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Post-harvest biocontrol of *Penicillium* rot of table grapes by using antagonist *Debaryomyces hansenii* Zopf.

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ABSTRACT: The yeast *Debaryomyces hansenii* Zopf. isolated from the surface of grape berries inhibited incidence of *Penicillium* rot of grape effectively. Efficacy of *D. hansenii* was maintained when applied simultaneously or prior to inoculation with *Penicillium chrysogenum* Thom. However, the efficacy was reduced when *D. hansenii* was applied after inoculation of the pathogen. The yeast antagonist also reduced the incidence of *Penicillium* rot of injured, naturally infected grapes stored at 25° C for 9 days.

KEY WORDS: Biocontrol, *Debaryomyces hansenii*, *Penicillium* rot

Large share of grape fruits are lost by the post-harvest decay caused by *Penicillium chrysogenum* Thom. (Sharma, 1989). Generally, fungicides are used for suppression of this disease. The concern on the fungicide resistant strains of the post harvest pathogens, presence of terminal residues in the fruits and the possible deregistration of some of the more effective fungicides have necessitated the development of safer alternative post-harvest disease management technologies. Biological control of the post-harvest pathogens using natural antagonist is one such technology.

Success in this area has been achieved with peaches (Wilson and Pusey, 1985; Pusey *et al.*, 1986), apples (Janisiewicz and Roitman, 1988) and citrus (Chalutz and Wilson, 1990; Sharma *et al.*, 1996). The efficacy of the antagonist is attributed to the release of antibiotics. The emphasis is now to find new biological control antagonists of rot pathogens, particularly those that did not produce antibiotic substances as part of their mode of action. The present communication describes the biocontrol of *Penicillium* rot of table grapes caused by *P. chrysogenum*.

MATERIALS AND METHODS

Isolation of antagonist

During a study conducted in Plant Pathology Laboratory of Botany Department, Lucknow University in 1996-97, potential antagonists were isolated from the surface of healthy grape and citrus fruits by washing them in distilled water. Wash water (0.1 ml) was then spread on Nutrient Yeast Dextrose Agar (NYDA) medium plates and allowed to incubate at 25±1° C for 24 h at 80 per cent relative humidity (RH). Single cell colonies were isolated and maintained in pure culture and stored in a refrigerator at a temperature of 5° C.

Screening of antagonist

Culture of individual isolates to be tested were grown on NYDA medium for 3 days. A loop of 3 days old culture was then transferred to flasks containing 50 ml of NYDB and incubated at 25 ± 1°C on a rotary shaker for 24 h at 200 rpm. After 24 h one ml from each flask was transferred into another flask containing 50 ml of NYDB and incubated for 48 h before testing them on fruits. Fresh and healthy grape berries (variety Thomson seedless) were surface sterilized by dipping in 95 per cent ethanol for 30 seconds and thereby killing all other fungal spores from the surface of grapes.

There were four experiments. In the first experiment, surface sterilized, fresh and healthy grapes were dipped in the yeast NYDB mixture, either as whole clusters with non-injured berries, or as individual berries which had been removed from the stalks causing a wound and were dipped in the pathogen spore suspension (10^6 spores ml^{-1}) 20h after antagonistic treatment. In the second experiment, fresh and healthy grapes were dipped in the yeast-NYDB mixture and then inoculated by dipping in the pathogen spore suspension (10^6 spores ml^{-1}).

In the third experiment, grapes were first dipped in the pathogen spore suspension and after 24h these were treated with yeast-NYDB mixture. In the fourth experiment, grapes were dipped in the sterile NYDB and then inoculated with pathogen spore suspension.

The treated berries were then placed in plastic baskets and covered with polythene sheets. Incubation was carried out in a BOD incubator at a constant temperature of 25 ± 1°C for 9 days. Decay incidence was determined by counting the number of infected berries. Each treatment consisted of 3 replicates of 20 berries.

To test whether the yeast antagonist was effective in reducing the incidence of *Penicillium* rot in injured, non-injured and naturally infected grape berries, grapes were dipped in sterile NYDB (control) or in a 48 h culture of yeast antagonist (protected) and then placed in plastic trays at 25 ± 1°C for 4 days. Incidence of decay was later calculated.

For evaluating the effect of water soluble nutrients present in grapes on the efficacy of *D. hansenii* in the inhibition of *Penicillium* rot, fresh grape berries were sterilised by 95 per cent ethanol, homogenized and then centrifuged at 9000

rpm for 10 minutes. The supernatant was diluted with water and filter sterilized for the preparation of pathogen spore suspension used for inoculation.

The interaction between antagonist and pathogen was tested by placing 4 mm agar discs of 7 days old culture of the pathogen in a petri dish containing 15 ml of NYDB with and without antagonist cells.

RESULTS AND DISCUSSION

Screening tests revealed that grape isolate of *Debaryomyces hansenii* was more effective than citrus isolate and isolate obtained from ATCC (American type Culture Collection) (Fig. 1).

Results of initial laboratory experiments indicated that *D. hansenii* was effective in reducing the incidence of *Penicillium* rot in injured and non-injured grape berries (Table 1). The time of application of the antagonist (before, simultaneous or after inoculation) clearly affected disease inhibition (Fig. 2). In case of post and simultaneous application of the pathogen, the disease incidence was reduced to 79.5 per cent and 70 per cent, respectively. But in case of preinoculation of pathogen, the efficacy of the yeast antagonist was reduced.

A culture filtrate of the yeast antagonist failed to provide any protection against *Penicillium* rot of table grapes (Table 2). *Debaryomyces hansenii* did not inhibit the pathogen by producing antibiotics. It had no effect on the growth of the pathogen in culture.

The results indicated an increased incidence of decay in the NYDB treated control fruit. This phenomenon resulted from the rich nutritional environment provided by the treatment to the berry, thus enhancing the development of rot causing grape pathogen.

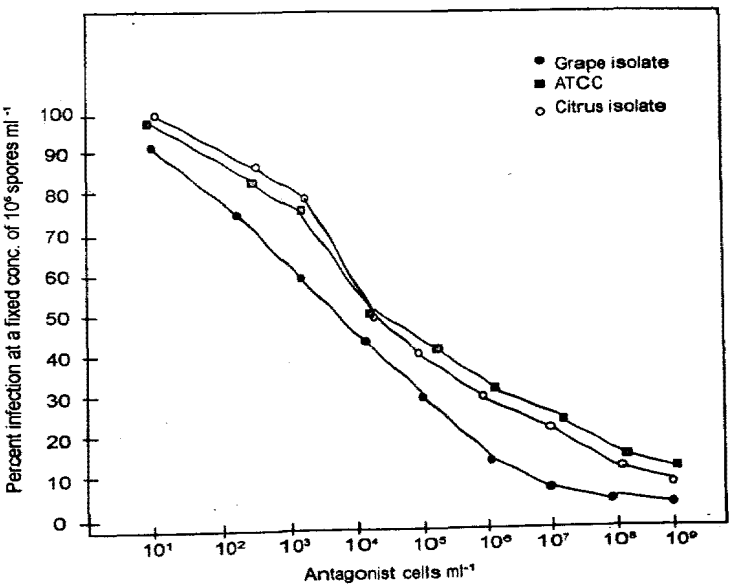

Fig.1 : Relationship between antagonist cell concentration and development of infection

Table 1. Inhibition of *Penicillium* rot of grapes by the yeast antagonist, *Debaryomyces hansenii*

Fruit	Treatment	Mode of inoculation	Per cent decay
Non injured grapes	I. Control (Dipping in sterile medium)	Pathogen spore suspension	16*
	ii. Protected (Dipping in yeast antagonist)	Pathogen spore suspension	12*
Injured grapes	i. Control (Dipping in sterile medium)	Pathogen spore suspension	58*
	ii. Protected (Dipping in yeast antagonist)	Pathogen spore suspension	11*
Infected grapes	I. Control (Dipping in sterile medium)	Natural infection	82*
	ii. Protected (Dipping in yeast antagonist)	Natural infection	44*

* significant (P=0.01)

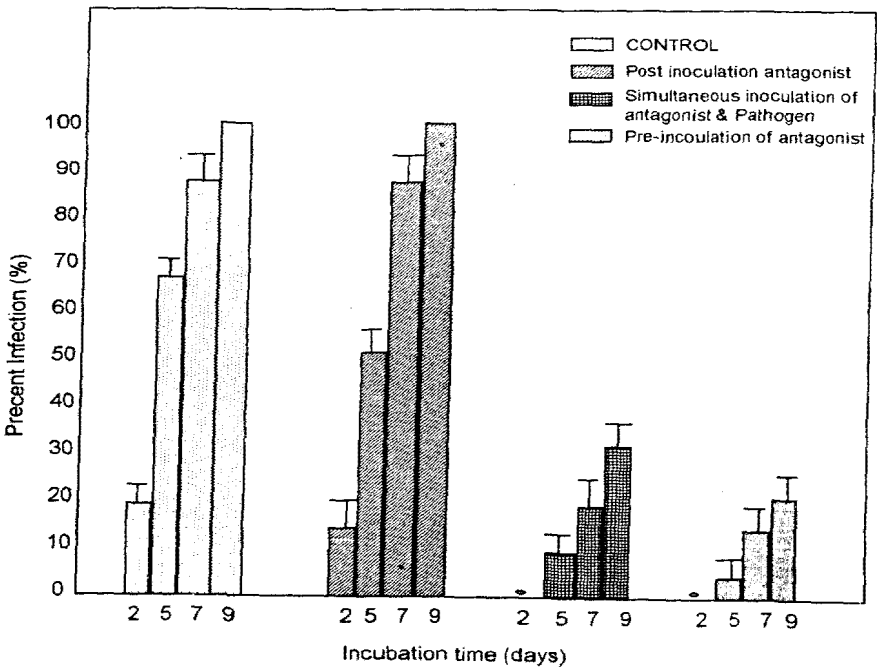

Fig.2 : Inhibitory activity of *Debaryomyces hansenii* with respect to the time of application and periods

Debaryomyces hansenii lost its antagonistic activity when the cells were killed and also the culture filtrate did not show any activity (Table 2). No inhibitory activity was observed against the fungal pathogen on the culture plates. Thus, the mode of action of this yeast in antagonising the grape rot pathogen is not through the production of antibiotic.

It is evident from the present results and also from earlier reports that competition for nutrients at the wound site could be the main mechanism by which *D. hansenii* inhibits (Chalutz *et al.*, 1990) fruit rotting pathogen. The culturing of antagonist cell with a pathogen on a synthetic medium resulted in marked reduction in the growth rate of pathogen only under limited nutritional condition. The growth of *D. hansenii* in the medium did not result in any residual inhibitory effect on the pathogen. Competition for nutrients as the mode of action for post-harvest disease of fruit has been suggested by Wisnewski *et al.* (1988). The lack of evidence of antibiotic production or any direct interaction between the yeast and the pathogen, suggests that competition for nutrients is likely the major mode of action by which the yeast antagonises artificially inoculated pathogens on grape berries.

Table 2. Inhibition of *Penicillium* rot of grapes by antagonist *D. hansenii* by pre-treatments of the antagonistic cells

Pre-treatment	Per cent infection days after treatment			
	2	5	7	9
None	0.0	4.9[a]	19.7[b]	19.9[b]
Autoclaved	18.8[b]	49.8[c]	78.2[d]	80.5[d]
Cultured filtrate	18.2[b]	50.2[c]	78.5[d]	80.0[d]
Sterile medium	25.5[b]	56.0[c]	90.0[e]	100.0[e]
Water control	18.5[b]	49.0[c]	78.0[d]	80.2[d]

Values followed by different letters are significantly different (P=0.05) according to DMRT

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