

Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print) http://esciencepress.net/journals/PP

ASSESSMENT OF RESPONSES OF PEACH CULTIVARS TO POSTHARVEST PATHOGEN *BOTRYTIS CINEREA* AND ITS MITIGATION USING PLANT ESSENTIAL OILS

^aZahra Ahmad, ^aHuma Abbas, ^aTamsal Murtaza, ^bAtta ur Rehman Khan, ^aAnsar Ali, ^aKhizra Zahid, ^cZunaira Tahir, ^bTahir Mahmood, ^aAmer Habib

^a Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

^b Department of Plant Pathology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

^c Department of Plant Protection, University of Applied Sciences, Isparta, Turkey.

ARTICLE INFO ABSTRACT

Article history Received: 29 th June, 2023 Revised: 26 th July, 2023 Accepted: 29 th July, 2023	In peaches, post-harvest losses occur due to injury during mishandling, storage, processing, and transportation, especially caused by pathogen infections that result in the reduction of quantity, quality, and market value of agricultural commodities. Numerous postharvest pathogens attack peaches, especially <i>Botrytis cinerea</i> , which causes grey mold disease, resulting in losses of about \$10 billion globally. The
Keywords Prunus persica Postharvest pathogens Plant essential oils Disease resistance Antifungal activity	current paper reports the results of a study that investigated the occurrence of different fungi and the effect of different essential oils on mold growth in five peach cultivars. The study found that <i>Penicillium expansum, Botrytis cinerea, Aspergillus flavus,</i> and <i>A. niger</i> had different preferences and distributions among the cultivars. The study also found that Florida King 6-A was resistant to <i>B. cinerea,</i> while Early Gold was highly susceptible. The study also found that cinnamon oil was the most effective essential oil in controlling mold growth in Florida King 6-A, while sesame oil was the least effective in Early Grand. The study also found that clove bud oil at 100% concentration had the highest antifungal potency among the tested essential oils and that different concentrations of essential oils were required for different cultivars. These findings highlight the varying levels of susceptibility among peach cultivars to fungal pathogens and the varying degrees of effectiveness associated with different essential oils in mitigating fungal growth. Such insights are invaluable for agricultural practices aiming to protect peach crops from fungal infections and optimize yield.

Corresponding Author: Amer Habib Email: amer.habib@uaf.edu.pk © 2023 EScience Press. All rights reserved.

INTRODUCTION

Fruits and vegetables are major horticultural crops and are considered an essential part of the diet for the citizens of Pakistan. Pakistan, being an agricultural country, cultivates approximately 22.2 million hectares of land for crop production and about 4.5 million hectares for growing fruits and vegetables. Pakistan not only meets its own domestic needs but also earns valuable foreign exchange by exporting fruits and vegetables to several countries, including India, the United Arab Emirates, Saudi Arabia, Afghanistan, and the United Kingdom (Syed et al., 2014).

According to econometric estimations, the handling, transportation, and storage of fruits have a significant

impact on their quantity, quality, and market value. Approximately 10-15% of fruits are wasted from tree to table due to mishandling, transportation issues, inadequate storage facilities, and a lack of technical expertise. The overall postharvest losses in fruits and vegetables are estimated to be around 25-40%, which is alarming in terms of food waste and results in significant financial losses (Ahmed et al., 2015; Aslam et al., 2019).

Postharvest losses not only reduce the availability of fruits but also contribute to increased transportation and marketing costs. These losses have a direct impact on both producers, as they experience a reduction in their share of consumer prices, and consumers, who face higher prices and reduced availability (Murthy et al., 2009).

For this reason, approximately 1000 new peach cultivars were introduced in the 20th century, and successful breeding programs were established through controlled crosses (Li et al., 2013). Peach is widely regarded as the queen of stone fruits due to its high nutritional value, delightful taste, and appealing color. Therefore, it ranks just behind apple in terms of popularity (Habib, 2015).

In Pakistan, the cultivated land dedicated to peaches spans approximately 7,600 hectares, resulting in an annual production of nearly 60,400 tons of fruit. Peach cultivation is particularly prominent in the Khyber Pakhtunkhwa (KPK) region, where an area of 3,500 hectares yields around 33,800 tons of fruit, as per Pakistan's statistics.

Peaches are known for their abundant content of carbohydrates, phenolics, antioxidants, volatiles, vitamins, and sufficient amounts of lipids and proteins. A single peach fruit consists of approximately 87% water and provides around 180 KJ (43 Kcal) of energy (Crisosto, 1994).

The primary postharvest diseases affecting peaches are caused by plant pathogens, including blue mold caused by *Penicillium expansum*, *Rhizopus* rot caused by *Rhizopus stolonifer*, brown rot caused by *Monilinia* spp, and grey mold caused by *Botrytis cinerea* (Arrebola et al., 2010; Ahmed et al., 2018). Grey mold is the most common and economically significant postharvest disease of peaches (Zhang et al., 2008). Globally, *B. cinerea* causes economic losses exceeding \$10 billion (Hua et al., 2018).

Currently, the primary and most practical method of controlling grey mold disease is the use of synthetic fungicides (Iqbal and Mukhtar, 2020a). However, these fungicides are hazardous to both the environment and human health. Prolonged and widespread use of synthetic fungicides and chemicals in packing houses has led to the development of pathogen strains that are resistant to these chemicals. These issues have prompted the search for alternative methods to control postharvest diseases in fruits and vegetables, specifically targeting grey mold disease in peaches (Zhang et al., 2008; Iqbal and Mukhtar, 2020b; Ahmed et al., 2021; Azeem et al., 2021; Haq et al., 2021; Mukhtar et al., 2021, 2023; Shahbaz et al., 2023; Shakoor et al., 2015).

An alternative and environmentally friendly approach to synthetic fungicides for postharvest applications is the use of plant-derived products and natural compounds. Essential oils have been explored as alternatives for the control of postharvest fruit diseases (Yazdani et al., 2011; Sales et al., 2016; Nabila and Soufiyan, 2019). So far, there has been limited research conducted in Pakistan on the biological management of postharvest losses in peaches (Saeed et al., 2021). Therefore, the present study was undertaken with the aim of investigating the association between postharvest fungal pathogens and peaches, as well as exploring safe management practices. The study focused on the following objectives: Firstly, to identify the primary fungus responsible for causing significant postharvest fungal losses in peaches. Secondly, to assess the effectiveness of various essential oils in controlling the postharvest fungal pathogens affecting peaches.

MATERIALS AND METHODS

Sampling, purification and identification of fungi

A survey of different markets in Faisalabad was conducted. Peach fruits showing signs of discoloration, rot, and deterioration were randomly collected and placed in polythene bags. The fruits were then sorted into infected and healthy ones and placed in zipper polythene bags with appropriate labeling. To facilitate further research, all collected samples were taken to the Seed Health Testing Laboratory in the Department of Plant Pathology at University of Agriculture, Faisalabad. Common symptoms of fungal diseases and disease incidence percentages were determined using the PDI formula (Abdulsalam et al., 2015).

Disease incidence (ID %)

 $\frac{\text{Number of infected peach fruits}}{\text{Total Number of collected Peach fruits}} \times 100$

Total Number of collected Peach fruits Disease index in percent was recorded by acquiring this formula:

Disease index (DI %)

Sum of all numerical ratings

 $= \frac{1}{\text{Detected no. of fruit sample } \times \text{Maximum disease rating}} \times 100$

The method employed for the identification and isolation of the fungus from peach fruits involved the following steps: Small pieces of peach fruits, bearing a rotted part measuring approximately 1-2 cm, were sliced and separated, after which a surface disinfection method was applied. Subsequently, the samples were carefully placed on dry filter paper to facilitate drying before being gently transferred to petri plates filled with sterilized PDA medium. The inoculated petri plates were then incubated at a temperature of 24°C, with regular monitoring of fungal growth on the PDA medium within the plates. Upon reaching a satisfactory level of fungal growth, a single hyphal tip was extracted from each colony, aided by a needle, and transferred onto another petri plate containing PDA. This plate was then subjected to another incubation period in an incubator set to a room temperature of approximately 25± 2°C, thereby enabling the attainment of a pure culture of the fungus.

The purification of the fungus was carried out within a laminar flow chamber. Cultures of *Botrytis cinerea* were prepared and stored in slants for future studies, with an incubator set at a temperature of 4°C. To identify the fungus, its morphological and cultural characteristics were carefully observed, along with microscopic examination under 100 × magnifications, using the hyphal tip method for fungus isolation as described (Rajasekar et al., 2020).

Artificial inoculation of fungus

Fungal cultures were prepared on PDA media for the purpose of inoculation. Fresh peach fruits were punctured with a needle and subsequently inoculated with the fungus. The inoculated samples were then placed in zipper polythene bags and incubated for a minimum of 48 hours to observe the development of symptoms.

Pathogenicity test

A pathogenicity test was conducted in the laboratory to confirm the presence of the fungus. Pure cultures were grown on PDA media. Fresh and healthy peach fruits were first washed with normal tap water. Then, a surface sterilization method was employed using 0.1% sodium hypochlorite (NaOCl) solution for 1-2 minutes. Following this, the fruits were rinsed twice with distilled water and dried on filter paper to remove any excess water. Subsequently, the peach fruits were inoculated using an inoculating needle and placed in zipper polythene bags, which were then placed in an incubator. Symptoms on the fruit surface were observed after a period of 2 days.

Management with essential oils

All the essential oils of the plants were collected from various Pansar and scientific stores in Faisalabad, Pakistan, and were used against *B. cinerea*. Fresh peach fruits were punctured in a circular shape using a needle and marked with a permanent marker. Subsequently, the fungus was applied to the fruits' surfaces using cotton swabs taken from the petri plates. Finally, the entire fruit surface was coated with selected oils (Sesame, Clove bud, and Cinnamon) at concentrations of 25%, 50%, and 100%. The entire setup was then placed in polythene bags, and the size of rotting lesions on the fruit surface was measured after intervals of 2, 3, and 4 days.

Statistical analysis

The experimental trials were conducted under laboratory conditions following a completely randomized design. The data were recorded and subjected to statistical analysis, following the guidelines outlined by Steel et al. (1997).

RESULTS

Identification of post-harvest fungi

Different fungi were isolated from different peach cultivars. *Penicillium expansum* was found to be present in Florida King 6-A, Early Grand, Golden Early, and Shah Pasand cultivars, while it was absent in the Shireen cultivar. *Botrytis cinerea* was observed in all five cultivars. *Aspergillus flavus* was absent in the Florida King 6-A cultivar but present in Early Grand, Golden Early, and Shireen cultivars. However, it was absent in the Shah Pasand cultivar. Lastly, *A. niger*, also known as black rot, was found in Florida King 6-A, Golden Early, and Shah Pasand cultivars, while it was absent in Early Grand and Shireen cultivars (Table 1).

Screening of different peach cultivars for resistance to *B. cinerea*

The study revealed that the cultivar Florida King 6-A exhibited resistance to *B. cinera*, whereas Early Gold demonstrated a high susceptibility. Moreover, Golden Early, Shah Pasand, and Shireen were identified as susceptible to mold development. Detailed symptoms for each cultivar can be found in Table 2.

Table. 1 Association of post-harvest fungi with different peach cultivars.	
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Postharvest fungus	Florida King 6-A	Early Grand	Golden Early	Shah Pasand	Shireen
Penicillium expansum	+	+	+	+	-
Botrytis cinerea	+	+	+	+	+
Aspergillus flavus	-	+	+	-	+
Aspergillus niger (Black rot)	+	-	+	+	-

+ Isolated - Not isolated

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Score	Symptoms	Rating Score	Remarks
Florida King 6-A	Mold growth covered about 2 cm area (less	1	Resistant
	than ¼ of peach surface)		
Early Gold	Mold growth covered about 13 cm area	4	Highly Susceptible
	(more than ½ of peach surface)		
Golden	Mold growth covered about 7 cm area	3	Susceptible
Early	(more than $\frac{1}{2}$ and less than $\frac{3}{4}$ of peach		
	surface)		
Shah Pasand	-DO-	3	Susceptible
Shireen	-DO-	3	Susceptible

Effect of essentials oils against B. cinerea

The ANOVA given in Table 3 presents the statistical results of the effect of fruit coating with essential oils on five cultivars of peaches. The effects of doses, treatments, and varieties had highly significant effects on the response variable (fruit coating with essential oils). Similarly, the interaction between dose and oils, dose and varieties and oil and varieties also had highly significant effects. Furthermore, the interaction between dose, oil, and variety was also significant.

The findings of the present investigation unveiled that among the applied essential oils; cinnamon oil demonstrated the most effective results when applied to the cultivar Florida King 6-A (0.256 Q). Conversely, sesame oil exhibited the least effective control (18.567 B), and the untreated control manifested the highest incidence of fungal growth (927.167 A) in the case of the cultivar Early Grand (Table 4). Clove bud oil at 100% concentration, followed by cinnamon and sesame oils, resulted in the lowest growth (2.753), while the highest growth (13.673) was observed in the un-treated control (Table 5). The minimum growth was controlled at 25% concentration in Early Grand and the maximum growth was controlled at 100% concentration in Shah Pasand (2.858) as shown in Table 6. The fungal growth at each concentration on each cultivar is given in Figure 1.

Table 2. Analysis of warian as	for furthe costing with	accountial ails on F	aultimore of moode
Table 3. Analysis of variance	IOP IPHIL COALING WILD	essential ous on 5	cultury ars of neach
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Source	DF	SS	MS	F	Р
Dose	2	42.04	21.02	445.10	0.0000
Treatment	3	2797.53	932.51	19747.3	0.0000
Variety	4	5890.77	1472.69	31186.4	0.0000
Dose × oils	6	13.75	2.29	48.54	0.0000
Dose × Variety	8	42.94	5.37	113.66	0.0000
Oil × Variety	12	440.88	36.74	778.03	0.0000
Dose × Oil × Variety	24	16.22	0.68	14.31	0.0000
Error	120	5.67	0.05		
Total	179	9249.80			
Total	35	637.250			



Figure 1: Response of fruit coatings with different essential oils on 5 cultivars of peach.

Table 4. Comparison of mean valu	es for fruit coatings with differen	t essential oils on 5 cultivars of pe	ach
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Cultivar	Sesame	Cinnamon	Clove bud oil	Control
Floida King 6-A	0.411 Q	0.256 Q	0.867 P	11.933 E
Early Grand	18.456 B	15.222 C	11.267 F	27.167 A
Golden Early	2.8 L	2.3 M	1.7 N	6.711 I
Shah Pasand	1.533 N	1.267 0	0.867 P	8.533 G
Shireen	7.067 H	5.067 J	3.067 K	14.067 D
LSD	0.2028			

Table 5: Comparison of mean values for fruit coatings with different essential oils (Treatment × Dose).

Essential oil		Concentratio	ns		
	25%	50%	100%		
Sesame	6.827 B	6.08 C	5.253 E		
Cinnamon	5.547 D	4.82 F	4.1H		
Clove bud	4.44 G	3.467 I	2.753 I		
Control	13.7 A	13.673 A	13.673 A		
LSD	0.571				

Table 6: Comparison of mean values for fruit coatings at different oil dosage on (Dose × Variety).

Cultivar		Concentrations			
	25 %	50 %	100 %		
Florida King 6-A	3.458 GH	3.358 HI	3.283 HIJ		
Early Grand	19.758 A	17.958 B	16.367 C		
Golden Early	3.608 G	3.375 HI	3.15 JK		
Shah Pasand	3.25 IJ	3.042 K	2.858 L		
Shireen	8.067 D	7.317 E	6.567 F		
LSD	0.1756				

DISCUSSION

The results of the study show that different peach

cultivars are susceptible to different fungi. Earlier, many workers reported postharvest fungi associated with

peach in different regions of the world (Thomidis et al., 2007; Villarino et al., 2016; Zhang et al., 2019; Li et al., 2021; Dao et al., 2023; Hrustic et al., 2023). *P. expansum* was found to be present in four of the five cultivars, while *A. flavus* and *A. niger* were only found in three of the five cultivars. *B. cinerea* was the only fungus that was found in all five cultivars. The absence of certain fungi in some cultivars may be due to genetic factors or to environmental conditions. For example, the absence of *A. flavus* in the Florida King 6-A cultivar may be due to a genetic resistance to this fungus. The absence of *A. niger* in the Early Grand and Shireen cultivars may be due to environmental conditions, such as lack of moisture (Villarino et al., 2016; Zhang et al., 2019).

The presence of different fungi in different peach cultivars has implications for disease management. For example, growers of the Florida King 6-A cultivar may need to focus on controlling *P. expansum*, while growers of the Early Grand cultivar may need to focus on controlling *A. flavus*. The study also provides some insights into the biology of these fungi. For example, the presence of *B. cinerea* in all five cultivars suggests that this fungus is a generalist that can infect a wide range of hosts. The absence of *A. flavus* in the Florida King 6-A cultivar suggests that this fungus may be more specialized and may require specific environmental conditions to survive (Thomidis et al., 2007; Dao et al., 2023; Hrustic et al., 2023).

In the study, Golden Early, Shah Pasand, and Shireen were identified as susceptible to mold development. According to previous studies, peach cultivars can be infected by various mold species, such as *P. expansum*, which causes blue mold rot, *A. flavus* and *A. niger*, which produce aflatoxins and cause black rot, and *P. pannosa* var. *persicae*, which causes powdery mildew (Pascal et al., 2010; Korba et al., 2013; Lalancette et al., 2014). Each of these mold species may have different preferences and distributions among peach cultivars and may interact with each other in complex ways (Pascal et al., 2010). Therefore, it is important to identify the mold species involved in the infection and to assess their relative importance and impact on the quality and yield of peach cultivars.

The study also did not consider the environmental factors that may influence the development and spread of mold in peach cultivars. Factors such as temperature, humidity, rainfall, irrigation, pruning, fertilization, and pesticide application may affect the susceptibility or resistance of peach cultivars to mold (Carrera et al., 2005; Byrne, 2010; Pascal et al., 2010; Lalancette et al., 2014; Szymajda and Zurawicz, 2016; Oh et al., 2017). For example, high humidity and low temperature may favor the growth of *B. cinerea* and *P. expansum*, while high temperature and low humidity may favor the growth of *A. flavus* and *A. niger* (Pascal et al., 2010). Therefore, it is necessary to take into account the environmental conditions under which the peach cultivars were grown and to compare them with the optimal conditions for mold development.

The results of this study also showed that essential oils have different effects on the control of mold growth in peach cultivars. Cinnamon oil demonstrated the most effective results when applied to the cultivar Florida King 6-A, while sesame oil exhibited the least effective control. This suggests that cinnamon oil has stronger antifungal properties than sesame oil and that Florida King 6-A is more responsive to essential oil treatment than other cultivars. Clove bud oil at 100% concentration, followed by cinnamon and sesame oils, resulted in the lowest growth, while the highest growth was observed in the untreated control. This indicates that clove bud oil has the highest antifungal potency among the tested essential oils and that higher concentrations of essential oils are more effective than lower concentrations. This is consistent with previous studies that have shown that clove oil has a broadspectrum antifungal activity against various mold species and that its efficacy increases with concentration (Carovic-Stanko et al., 2013; Elshafie et al., 2015a; Taheri et al., 2021; Yan et al., 2021; Lin et al., 2022). However, the study did not consider the possible adverse effects of high concentrations of essential oils on the peach quality and safety. Essential oils may cause phytotoxicity, oxidative damage, or allergic reactions in some cases (De Corato et al., 2010; Elshafie et al., 2015b; Fontana et al., 2021; Taheri et al., 2021; Xiong et al., 2021; Cao et al., 2022; Lin et al., 2022). Therefore, it is important to evaluate the optimal concentration and application method of essential oils that can balance between antifungal efficacy and peach quality and safety.

The minimum growth was controlled at 25% concentration in Early Grand and the maximum growth was controlled at 100% concentration in Shah Pasand. This implies that there is a variation in the sensitivity of different peach cultivars to essential oil treatment and that different concentrations of essential oils are

required for different cultivars (Fontana et al., 2021; Mou et al., 2021; Oliveira Filho et al., 2021; Xiong et al., 2021; Cao et al., 2022). It may be related to the genetic, physiological, or morphological characteristics of different peach cultivars or to the environmental conditions under which they were grown (De Corato et al., 2010; Carovic-Stanko et al., 2013; Oliveira Filho et al., 2021; Taheri et al., 2021; Yan et al., 2021; Cao et al., 2022; Lin et al., 2022). Therefore, it is necessary to investigate the factors that affect the sensitivity of peach cultivars to essential oil treatment and to customize the concentration and application method according to each cultivar.

CONCLUSIONS

The findings of the current study highlight the varying levels of susceptibility among peach cultivars to fungal pathogens and the varying degrees of effectiveness associated with different essential oils in mitigating fungal growth. Such insights are invaluable for agricultural practices aiming to protect peach crops from fungal infections and optimize yield.

AUTHORS' CONTRIBUTIONS

ZA and HA conceived the idea; ZA, HA and TM designed the study; ZA, AA, KZ collected diseased samples; ZA, ZT and TM helped in the isolation and identification of the fungi; ZA, AA and KA conducted screening trial; ZA, ARK analyzed the data statistically; ZA wrote the manuscript; ARK and AH proofread the manuscript; AH supervised the work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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