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DEGRADATION OF FRUITS LAYERS BY ENZYMATIC ACTIVITY OF FUNGI

GULNAZ PARVEEN[1*], NAILA MUKHTAR[2], IRFAN ULLAH[3], AMTUL SAMI[4], SHAMAILA IRUM[5], NAIN TARRA BUKHARI[6], ANEELA NAZ[7] AND TAHIRA BATOOL[8]

[1]*Department of Botany, Women University, Swabi, KP Pakistan*
[2]*Department of Botany, University of OKARA, Pakistan*
[3]*Department of Zoology Karakoram International University, Ghizer Campus 15200, Pakistan*
[4]*Department of Molecular Biotechnology Women University Swabi*
[5]*Department of Zoology, University of Gujrat. Clinical Laboratory Sciences, Women University, Swabi, Pakistan*
[6]*Department of Microbiology, Women University Swabi, Pakistan*
[7]*Department of Botany, Khushal Khan Khattak University Karak, Pakistan*
[8]*Department of Biotechnology, Women University of Azad Jammu and Kashmir Bagh, Pakistan*
**Corresponding author's email: gulnaz.malik3@gmail.com/ gulnaz.parveen@wus.edu.pk*

Abstract

Fruits losses are the foremost reason for declining the export income of a country. These losses encompass many reasons. Among these, post harvest losses are one of the ample causes discussed in the present study. However, microorganisms are the foremost cause for post harvest losses. This study is based on the isolation and identification of fungi which are responsible for post harvest loss by degrading cell wall of fruits by producing ezymes. Because fruits are the paramount source for colonization of fungi. Total 10 different fruits samples from District Swabi were taken under consideration to identify 13 different species of fungi included (*Cladosporium cladosporioides, Alternaria sp., Fusarium solani, Geotrichum candidum, Penicillium notatum., P.expansum Botrytis* sp*., Aspergillus* sp*., Drechsler* sp*., Rhizopus stolonifer, Phytopythora* sp*., Cladosporium* sp*., Colletotrichum* sp*., Mucor* sp.,) that were predisposed to produce post harvest losses of different fruits. The most common fungi were isolated was *Alternaria* species, *Penicilium* and *Fusarium* spp. All isolated fungi were cultured on fruits peel and screened the Cell wall degrading enzymes of plants, like cellulase, polygalacturonase, a-amylase and xylanase. Highest level of polygalacturonase and xylanase were recorded compared to the others two. In decision, *Penicilium* sp., are common and the polygalacturonases and xylanses are the main fungal enzymes that accountable for rotting of fruits.

Key words: Disease; Losses; Pathogens; Postharvest; Rotting.

Introduction

Fruits and vegetables are the paramount food resources all over the world as well as in developing countries and highly populour countries in Asia like Pakistan. According to an estimate, the world population will be projected around 11.2 billion by the end of 2050 (Anon., 2011) and the same immense increase is being expected in the population of Pakistan. Pakistan's population (20.6 million) is expected to be increased 300 million by the end of 2050 (Anon., 2019).

United Nations has set some fundamental goals in the form of Millennium Development Goals (MDGs)to overcome the hunger and global poverty (Anon., 2011).

Among a number of global issues, food waste and food losses are one of the serious problems with an annual loss of about 1.3 billion (Dos Santos *et al*., 2020). Storage of food without refrigeration, shipment constraints like mechanical injuries, temperature fluctuations, exceeding purchase volume, and careless handling by vendors and consumers are also among major causes of food losses (Dos Santos *et al*., 2020, Khan *et al*., 2019). Ample losses of fruits both at postharvest and pre- harvest stages were caused by pathogens (Singh & Sharma, 2007). Fungi among all pathogens were found most lethal with severe postharvest losses of vegetables and fruits due to their high sugar and moisture contents and most eminently low pH lead to fruit decay and deterioration ultimately resulting in economic losses (Abdullah *et al*., 2016, Parveen *et al*., 2021). Different pathogenic fungi are responsible for the post harvest losses of fruits (Gong *et al*., 2022). Degradation of fruits layers by producing different ezymes by pathogenic fungi are mainly Aspergillus spp. and the main ezymes produced by this pathogen are polygalacturonases and xylanses that was responsible for the spoilage of fruits (Al-Hindi *et al*., 2011; Kirana *et al*., 2016). Gen editing can reduce the loss of fruits that cause by fungi (Shipmen *et al*., 2021). Post-harvest losses are the most common issue in Pakistan as well as all over the world. Due to lack of awareness, and research done on post-harvest losses of fruits are very less in Khyber Pakhtunkhwa and especially in District Swabi. Therefore, the purpose of the study is to find out the pathogens which are responsible to cause diseases losses of fruits.This study also indicates the need for improvements both in the infrastructure and in the hygienic care, management and postharvest conservation of fruits.

Materials and Methods

During the year of 2021, a survey was conducted on selected diverse rotted fruits from local markets of Swabi, jalsai, and Ghazi situated in Swabi District (Khyber Pakhtunkhwa, Pakistan). Following are the

fruits which have been selected for the quality judgment such as melon (*Cucumismelo L.*), apple (*Malus pumila Mill*), guava (*Psidium guajava L.*), Sweet orange (*Citrus sinensis* Osbeck), banana *(Musa)*, lemon (*Citrus limon Osbeck*), persimmon (*Diospyros kaki*) bear (*Ziziphus mauritiana*), pomegranate (*Punica granatum*) and grapes (*Vitis vinifera*).

Five samples of each fruit were collected from fruit markets and then shifted the rotted fruits in sterile polythene bags and safely transported to laboratory. Infected tissues were cut with the help of a sterile sharp scaliper. Tissue surface was sterilized with 70% alcohol and dried in a fuming chamber. The selected samples were applied aseptically onto potato dextrose agar plates containing antibiotics (Penicillin: 10^5 units/L and streptomycin 0.2 g/L). Percent infection and percent colonization were evaluated by Parveen *et al*., (2020) with the help of given formula:

$$\% \text{ Infection of fruits} = \frac{\text{No. of infected fruits}}{\text{Total no. of fruits}} \times 100$$

However, temporary mountings were detected with lactophenol mounting method and were observed under a compound microscope. Identifications of pathogenic fungi were evaluated on the basis of morphology and their spore arrangement pattern. Moreover, pure culture study was done by observing the following characteristics, i.e., colony morphology, biochemical properties, microscopic morphology, staining reaction etc (Aneja, 2007). Isolations of pure fungal cultures were done by "single spore isolation method on Potato Dextrose Agar slants for supplementary studies.

Cell wall degrading enzymes Production: Different Cell wall degrading enzymes were produced from test fungi isolated from spoil fruits were cellulases, Polygalacturonase, amylases and xylanases, in agitinate phases. Fungi were injected under sterile conditions in 500 ml flasks containing 5% fruit peels and incubated at 27ºC in shaking incubator at 150 rpm for 5 days (Rashad *et al*., 2011).

Enzyme assays: a-amylase, xylanase, cellulase, and Polygalacturonase activity were determined by using maltose, xylose, glucose and galacturonic acid as standards, respectively (Miller, 1959, & Al-Hindi *et al*., 2011). The mixture were prepared by adding, a suitable amount of crude extract, 0.05 M sodium acetate buffer pH 5.5, 1% (0.5 ml) substrates (Substrates used were starch, xylan, CM-cellulose, and polygalacturonic acid) for amylase, xylanase, cellulase, and polygalacturonase respectively, and kept for 1hour at 37°C. Then each tube was added by 0.5 ml dinitrosalicylic acid reagent and heated for 10 min in a boiling water bath. After cooling to room temperature, the absorbance was measured at 560 nm. One unit of enzyme activity was defined as the amount of enzyme which liberated 1 µmol of reducing sugar per h under standard assay conditions.

Statistical analysis

Data of infection % and colonization % were statistically analyzed by standerd daviation by using IBM SPSS STATISTICS 20 programe (Sokal & Rohlf, 1995). And aach value of enzyme activity represents the mean of three runs ±S.E.

Results and Discussion

Fruit are a considerable source of vivacious micronutrients, fibres, and phytochemicals. Alot of significant research and reports have been published that the impact of fruit and Post-harvest losses among fruits and vegetables may occur at any time while handling as the management related to postharvest done to maximize the storage value and quality of vegetables and most of the genera found involved acting as pathogens like Geotrichum, Aspergillus, *Penicillium, Alternaria, Fusarium, Phomopis* etc (Adaskaveg *et al*., 2002; Rahul *et al*., 2015).

Fungi like pathogens secrate numerous cell wall degrading enzymes to worsen and digest fruit layers as nutritional sources, ultimately falling postharvest life which ultimately lead to noxious and adverse effects on fruits and vegetable taste. Therefore, there is dire need for ample storage and protective measures to lessen postharvest damage.

In the present study, fruits such as melon (*Cucumismelo* L.), apple (*Malus pumila* Mill), guava (*Psidium guajava* L.), Sweet orange (*Citrus sinensis* Osbeck), banana (*Musa*), lemon (*Citrus limon* Osbeck), persimmon (*Diospyros kaki*) bear (*Ziziphus mauritiana*), pomegranate (*Punica granatum*) and grapes *(Vitis vinifera)* were collected from markets located in Swabi District and various species of fungi were isolated and identified from different fruits which showed infections (Fig. 1). *Cucumis melo* L. (Melon) was found to be infected with *Fusarium solani* (66%), *Cladosporium cladosporioides* (35.66%) in Ghazi market. However, *Alternaria alternata* (64.33%) and *Fusarium solani* (98.33%) species were observed in infected fruits collected from swabi market. Although *Geotrichum candidum* produced (32.66%) infection in *Cucumis melo* L. (Melon) collected from jalsai market which are responsible for watery rot disease in fruits. *Penecilium* spp., were found as a causal agent in M*alus domestica* (Apple) from jalsai and Ghazi sites which is responsible for 100% grey mold infection. *Psidium gujava* (Guava) was observed with dark brown lesions due to *Alternaria* sp., *Drechslera* sp., and *Phytopythora* sp., with 99%, 0% and 65% infection in swabi, jalsai and ghazi markets respectively. Moreover, *Citrus sinensis* was 99.66% infected with *Penicillium* sp., in Ghazi and swabi market, which are responsible for blue green mold infection in fruits (Table 1). Similar study was done by Zang *et al*., 2019, Isolated and identified the most virulent species of *Alternaria tenius* and *Botrytis* in fruits (peach) that destroyed the field of peach completely. Different major genera of fungi included *Cladosporium*, *Aspergillus*, *Alternaria*, *Penicillium*, *Colletotrichum* and *Fusarium*, that were the major cause of destroying the citrus fruits (Saif *et al*., 2021).

Fig. 1. Different infected fruits samples were collected from different markets of Swabi.

Penicillium notatum species was responsible for Fruit rot disease having 65.33% and 34% infections were perceived in *Musa* (Banana) collected from Swabi and Ghazi market, respectively. While *Fusarium* spp., were responsible for 66% fruit infection in *Citrus limon* (Lemon) throughout area of Ghazi. *Alternaria* was detected with 99.33% infection in *Punica granatum* (Pomegranate) in swabi market, whereas 67.66% and 33% infections were detected in Jalsai and Ghazi markets. *Vitis vinifera* (Grapes) were infected with *Botrytis* (67%) and *Penicilium* spp., (64.66%). Black spot disease was found in *Diospyros kaki* (Persimmon) caused by *Alternaria* spp., (66%), from Ghazi. Whereas the most frequent *Fusarium* sp (33.33% and 66.66% infection) were recorded from Swabi and jalasai markets and the same species were also isolated from *Ziziphus mauritiana* (Ber) Jalsai market (Table 1, Fig. 1). Standard deviation has been calculated in case of percentage infection and percent colonization of fungi isolated from postharvest infected fruits collected from different markets of swabi which is range between 0.00 and 3.05 as shown in Tables 1 and 2. The highest standard deviation value has been observed in the case of percent infection of two fungal species, i.e., *Cladosporium* spp. and *Fusarium* spp., which were 3.78 and 3.05 respectively. The highest standard deviation value have been observed in case of percent colonization of fungal species, i.e., *Penecellium* spp., *Butrytus* spp., and *Fusarium* spp., which were 4.163, 2.645 and 3.055 respectively. *Penicillium*, *Fusarium*, and *Alternaria* species were most frequently found in various species of fruits collected from different markets. The disease causing agents like *Fusarium solani* and *Cladosporium cladosporioides* in Ghazi market. However, *Alternaria alternata* were found to be frequent in melon which also has been previously reported by (Fatima *et al*., 2009). Tolulope *et al*., (Ewekeye *et al*., 2016) was also reported the same species of *Aspergillus* sp., in apple infection. The pathogens that infected persimmon *(Diospyros kaki)* are *Alternaria* spp., *Fusarium* sp., and) (Palou *et al*., 2012) also reported that *Alternria alternate* attack on persimmon. Various species (*Aspergillus niger*, *Geotrichum candidum*, *Diplodianatalensis*, *Trichoderma viride*, *Penicillium* sp., *Fusarium* sp., *Alternaria alternata*, *Aspergillus ochraceous* and *Aspergillus fumigatus*) of pathogen were found to be common in sweet orange as earlier reported by (Rasool *et al*., 2014). *Alternaria alternata*, Drecshlera sp., was

observed in guava as infected specie and the similar results was reported by (Fatima *et al*., 2009). The brown spots appeared on Ber (*Ziziphus mauritiana*) which are due to the attack of fungal pathogens, like *Penecillium* sp., and *Fusarium* sp., and has been reported by (Pallavi *et al*., 2014). Sarkar *et al*., (2013) reported that Banana was found infected with fruit rot. The pathogens identified on Grapes *(Vitis vinifera)* were *Rhizopus stolonifer, Penecillium* sp., *Botrytis* sp., was also documented by (Singh *et al*., 2017). Lemon (*Citrus limon)* was infected by *Alternaria* sp., *Drechslaria* sp., *Penecillium niger, Fusarium* sp., and *Rhizopus stolonifer* were also identified and reported by (Pallavi *et al*., 2014). Some pathogens infect the pomegranate (*Punica granatum)* and cause dark lesions due to *Aspergillus niger, Botrytis* spp., *Penicillium* sp., *Fusarium* sp., and *Alternaria* sp. While (Fatima *et al*., 2009) reported *Penecillium* sp., an infection in pomegranate. Ahmad *et al*., (2021) reported the deterioration of fruits are caused by fungi and responsible the huge loss.

In the present study, the highest level of xylanase was recorded in *Penicilium* sp., (2876 ± 50 units/100 ml) was grown on Banana, the level of PGase in *Alternaria alternata* (1075±32 units/100 ml) grown on Persimon and Guava with agitation, The levels of cellulase and Amylase activity were very low in all tested fungi grown on all fruits peel as compared to PGase and xylanase (Table 3). Similarlry *Fusarium solani* produced high level of PGase in Lemmon peel and Earlier research described that the several plant cell wall degrading enzymes were produced from same tested fungi and *Fusarium* sp., was able to secrete several cell wall degrading enzymes like xylanase, cellulase, pectinase and a-amylase and (Di Pietro *et al*., 2003). High level of xylanase was produced by *F. oxysporum* (Simoes *et al*., 2009).

Table 1. Average infection% of fungi isolated from postharvest infected fruits collected from different markets of Swabi.

No.	Common name	Botanical name	Pathogen	Average of infection %	Standard deviation	Collection market
1.	Melon	*Cucumis melo* L.	*Alternaria alternata*	64.33	2.081	Swabi
			Fusarium solani	98.33	1.527	
			Geotrichum candidum	32.66	0.577	Jalsai
			F. solani	66	1.0	Ghazi
			Cladosporium sp.	35.66	3.785	
2.	Apple	*Malus domestica*	*A.alternata*	67.33	1.154	Swabi
			Aspergillus flavous,	32.66	0.577	
			Penicillium expansum	99.66	0.577	Jalsai
			A. flavous	33.33	0.577	Ghazi
			Penicillium sp.	99.33	0.577	
3.	Guava	*Psidium guajava* L.	*A. alternata*	99	1.00	Swabi
			Drechslera sp.	99.66	0.577	
			Nil	0.00	0.00	Jalsai
			Phytopythora	65.66	0.577	Ghazi
4.	Sweet orange	*Citrus sinensis* Osbeck	*Penicillium* sp.	99.66	0.577	Swabi
			Nil	0	0.00	Jalsai
			Penicillium sp.	99.33	1.154	Ghazi
5.	Banana	*Musa* sp.	*P. notatum*	65.33	1.154	Swabi
			Alternaria sp.,	34	1.00	
			Nil	0	0.00	Jalsai
			P. notatum	35	2.645	Ghazi
6.	Lemon	*Citrus limon*	Nil	0	0	Swabi
			Nil	0	0	Jalsai
			Fusarium solani	66	2.0	Ghazi
7.	Pomegranate	*Punica granatum*	*Alternaria* sp.	99.33	0.577	Swabi
			Botrytis sp.	33.33	0.577	Jalsai
			Penecillium sp.	67.66	1.527	Ghazi
			Fusarium sp.	35.66	3.055	
8.	Grapes	*Vitis vinifera*	*Penecillium notatum*	64.66	4.163	Swabi
			Nil	0	0	Jalsai
			Botrytis sp.	67	2.645	Ghazi
9.	Persimmon	*Diospyros kaki*	*Fusarium* sp.	35.66	3.055	Swabi
			Fusarium sp.	33.33	0.577	Jalsai
			Alternaria sp.	66.66	1.154	Ghazi
10.	Ber	*Ziziphus mauritiana*	*Penecillium notatum*	65	2.645	Swabi
			Fusarium sp.	33.33	0.577	Jalsai
			Penecillium notatum	34.66	1.527	Ghazi

Table 2. Average percent colonization of fungi isolated from postharvest infected fruits collected from different markets of Swabi.

No.	Common name	Botanical name	Pathogen	Average	Standard deviation	Collection market
1.	Melon	*Cucumis melo* L.	*Alternaria alternata*	39.33	0.768	Swabi
			Fusarium solani	41.33	0.509	
			Geotrichum candidum	19.66	0.693	Jalsai
			F. solani	40.33	0.693	Ghazi
			Cladosporium sp.	20.33	0.577	
2.	Apple	*Malus domestica*	*A.alternata*	54.00	0.577	Swabi
			Aspergillus flavous	25.00	0.509	
			Penicillium expansum	66.33	1.154	Jalsai
			A. flavous	26	0.577	Ghazi
			Penicillium sp.	80	0.192	
3.	Guava	*Psidium guajava* L.	*A. alternata*	72.66	0.693	Swabi
			Drechslera sp.	25.66	0.00	
			Nil	0	0.577	Jalsai
			Phytopythora	53	1.00	Ghazi
4.	Sweet orange	*Citrus sinensis* Osbeck	*Penicillium* sp.	81	0.00	Swabi
			Nil	0	0.509	Jalsai
			Penicillium sp.	65.66	1.00	Ghazi
5.	Banana	*Musa* sp.	*P.notatum,*	41	0.508	Swabi
			Alternaria sp.	19.66	0	
			Nil	0	0.192	Jalsai
			P. notatum	19.66	0	Ghazi
6.	Lemon	*Citrus limon*	Nil	0	0	Swabi
			Nil	0	1.575	Jalsai
			Fusarium sp.	52.33	1.527	Ghazi
7.	Pomegranate	*Punica granatum*	*Alternaria* sp.	65.00	0.00	Swabi
			Botrytis sp.	20.00	0.509	Jalsai
			Penecillium sp.	52.66	0.577	Ghazi
			Fusarium sp.	32.00	0.509	
8.	Grapes	*Vitis vinifera*	*Penecillium notatum*	79.66	0.00	Swabi
			Nil	0.00	0.192	Jalsai
			Botrytis sp.	80.33	1.00	Ghazi
9.	Persimmon	*Diospyros kaki*	*Fusarium* sp.	65.00	0.769	Swabi
			Fusarium sp.	64.66	1.730	Jalsai
			Alternaria sp.	80.00	1.575	Ghazi
10.	Ber	*Ziziphus mauritiana*	*Penecillium notatum*	53.66	0.192	Swabi
			Fusarium sp.	20.33	0.509	Jalsai
			Penecillium notatum	19.66	0.508	Ghazi

Table 3. Enzyme responsible for cell wall degradation isolated from spoilage fungi cultures on different fruits peel.

Fruits peel	Fungi isolated	Units / 100 ml			
		PGase	Xylanase	Cellulase	Amylase
Mellon	*Fusarium solani*	878 ± 15	110 ± 2	21 ±12	22 ± 1
Apple	*Penicilium* sp.	2930 ± 61	166 ± 12	31± 2	70 ± 3
Guava	*Alternaria alternata*	812 ± 26	713 ± 27	40 ± 3	206 ± 4
Sweet orang	*Penecilum* sp.	530 ± 4	518 ± 51	169 ± 4	25 ± 1
Banana	*Penecilium* sp.	824 ± 15	2876 ± 50	65 ± 2	40 ± 1
Lemmon	*Fusarium solani*	1173 ± 31	325 ± 15	129 ± 13	26 ± 2
Pome granate	*Alternaria alternata*	390 ± 10	225 ± 20	58 ± 2	35 ± 2
Grapes	*Botrytis* sp.	210 ± 11	152 ± 11	67 ± 3	47 ± 2
Persimon	*Alternaria alternaria*	1075 ± 32	113 ± 5	89 ± 5	48 ± 3
Ber	*Penicilium notatum*	567 ± 25	122 ± 6	57 ± 4	17 ± 1

Each value represent the three runs of ± S.E

Conclusion

The area under consideration (District Swabi) and nearby areas are very well known for the production of various fruits and vegetables. However, the fruits were infected with different pathogenic fungi that are responsible to damage the fruits at market place. It is because not having much awareness on the identification of disease causing agents and precaution that can prevet the such huge losses. Therefore, a tremendous need to manage and develop a strategy to control these effects, to reduce the fungal pathogen affect.

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