identification of *Aspergillus* species using morphological characteristics. Pakistan Journal of Medical Sciences, 23, 6–867.

Droby, S., & Lichter, A. (2007). Post-harvest Botrytis infection: Etiology, development and management. In *Botrytis*: Biology, pathology and control (pp. 349–367). Springer.

Elsayed, M. I., Al-Qurashi, A. D., Almasaudi, N. M., & Abo-Elyousr, K. A. (2022). Efficacy of essential oils against gray mold and effect on fruit quality during cold storage in table grapes. South African Journal of Botany, 146, 481–490.

Enrico Drioli, Lidietta Giorno, & Enrica Fontananova. (2017). Comprehensive Membrane Science and Engineering Reference Work (2nd ed., pp. 4:48–7).

Esteban, A., Abarca, M. L., Bragulat, M. R., & Cabañes, F. J. (2004). Effects of temperature and incubation time on production of ochratoxin A by black *Aspergilli*. Research in Microbiology, 155, 861–866.

FAO. (2021). Food and Agriculture Organization of the United Nations. Statistical database production/yield quantities of grapes in Pakistan. https://www.fao.org/3/cc3751en/cc3751en.pdf

FAOSTAT. (2021). Food and Agriculture Organization of the United Nations. Statistical database production/yield quantities of grapes in the world. https://www.fao.org/3/cb4477en/cb4477en.pdf

Gabler, F. M., Mansour, M., Smilanick, J. L., & Mackey, B. E. (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.

Gatto, M. A., Ippolito, A., Linsalata, V., Cascarano, N. A., Nigro, F., Vanadia, S., & Di Venere, D. (2011). Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. Postharvest Biology and Technology, 61, 72–82.

Ippolito, A., Nigro, F., Linsalata, V., Cascarano, N., & Lima, G. (1997). Marciumi dell'uva da tavola in post-raccolta, Risultati di trattamenti preraccolta con cloruro di calcio. Italus Hortus, 4, 27–31.

Janisiewicz, W. J., & Korsten, L. (2002). Biological control of postharvest diseases of fruit. Annual Review of Phytopathology, 40, 411–441.

Li, C., Ma, X., Deng, J., Li, J., Liu, Y., Zhu, X., & Zhang, P. (2021). Machine learning-based automated fungal cell counting under a complicated background with ilastik and ImageJ. Engineering in Life Sciences, 21, 769–777.

Lichter, A., Zutchy, Y., Sonego, L., Dvir, O., Kaplunov, T., Sarig, P., & Ben-Arie, R. (2002). Ethanol controls postharvest decay of table grapes. Postharvest Biology and Technology, 24, 301–308.

Lisker, N., Keren-Shacham, Z., Sarig, P., Zutkhi, Y., & Ben-Arie, R. (1996). The biology and pathology of the fungus *Rhizopus stolonifer*, cause of black mould disease of table grapes in Israel. Plant Pathology, 45, 1099-1109.

Lopez-Malo, A., Barreto-Valdivieso, J., Palou, E., & Martín, F. S. (2007). *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. Food Control, 18, 1358–1362.

Nashwa, S. M. A., & Abo-Elyousr, K. A. M. (2012). Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Protection Science, 48, 74–79.

Nelson, K. E. (1979). Harvesting and Handling California Table Grapes for Market. University of California Bulletin no. 1913.

Okigbo, R. N., & Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam (Dioscorea spp.) rot. African Journal of Biotechnology, 5, 727-731.

Perdones, T., Vargas, M., Atarés, L., & Chiralt, A. (2014). Physical, antioxidant and antimicrobial properties of chitosan-cinnamon leaf oil films as affected by oleic acid. Food Hydrocolloids, 36, 256–264.

Perrone, G., Susca, A., Cozzi, G., Ehrlich, K., Varga, J., Frisvad, J. C., & Samson, R. A. (2007). Biodiversity of *Aspergillus* species in some important agricultural products. Studies in Mycology, 59, 53–66.

Riaz, T., Khan, S. N., & Javaid, A. (2008). Antifungal activity of plant extracts against *F. oxysporum* the cause of corm-rot disease of Gladiolus. Mycopathology, 6, 13–15.

Rousseaux, S., Diguta, C. F., Radoï-Matei, F., Alexandre, H., & Guilloux-Bénatier, M. (2014). Non-Botrytis grape-rotting fungi responsible for earthy and moldy off-flavors and mycotoxins. Food Microbiology, 38, 104–121.

Sanzani, S. M., Montemurro, C., Di Rienzo, V., Solfrizzo, M., & Ippolito, A. (2013). Genetic structure and natural variation associated with host of origin in *Penicillium expansum* strains causing blue mould. International Journal of Food Microbiology, 165, 111–120.

Sanzani, S. M., Reverberi, M., & Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: Insights into producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. Postharvest Biology and Technology, 122, 95–105.

Satish, S., Mohana, D. C., Ranhavendra, M. P., & Raveesha, K. A. (2007). Antifungal activity of some plant extracts against important seed-borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology, 3, 109–119.

Satish, S., Raveesha, K. A., & Janardhana, G. R. (1999). Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. Letters in Applied Microbiology, 28, 145–147.

Shariff, N., Sudarshana, M. S., Umesha, S., & Hariprasad, P. (2006). Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and *callus extracts*. African Journal of Biotechnology, 5, 946–950.

Singh, G., Maurya, S., DeLampasona, M. P., & Catalan, C. A. (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology, 45, 1650–1661.

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. Agriculture and Natural Science, 42, 169–174.

Shamsullah, S., Ibrahim, M., Abbas, A., Ullah, M. A., Aslam, M., Kamran, M., & Hussain, S. (2020). Grapes Post-harvest Decaying Process, Associated Fungal Pathogens and their Ecofriendly Control by Plant Extracts and Oils. International Journal of Biosciences, 16, 197-204.

Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Postharvest Biology and Technology, 32, 235-245.

Tripathi, P., Dubey, N. K., & Shukla, A. K. (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.

Tzortzakis, N. G. (2009). Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. Innovative Food Science and Emerging Technologies, 10, 97–102.

Velluti, A., Sanchis, V., Ramos, A. J., Egido, J., & Marín, S. (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Journal of Food Microbiology, 89, 145–154.

Walker, A. S., Gautier, A., Confais, J., Martinho, D., Viaud, M., Le Pêcheur, P., & Fournier, E. (2011). *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. Phytopathology, 101, 1433–1445.

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.