International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 25-01-2021; Accepted: 22-02-2021: Published: 25-02-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 626-630

Management of postharvest fungal rot of apricot, *Prunus Armeniaca, marsh* in Kashmir valley, India

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Abstract
The relatively short shelf life caused by pathogen attacks is one of the limiting factors that affect the fruits economic value. The estimated degradation by pathogenic agents in post-harvest care is between 20-25 percent of fruits. Management technique for the control of pathogenic fungi has been used for various purposes, including cultural, physical, chemical, biological and regulatory approaches. In terms of environmental and economic concerns, organic and traditional fruit processing systems, biological regulation would seem to have a major potential. The purpose of this research is biocontrol agents (Trichoderma sps) for the control of Apricot fruit postharvest diseases caused by various fungi known worldwide, e.g. Alternaria alternate, Aspergillus flavus, and Penicillium expansum. Biological control has been proposed as an effective, safe strategy for monitoring major fruit declines post harvests and improving crop production. Several plants and plant products have been used for plant disease control and have been found to be harmless and non-toxic. The methodology used in this research is Dual culture technique that analysed the efficacy of the biochemical agent against phytopathogens under laboratory conditions and measured the results of percentage inhibition of the pathogen by the bioagent. The conclusion of this study revealed that the Trichoderma sp caused significant inhibition of the colony diameter of fungal pathogens.

Keywords: apricot, fungal attack, colony diameter, biocontrol strategy, storage

Introduction

Apricot (Prunus armeniaca L.) is a member of the Rosaceae family. Rosaceae are one of the largest angiosperm families, with about 3,400 species in temperate northern parts of the World, including almonds, fish, pommes, feather, cherries and berries. It is a temperate fruit grown in well-differentiated seasonal climates. According to FAO statistics (2010), the world's largest producers are Turkey and Iran accounting for 21.6% and 14.7% of world apricot production respectively, followed by Pakistan, Uzbekistan, Italy, Algeria, Japan, Morocco, Egypt and Spain. Apricot is a carbohydrate-rich commodity among stone fruits and is a good source of fibres, minerals and vitamins. These fruits are attacked by many fungal and bacterial pathogens causing heavy losses to the growers. (Parpia, 1976; Agarwal and Sharma, 1968; Abbas *et al*., 1981) [1, 1, 4, 1]. Several fungal pathogens are responsible for decaying of fruits both under field and storage conditions. These rot causing fungi are; *Alternaria alternata*, *Glomerel lacingulata*, *Penicillium expansum*, *Monilinia*spp. *Botrytis cinerea* and *Rhizopus stolonifer* causing a considerable damage to these fruits. (Hall, 1971; Baxler*etal*., 1974; Ogawa*et al*., 1975; Tamm*et al*., 1995; Leponis*et al*., 1987) [2, 2, 2, 2, 2]. Several management strategies such as cultural, physical, chemical, biological and regulatory methods have been used for the control of pathogenic fungi. Several biocontrol agents such as bacteria and fungi have been identified for the purpose of controling post-harvest diseases of a large number of berries, and have been commercialized worldwide. (Wisniewski and Wilson, 1992; Ragsdale and Sisler, 1994; Montesinos, 2003; Sobowaleet al., 2008; Montesinos and Bonaterra, 2009; Junaidet al., 2013) [2, 2, 2, 2, 2, 2, 2, 2]. Biological control has been proposed to control major fruit degradation after harvest and increase crop production as an efficient and safe strategy.
(Janisiewicz and Kerstin, 2002; Dalal and Kulkarni, 2013) [3, 20].

Therefore, the present study was carried out with the main objective of identifying the fungal rot pathogen that causes decaying in apricot and the control measures by using local isolate of *Trichoderma* sp. under storage conditions in Kashmir Valley.

Materials and Methods

To evaluate the fungi which cause decay and rotting of apricot fruits. These fruits were collected in a polythene bags from godowns, markets and storage houses. These samples were either used immediately or stored at 10ºC in the laboratory for different pathological studies. Various sterilization techniques physical as well chemical methods were carried out for the removal of potentially infective microbes. Under physical methods Autoclaving were carried out to destroy micro-organisms at the temperature of 121^0C or 15lb pressure for 15 to 20 minutes. In chemical methodology sterilization was done by using various chemicals like 70% ethanol, mercuric chloride (1-3%), Sodium hypo chloride (3%). The media used for the isolation, culturing and propagation of soil fungi in the present study was Potato dextrose agar (PDA) and Richard's synthetic agar medium. Isolation were carried out from surface infected disinfected apricot fruits done by 1% sodium hypo chloride solution. Small diseased fruit tissues were then inoculated on plates containing PDA medium. Petri plates were incubated at 25±2ºC in an incubator. After 8 days of incubation the fungal pathogens were identified by cultural, and microscopic characteristics. (5), (6). In order to create a pure culture of each isolate, individual colonies from mixed and mother cultures were subculturated on the fresh PDA plates. The pure culture was isolated for further identification through lactophenol cotton blue staining technique. The fungi are classified on the basis of colonial characteristics and morphology. Blue cotton and lactophenol were used to make fungal slides. These slides were observed under microscope and identification was done by monographs and relevant literature. The dual culture technique method was used to study the efficacy of the biocontrol agent against phytopathogens under laboratory conditions.

In the present study an attempt was made to study the effect of isolated local isolate of *Trichoderma* sp. under *in vitro* conditions for the control of *Penicillium* rot of apricot caused by *Penicillium expansum*, *Aspergillus* rot of apricot caused by *Aspergillus niger*,

***Alternaria* rot of apricot caused by *Alternaria alternata*.**

Table 1: Potato dextrose agar (PDA)

Compound	Quantity
Potato	250 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Table 2: Richard's synthetic agar medium

Compound	Quantity
Potassium nitrate	10 g
Potassium dihydrogen phosphate	5 g
Magnisium sulphate	2.5 g
Ferric choloride	0.02 g
Sucrose	50 g
Agar	15 g
Distilled water	1000 ml

Raising and Maintainance of Pure Culture

During isolation from petriplates, pure culture of the fungi was on PDA medium. In order to establish the fungi of interest, isolation was performed to isolate the strain from a mixed population. Spores or mycelia or agar blocks comprising these structures have been converted into plateaux with solidified agar media aseptically. During 5-7 days, the plaques were incubated and fungal growth observed.

Dual culture technique

The efficacy of the biocontrol agent against phytopathogens under laboratory conditions was studied using this approach. PDA medium was prepared in this process and then medium (20 ml) was poured into sterilized petriplates (9 cm diameter) and medium was allowed for 15 minutes to solidify at room temperature. (Plate 3). Pathogen and bioagent (antagonist) strains were moved aseptically to the periphery of the medium containing petriplates opposite each other.

In the medium containing petriplates, which acted as control, the pathogen was inoculated alone. In the incubator, the inoculated petriplates were transferred and incubated at 25+20C.

The growth of the pathogen and antagonist was observed to test colony growth in each petriplate. By using the following formula, the percent inhibition of the pathogen by the bioagent (antagonist) was calculated:

I = C – T/C x 100

Where,

I = Percent inhibition of mycelial growth.

C = Colony growth of pathogen in control.

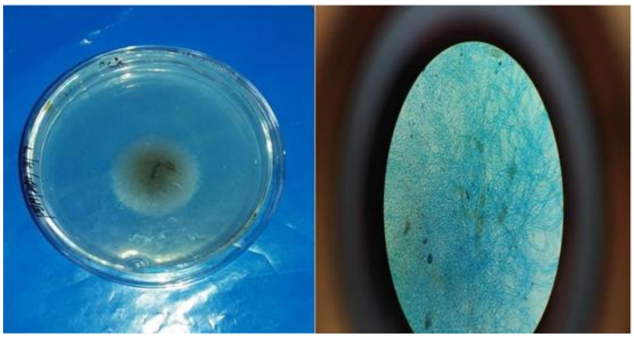
T = Colony growth of pathogen in dual culture

Results

In the present study the casual pathogens infecting apricot fruits were identified as *Penicillium expansum* Link ex Thorn and *Aspergillus niger* Van Tiegh resulting in blue mold or *Penicillium* rot of apricot and black mold or *Aspergillus* rot of apricot and *Alternaria alternata* resulting in core rot or black spot of apricot. These two fungi were identified on the basis of symptoms caused by the fungus on apricot fruits and on the basis of cultural and microscopic characteristics. On PDA, the alternaria fungus created profuse mycelial growth. The colonies of Alternaria were black or greyish, circular with an entire margin and floccose (Plate-1 Fig.a). Under microscopic characters Conidiophores, 25-60µmin in length, brown in colour were seen. Individual conidiophores derive directly from a substrate that forms bushy heads made up of chains of conidia. The conidia are pale brown to light brown, with a short conical beak at the tip, or 20-63 µm in height, without a beak. There are several vertical and transverse septas. (Fig. b of Plate-1). Aspergillus flavus has shown rapid colonial growth rate and texture varying from downy to powdery base. The colony color is initially white with the conidial development being black. The margins of the colony are entire. (Fig. c of Plate-1). Under microscopic characteristics septate and hyaline are hyphae. The conidial heads are broad in, globose, dark brown in colour. Conidiophores are smooth walled, hyaline, about 550µm long in length, darker towards the apex and ending in globose vesicles of about 58µm in diameter in size (Plate-1 Fig. d). The colony of Penicillium appears white on Potato Dextrose Agar (PDA) medium, at first velvety and then turns bluish green due to the development of conidiophores and conidia. (Fig. a of Plate-2). Microscopic observation revealed that mycelium is septate with about 48 µm-120µm long branched conidiophores, terverticilate (two stage branching) with three branch forms, *viz*. Stipe, Phialides, and Metula. Metulae have a length of 3.7µm to 7.8µm. Metulae form sterigmata or phialides, called conidiogenous cells. Phialides have a length of 3.5µm-7.5µm and give rise to ellipsoid or globose conidia with a diameter of 1.48µm-3.7µm. (Fig.b of Plate-2).

Plate 1

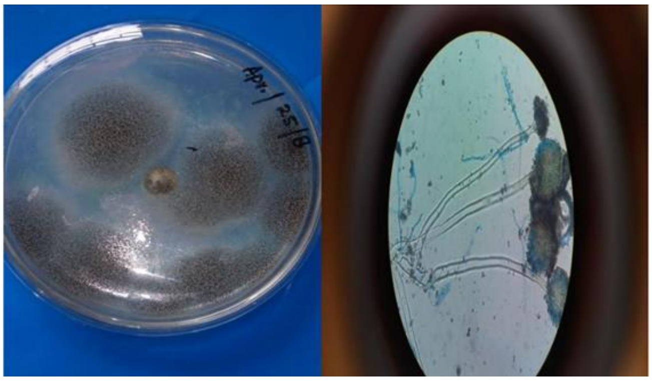
Alternaria alternata



a b

Fig 1: a. Showing culture on PDA. Fig. b. Showing conidia

Aspergillus flavus

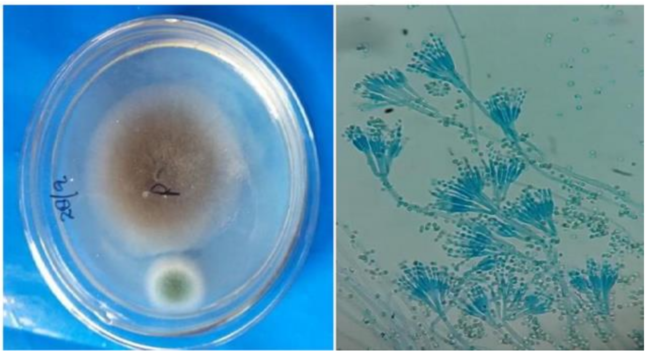


c d

Fig 2: C. showing culture on PDA. Fig. d. Conidiophore and conidia of Aspergillu flavus

Plate 2

Penicillium expansum

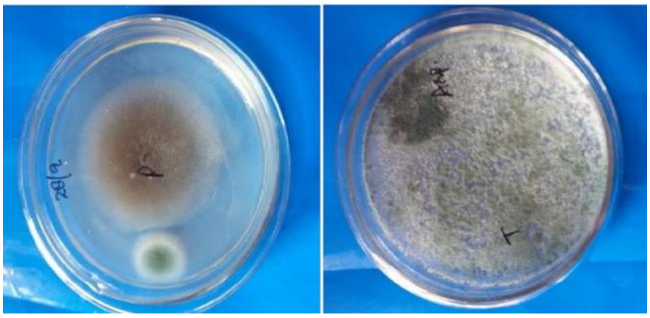


a b

Fig 3: a. Showing Culture on PDA. Fig. b. Conidiophore and conidia of *Penicillium expansum*

Plate 3

Dual culture technique



c d

Fig 4: c. Dual culture of *Pancillium expansum* and *Trichoderma* Fig. d. Dual culture of *Aspergillus flavus* and *Trichoderma.*

Control measures

Control of rot of apricot by local isolate of *Trichoderma* sp.

An attempt was made during the present study to verify the efficacy of local Trichoderma sp. isolate. Isolated against pathogenic fungi from apricots, *viz*. Expansion of *Alternaria alternata and Penicillium expansum* and *Aspergillus flavus* that cause rot in apricots.

Effect of *Trichoderma* isolate on colony diameter of *Alternaria alternata.*

The findings (Table 1) showed that the Trichoderma isolate used during the present study induced a major inhibition of Alternaria alternata's colony diameter. The highest colony-diameter inhibition was recorded after 7 days of incubation. The percentage inhibition in colony diameter was recorded as 22.22 percent after 3 days of inoculation, 29.09 percent after 5 days of inhibition and 31.00 percent after 7 days of percentage inhibition in colony diameter. (Table 1, Fig. a of Plate-1).

Table 3: Effect of *Trichoderma* sp. on colony diameter of *Alternaria alternata:*

Days	after	Colony	diameter	of	Colony diameter of		Percentage inhibition
inoculation		pathogen in control			pathogen	in	of colony diameter
					interaction		
3 days		13.5			10.5		22.22
5 days		27.5			19.5		29.09
7 days		41.5			28.5		31.00

Data are the mean of three replicates

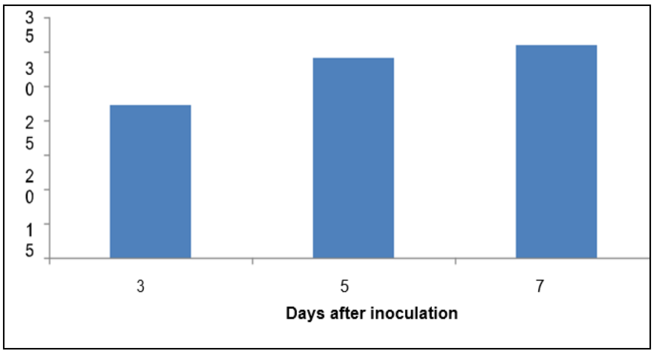


Fig 5: *Percentage inhibition in colony diameter of Alternaria alternata by Trichodermasp*

Effect of *Trichoderma* isolate on colony diameter of *Aspergillus flavus.*

From the results (Table 2), it was shown that the Trichoderma isolate used during the present study induced a major inhibition of the Aspergillus niger colony diameter. The highest inhibition was recorded in colony diameter after 7 days of incubation. The percentage inhibition in colony diameter was recorded as 18.18 percent after 3 days of inoculation, 25.49 percent after 5 days of inhibition and 34.62 percent after 7 days of percentage inhibition in colony diameter (Table 2, Plate-1 Fig. c)

Table 4: Effect of *Trichoderma* sp. on colony diameter of *Aspergillus flavus.*

Days	after	Colony	diameter	of	Colony diameter	of	Percentage inhibition
inoculation		pathogen in control			pathogen	in	of colony diameter

interaction							
3 days		12.00			5.50		18.18
5 days		25.50			19.00		25.49
7 days		39.00			25.50		34.62

Data are the mean of three replicates

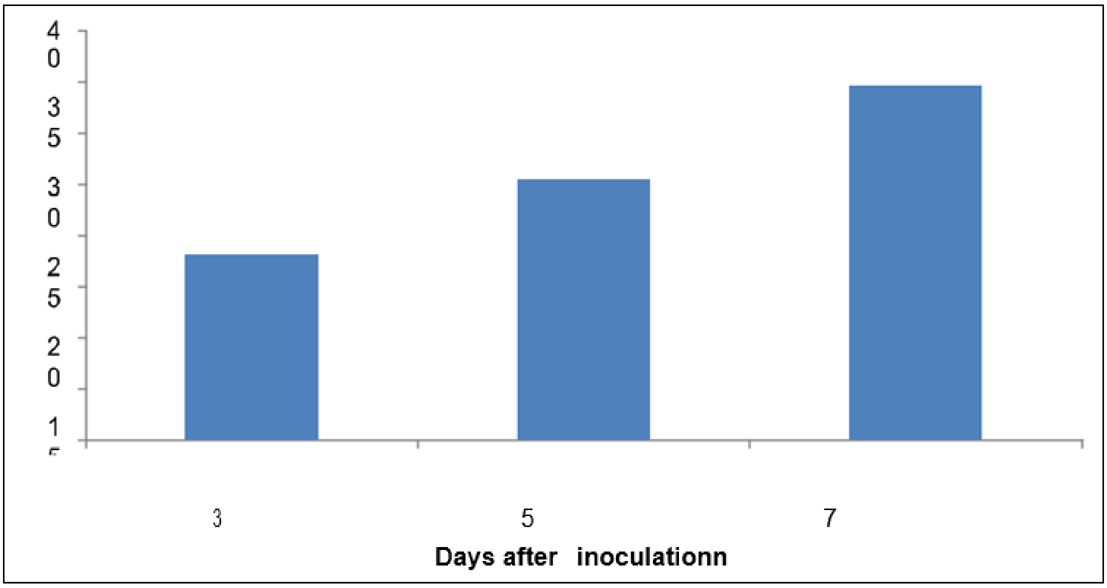


Fig 6: Percentage inhibition in colony diameter of *Aspergillusflavus* by*Trichoderma sp.*

Effect of *Trichoderma* isolate on colony diameter of *Penicillium expansum.*

The findings (Table 3) showed that the Trichoderma isolate used during the current study induced a major inhibition of the Penicillium expansum colony diameter. The highest colony-diameter inhibition was recorded after 7 days of incubation. The percentage inhibition in colony diameter was recorded as 219.23 percent after 3 days of inoculation, 24.44 percent after 5 days of inhibition and 41.11 percent after 7 days of percentage inhibition in colony diameter. (Table 3, Fig. d of Plate-2).

Table 3: Effect of *Trichoderma* sp. on colony diameter of *Penicillium expansum.*

Days inoculation	**after**	**Colony pathogen in control**	**diameter**	**of**	**Colony diameter pathogen**	**of in**	**Percentage inhibition of colony diameter**
interaction							
3 days		13.00			10.50		19.23
5 days		22.50			17.00		24.44
7 days		45.00			26.50		41.11

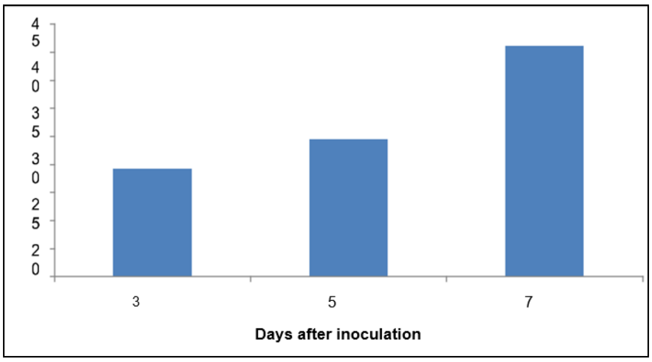


Fig 7: Percentage inhibition of colony diameter of *Penicillium expansum* by *Trichoderma* sp.

Discussion and Conclusion

The present work revealed that Penicillium expansum, Alternaria alternata and Aspergillus flavus are causative agents of apricot fruit deterioration. Several workers have confirmed the occurrence of fungal rot of apricot and other fruits by different fungi throughout the globe. The current study also found that Penicillum expansum causes apricot blue mold, Alternaria alternata causes apricot core rot (Combrinket al., 1985a; De Kocket al., 1991; Ntasiouet al., 2015) [3, 3, 3]. Cherry black spot disease (Zhao and liu, 2012). Aspergillus flavus causes apricot core and moldy rot (Gaoet al., 2013). Attempts were made during the present study to verify the effectiveness of the local isolate of Trichoderma sp. Isolated against pathogenic fungi from apricots, *viz*. Extension of Alternaria alternata and Penicillium that cause apricots to rot. The current investigation revealed that the Trichoderma isolate induced major inhibition after (7) days of the colony diameter of Penicillium expansum compared to Aspergillus flavus and Alternaria alternata. Our outcome is in line with Sobowalaet al. (2008), who identified Trichoderma as successful in controlling many diseases caused by fusarium verticollides, such as maize stem rot. The present study will help to establish a management plan by integrating the use of these indigenous Trichoderma sp into the apricots' integrated pest management strategy.

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