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Efficacy of different bio-agents against major postharvest pathogens of grape under *in vitro conditions*

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Abstract
The present study was to determine the efficacy of bio-agents against postharvest diseases of grapes. The agents *Trichoderma, Bacillus, Pseudomonas* and yeast isolates were individually screened against postharvest pathogens including *Aspergillus niger* and *Penicillium* spp. The fungal antagonist *Trichoderma harzianum* was the most effective, inhibiting the mycelial growth of *Aspergillus niger* and *Penicillum* spp. by 71.55 and 80.55 per cent respectively followed by *Trichoderma viride* (63.66 and 67.22) per cent respectively.

Keywords: *Aspergillus niger*, *Penicillum* spp. *Trichoderma* and grapes

Introduction
Grapevine (*Vitis vinifera* L.) is one of the most important fruit crop in the world, but between 20 to 30% of fresh grapes are lost every year due to inadequate postharvest storage (El-Ghouth and Wilson, 1995) [2]. It is widely grown fruit crop in the world and originated in Asia minor, Iran, Afghanistan, Caucasus in Russia (Khanduja, 1974) [3]. The fresh grapes are one of the most delicious, refreshing and nourishing fruits which is good source of sugars, carbohydrates, vitamins, proteins and minerals. They are used for table purpose, wine, juice, raisins and canning fresh as well as dried fruits. However, grape is grown in India mostly for table purpose. It is dollar earning crop as India is fast emerging as one of the major grape exporting countries in the world. Generally Grapes require a hot and dry climate during its growth and fruiting periods. It is successfully grown in areas where the temperature ranges from 15-40^{0}C. Grape is prone to a number of fungal, bacterial and viral diseases which significantly affect its quality and production. However, fungal diseases inflict huge losses to the crop. Grapes are highly susceptible to post harvest spoilage because of highly perishable in nature.
During the storage conditions numbers of fungi are known to cause spoilage of grape berries. Under tropical conditions considerable losses are occurred due to rots caused by *Penicillium* spp., *Aspergillus* spp. *Curvularia* spp., *Alternaria alternata* and becoming limiting factor for successful viticulture (Thomas, 1986) [5].

Material and Methods
Isolation of pathogens associated with postharvest diseases of grape
The microorganisms responsible for spoilage of grapes were isolated on PDA medium by employing tissue isolation method from diseased berries collected from rahuri market.The infected portion of berries were cut into small pieces. These pieces were disinfected by surface sterilization with 0.1 percent mercuric chloride solution for 30 seconds followed by three washings with sterilized water in order to remove traces of corrosive sublimate. These small pieces of infected tissues were transferred aseptically to sterilized petri plates containing PDA medium (3 to 4 pieces/plates).These plates were then incubated at room temperature (27^{o}C) for seven days. The plates were critically observed for the typical growth of the fungus. The fungal colonies showing different colouration and sporulation were separated and sub-cultured in separate plates by single spore isolation method. The fungal colonies were then transferred on PDA slants for further investigation.

In vitro evaluation of antagonists against postharvest diseases of grape
Trichoderma viride, T. harzianum, P. fluorescens, B. subtilis and yeast strain-I, yeast strain- II, were tested for their efficacy against postharvest diseases by the dual-culture technique

(Dennis and Webster, 1971) [1] In PDA. Three replications were done with each antagonist. Suitable control was maintained by placing only the pathogen on culture medium. The plates were incubated at 28±2°C. Petri plates were observed daily for recording antagonistic interactions between the pathogen and bio-control agent. The per cent inhibition (I) of the test pathogen was calculated when the growth of the pathogen was full in the control plate by using the formula as given below

$$I\ \% = C - T / C \times 100$$

Where

I = Inhibition of pathogen growth

C= Pathogen growth in control

T= Pathogen growth in treatment

Results and Discussion

Effect of bio-control agents against mycelial growth of postharvest pathogens of grape

The results revealed in the Table 1, among all the treatments, The fungal antagonist *Trichoderma harzianum* was the most effective, inhibiting the mycelial growth of *Aspergillus niger* and *Penicillum* spp. by 71.55 and 80.55 per cent respectively followed by *Trichoderma viride* (63.66 and 67.22) per cent respectively. Whereas least inhibition was observed in Yeast strain-I (7.44 and 25.44 per cent respectively).Similar findings were made by Ramu Senthil *et al*. (2011) [4] who reported that *T. harzianum* inhibited the growth of the postharvest rots of grapes.

Table 1: Efficacy of bio agents against postharvest pathogens of grapes *in vitro*

Treatments	*Aspergillus niger*		*Penicillium* spp.	
	Radial growth of the pathogen (mm)	Inhibition over Control (%)	Radial growth of the pathogen (mm)	Inhibition over Control (%)
Trichoderma viride	32.7	63.66	29.5	67.22
Trichoderma harzianum	25.6	71.55	17.5	80.55
Bacillus subtilis	80.8	10.22	65.9	26.77
Pseudomonas fluorescens	85.2	5.33	87.9	2.33
Yeast strain-I	83.3	7.44	67.1	25.44
Yeast strain-II	88.1	2.11	82.6	8.22
Control	90	00.00	90	00.00
S.E.(±)	0.075		0.138	
C.D @ 1 %	0.229		0.420	

Plate 1: *In vitro* evaluation of bio-control agents on growth of *Aspergillus niger*

T_1: *Trichoderma viride*
T_2: *Trichoderma harzianum*
T_3: *Bacillus subtilis*
T_4: *Pseudomonas fluorescens*
T_5: Yeast strain-I
T_6: Yeast strain-II
T_7: Control

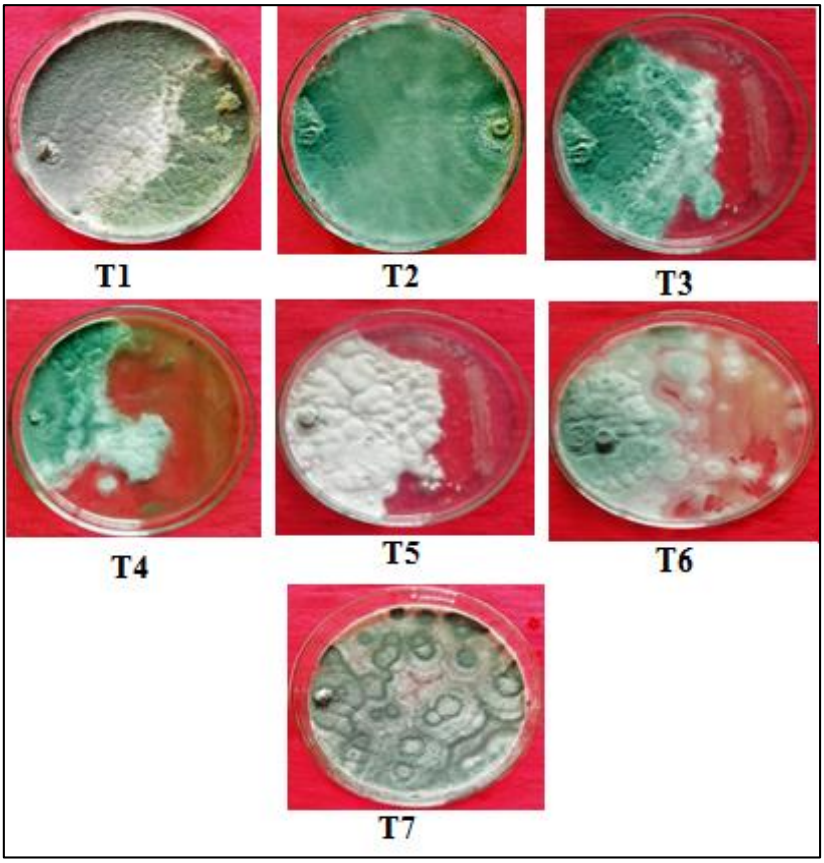

Plate 2: *In vitro* evaluation of bio-control agents on growth of *Penicillium* spp

T1: *Trichoderma viride*
T2: *Trichoderma harzianum*
T3: *Bacillus subtilis*
T4: *Pseudomonas fluorescens*
T5: Yeast strain-I
T6: Yeast strain-II
T7: Control

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