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BIOLOGICAL CONTROL OF *PENICILLIUM EXPANSUM* DURING STORAGE OF PEARS USING SOME MICROORGANISMS

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ABSTRACT

Total sixteen microorganisms were tested antagonistically to fludioxonil resistant (Pe-EMS-11) of *Penicillium expansum* (blue mold of pear) when tested individually and in mixture with fludioxonil fungicide. *In vitro Bacillus subtilis* gave higher inhibition zone (29.20) and others also inhibited the growth of *Penicillium expansum. In vivo* the culture filtrates of sixteen microorganisms were tested individually and in mixture with fludioxonil resistant (Pe-EMS-11) of *Penicillium expansum. Bacillus subtilis* gave significant PCE individually and followed by other microbes PCE ranged from (0.61-65.80) while in mixture with fludioxonil the highest PCE showed *Bacillus subtilis* and followed by other microbes ranged from (15.05-80.00). In mixture with fludioxonil the PCE increased as compared to individual. *Bacillus subtilis, Pseudomonas fluorescens,* and *Trichoderma harzianum, T. viride* have more effective for the controlling of *Penicillium expansum*.

KEYWORDS: Pear, blue mold, fludioxonil, *Penicillium expansum*, biocontrol agents.

INTRODUCTION

Pear (*Pyrus communis* L.) belongs to Rosaceae family are stored after harvest to provide nutritious fruit throughout the year.[5] More than 90 fungal species have been described that cause decay of pears during storage[8], but postharvest fungal diseases of pears are caused by *Penicillium expansum.*[14] Postharvest treatment of fruits by fungicides is traditional method of blue mold control. The residue of fungicides on agricultural products, concerns about human health and environmental pollution are the reason of found alternatives. Biological control using some antagonistic microorganisms is the safe method for control of postharvest

pathogens.[17,13,6] To minimize the chemical application biological management of plant pathogens is advocated now and then. The idea came through the first experiment by Millard and Taylor (1927) who showed that potato grown in sterilized soil and inoculated with *Streptomyces scabies* with *Streptomyces praecox*. Prior to this Sanford (1926) placed the hypothesis, saprophytic microorganisms can control the activity of plant pathogens and the microbiological balance of soil can be altered through the addition of fresh organic matter or other agronomic treatment for the management of pathogenic organisms.[2,3] Importance of *Trichoderma* is so increased that 113 species are known all over the world. Of these 13 species are reported in India.[10] Nearly 32 crop diseases have been controlled by different species of *Trichoderma* reported by Ade *et al*., (2005). Population dynamics of biocontrol agents and plant pathogens have very well been discussed by Paulitz and Belangr, (2001). Similarly, Suryawanshi and Gangawane, (1998) *Xanthomonas oxonopodis pv. citri.* managed with *Trichoderma viride* and *Bacillus subtilis.* Integrated management of web blight (*Rhizoctonia solani*) of urd and mung bean was controlled by applying *Gliocladium virens* and extracts of *Pongeamia glabra,* fungicide carboxin and *Rhizobium* as foliar spray and found that disease intensity was reduced up to 93.7%. Similarly many reports are available on use of different bacteria. These bacteria are various species of *Bacillus, Enterobacter* and *Azotobactor.* Interestingly use of *Pseudomonas solanacerum, Pseudomonas auruginosa, Pseudomonas aureofaciens* and *Pseudomonas fluorescens* for the biological control of different pathogens is increasing day by day.

MATERIALS AND METHODS

Post-harvest pears were collected from different local markets of Maharashtra, India. The antagonistic microorganisms were isolated from pear. Approximately 20gms of fruit sample were added with100 ml of sterile distilled water in 500 ml conical flask and incubated for 10 mins. The resultant suspension 0.1 ml was used to incubate at PDA and nutrient agar plates. All plates were incubated at room temperature 28±2ºC and 2 days microbial colonies were picked and restreaked until the pure culture was obtained. The isolates were maintained on PDA and nutrient agar slants for routine use. Total of 16 microorganisms were selected for this study. 23 isolates of *Penicillium expansum* from pear fruits were tested against fludioxonil on agar plates and on fruits. The food poisoning techniques[11] used for testing microorganisms effect on *Penicillium expansum*. For detection of antagonistic activity of bacterial isolates towards the growth of *Penicillium expansum* streak plate or cup plate methods were used. For the traditional streak method 40mm streak was made from 24 hours

old culture of bacteria, 23mm away from the center of petri dish. Using a 7mm diameter sterile cork borer, the growing edge of 7 days old fungal culture was aseptically cut and placed at the middle of the plates already inoculated with the test antagonistic plates were incubated at 28±2ºC and monitoring for 7 days. Measurement of the inhibition zone in mm and determine the percentage control efficacy.

RESULTS AND DISCUSSION

Total 16 microorganisms were tested, nine were antagonistic to fludioxonil resistant (Pe-EMS-11) of *Penicillium expansum*. *Bacillus subtilis* gave higher inhibition zone (29.20) followed by *Pseudomonas fluorescens, Corynebacterium diptheriae, Pseudomonas solanacearum, Fusarium oxysporum, Trichoderma harzianum, Trichoderma viride, Bacillus coagulans* in decreased order (zone of inhibition 16.00-23.22) while other microorganisms were tested and found negative results (Table 1). *In vivo* the culture filtrates of sixteen microorganisms were tested individually and in mixture with fludioxonil resistance (Pe-EMS-11) *Penicillium expansum*. The PCE ranged from (0.61-65.80) while in mixture with fludioxonil the PCE range from (15.05-80.00) in order. *Bacillus subtilis* (65.80) gave significant PCE individually and followed by *Bacillus coagulans, Trichoderma viride, Pseudomonas fluorescens, Trichoderma harzianum, Pseudomonas solanacearum, Fusarium oxysporum, Botrytis cinerea, Corynebacterium diptheriae, Aspergillus niger, Aspergillu stamari, Aspergillus flavus, Proteus vulgaris, Proteus mirabilis* and *Aspergillus swydowii* while in mixture with the highest PCE of *Bacillus subtilis* (80.00) and followed by other microbes ranged from (15.05-80.00). In mixture with fludioxonil the PCE increased as compared to individual. Therefore, in fludioxonil resistant biological control culture filtrates of some microorganisms were significant for the management of blue mold caused by *Penicillium expansum*. *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma viride* and *Trichoderma harzianum* have more antagonistic to the *Penicillium expansum* (Table 2). Similar results was also reported by Giuseppe Lima *et al.*, (2006) fungal decay on apple treated twice in semi commercial condition with the biological yeasts *Rhodotorula glutinis, Cryptococcus lawrentii, Aureobacterium pullulans* used against *Botrytis cenerea* and *Penicillium expansum* for the management of apple diseases. Kreuawan Thonglem *et al.*, (2007) reported that *Penicillium digitatum* is the major cause of green mold disease in orange, the strain *Bacillus pumilus* isolated from healthy orange can inhibit the growth of *Penicillium digitatum in vitro*, thus contributing the literature on biological control of this

pathogen. And also further evidences that antagonistic microorganisms are good sources of potential biocontrol agents against plant pathogenic fungi.

Table 1: Antagonistic effect of some microorganisms against fludioxonil resistant (Pe-EMS-11) of *Penicillium expansum* on agar plate.

Sr. No.	Micro-organisms	Inhibition zone (mm)
1.	*Aspergillus flavus*	0.00
2.	*Aspergillus niger*	0.00
3.	*Aspergillus swydowii*	0.00
4.	*Aspergillus tamarii*	0.00
5.	*Botrytis cinerea*	17.75
6.	*Fusarium oxysporum*	21.50
7.	*Trichophyton mentagrophytes*	0.60
8.	*Trichoderma harzianum*	19.50
9.	*Trichoderma viride*	18.75
10.	*Bacillus subtilis*	29.20
11.	*Bacillus coagulans*	17.25
12.	*Corynebacterium diptheriae*	23.15
13.	*Pseudomonas fluorescens*	24.50
14.	*Pseudomonas solanacearum*	22.20
15.	*Proteus vulgaris*	0.00
16.	*Proteus mirabilis*	0.00
17.	Fludioxonil (µg/ml)	26.0
18.	Control	61.05
	S.E.	5.833
	C.D. at 0.05	10.702
	0.01	15.35

*Means of three replications.

Table 2: Antagonistic effect of some microorganisms against fludioxonil resistant (Pe-EMS-11) of *Penicillium expansum* on pear.

Sr. No.	Micro-organisms	Percentage Control Efficacy (PCE)	
		Individual	Mixture with Fludioxonil
1.	*Aspergillus flavus*	3.50	22.75
2.	*Aspergillus niger*	10.75	30.35
3.	*Aspergillus swydowii*	0.61	23.50
4.	*Aspergillus tamarii*	6.50	15.05
5.	*Botrytis cinerea*	25.75	42.20
6.	*Fusarium oxysporum*	29.38	49.36
7.	*Trichophyton mentagrophytes*	7.90	18.80
8.	*Trichoderma harzianum*	42.98	50.00
9.	*Trichoderma viride*	46.79	60.75
10.	*Bacillus subtilis*	65.80	80.00
11.	*Bacillus coagulans*	60.61	66.00

12.	*Corynebacterium diptheriae*	24.46	31.90
13.	*Pseudomonas fluorescens*	44.08	68.81
14.	*Pseudomonas solanacearum*	41.86	61.30
15.	*Proteus vulgaris*	1.50	27.80
16.	*Proteus mirabilis*	1.75	33.50
17.	Fludioxonil (µg/ml)	52.75	--
	S.E.	1.906	5.251
	C.D. at 0.05	3.806	11.605
	0.01	3.981	13.967

*Means of three replications.

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