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Post Harvest Biological Control of Citrus Fruit Rot

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ABSTRACT

***Bacillus sphericus* and *Candida* sp. were tested for their ability to reduce disease development in citrus fruits after challenge with post-harvest pathogens *Alternaria citri* and *Penicillium italicum*. Lesion diameter and frequency of *Alternaria* rot were significantly less in fruits pre-treated with aqueous suspension of both the antagonists as compared with controls pretreated with water. Lesion development was related to pathogen spore concentration and the concentration of antagonists. The control of disease was proportional to the time allowed to the fruit for its submersion in the antagonistic suspension.**

KEY WORDS: Citrus, fruit rot, *Alternaria citri, Penicillium italicum,* biological control, *Bacillus sphericus, Candida* sp.

An increasing number of fungicide-tolerant strains of pathogens associated with fruits and vegetables and the pressure to reduce residual toxicity of fungicides has emphasised the need to develop alternative methods to control post harvest diseases. Biological control of pathogens of grapes (Dubos, 1984) and apples (Jainsiewiez, 1987) were achieved. Control of post harvest diseases of citrus fruits caused by *Alternaria citri* and *Penicillium italicum* through the application of antagonists is presented here.

MATERIALS AND METHODS

Screening of potential antagonists on fruits was done by treating wounded oranges with aqueous suspensions of potential antagonists and an aqueous suspension of pathogen spores (1×10^3 spores / ml). Lesion diameter was measured after 7 days of incubation at 25°C. There were three replicates per treatment.

In vitro testing for inhibition of spore germination of pathogen was conducted in ceramic dye plates; 0.5 ml each of a spore suspension of the antagonist and pathogen (10^6/ml) were mixed and after incubation at 25°C for 72 h, germination was determined. Equal volumes of freshly squeezed orange juice were added and after additional 24 h incubation, the examinations were made.

To standardise the period of treatment with antagonist, injured fruits were dipped in aqueous suspension of the antagonist for 5 and 10 min and immediately inoculated with spore suspension of *A. citri* and *P. italicum* (10^5/ml). The lesion diameter was measured after incubation at 25°C for a week.

To standardise the concentration of antagonist spore suspension, orange fruits were injured and dipped in water suspension of *Bacillus sphericus* and *Candida* sp. (10^3 to 10^8/ml) and inoculated at the wound site with a spore suspension of *A. citri* and *P. italicum* (10^5/ml). Lesion diameter was measured after six days of incubation at 25°C.

RESULTS AND DISCUSSION

Candida sp. inhibited spore germination of *A. citri* and *P. italicum* in aqueous suspension (Table 1). The addition of orange juice eliminated the inhibitory effect partially in the case of *A. citri* and completely in the case of *P. italicum*. *B. sphericus* also inhibited the germination of *A. citri* and *P. italicum* spores. The inhibition was eliminated after the juice supplement in case of *P. italicum* but not in

Table 1. Influence of Antagonists on germination of spores of *A.citri* and *P.italicum*

Antagonist	Hours	*A. citri* 10^4	*A. citri* 10^5	*A. citri* 10^6	*P. italicum* 10^4	*P. italicum* 10^5	*P. italicum* 10^6
B. sphericus water	72	-	-	Nil	-	-	-
Fruit Juice	96	-	-	+	-	-	+
Candida sp. water	72	-	+	+	-	+	+
Fruit Juice	96	-	+	+	-	-	+
Control	72	+	+	+++	+	+	+
	96	+++	+++	+	+++	+++	+

\- = Nil (No germination)
\+ = 1-34% spore germination
++ = 35-74% spore germination
+++ = 75-100% spore germination

case of *A. citri*. Water controls for both fungi showed inhibition in spore germination at higher spore concentrations.

The period of time oranges were submerged in antagonists suspension was an important factor influencing biocontrol effectiveness. There was no difference in inoculated orange fruit with *P. italicum* and in 5 min dipped fruits in both the antagonists suspension. However, 10 min dip resulted in good protection (Table 2). No lesions were observed on fruits by both the pathogens when the time for submersion was 10 min in both the antagonists. However, lesions of smaller size were observed in case of fruits treated with *Candida* sp. as compared to that of control.

Table 2. Effect of duration of antagonist dip on lesion size

Antagonist	Pathogen	Time (MIN)	Lesion Diameter (mm)
B. sphericus	*A. citri*	5	6
		10	0
	P. italicum	5	8
		10	0
Candida sp.	*A. citri*	5	12
		10	2
	P. italicum	5	15
		10	2
Control	*A. citri*	0	30
	P. italicum	0	38

Table 3. Effect of spore concentration on oranges after protection with different concentrations of antagonists

Antagonist	Spores/ml	Lesion diameter (mm) *A. citri* 10^5	Lesion diameter (mm) *P. italicum* 10^5
B. sphericus	10^8	0.0	0.0
	10^7	0.0	0.0
	10^6	0.0	0.0
	10^5	0.0	6.4
	10^4	9.0	11.8
	10^3	13.0	19.0
Candida sp	10^8	0.0	0.0
	10^7	0.0	0.0
	10^6	0.0	0.0
	10^5	0.0	2.0
	10^4	8.8	10.2
	10^3	12.0	25.6
		24.0	30.4

B. sphericus was found to be a very effective antagonist against *A. citri* and *P. italicum* as compared to *Candida* sp. (Table 3). Lesion size on citrus fruits depended on the spore concentration of the antagonists. *B. subtilis* and *Candida* sp. are a major component of the epiphytic microbial community on mature fruits (Beech and Davenport, 1970). It is possible that yeasts isolated from fruit surfaces may be more effective biocontrol agents because they are phenotypically adapted to this niche and, thus are able to colonize and establish on fruit surfaces and are effective protectants.

B. sphericus has been successfully used for the first time as the biocontrol agent against post harvest diseases. Due to its non-pathogenic and non-toxic characteristics it can be employed as an effective protectant against post harvest diseases.

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