J. Biol. Control, 20(2): 241-246, 2005

Research Note

Isolation, identification and screening of potential antagonistic microorganism for the biocontrol of *Phytophthora meadii* McRae causing fruit rot of arecanut (*Areca catechu* L.)

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ABSTRACT: **Fruit rot of arecanut caused by *Phytophthora meadii* McRae is a major constraint in arecanut production causing substantial loss in yield. Use of biocontrol agents is effective, ecofriendly and nonhazardous possibility for fruit rot management. The present investigation focuses on the screening of potential biocontrol agents against the fruit rot pathogen, *Phytophthora meadii* on arecanut. Among the 14 isolates screened on dual culture method and bioassay experiments, *Trichoderma viride* (04), *Bacillus cereus, B. polymyxa* and *B. licheniformis* were emerged as potential biocontrol agents.**

KEY WORDS: *Bacillus* sp., biocontrol, mycoparasites, *Phytophthora meadii*

Arecanut is grown largely in humid tropics. Fruit rot disease caused by *Phytophthora meadii* McRae is one of the major diseases prevalent in almost all arecanut growing areas incurring considerable economic loss. Though the disease is mainly controlled by spraying Bordeaux mixture (1%), the fungicide is unable to check the internal infection of the kernel, as proved by the isolation of the fungus from Bordeaux treated nuts (Sastry and Hegde, 1985). Since arecanut is a plantation crop, the inoculum concentration would increase year after year with a possibility of pathogen acquiring resistance to the fungicide (Islam and Dubey, 2003). It is felt that biocontrol may prove a more sustainable and effective. The objective of this study is to isolate and identify antagonistic microorganisms against *P. meadii* of the fruit rot disease in the Western Ghat region of Karnataka and Kerala, which could be developed as biocontrol agents.

For the isolation of the pathogen, diseased arecanut showing initial stages of infection were used. The pathogen was isolated by using plain carrot agar medium. The pathogen was identified as *P. meadii* and pathogenicity was confirmed by inoculating the healthy nuts with *P. meadii*.

Fourteen local isolates belonging to *Trichoderma viride*, and *Aspergillus niger* and seventeen bacterial isolates were isolated from the field inoculum (leaves, nuts and inflorescence) collected from different locations of Karnataka and Kerala States where fruit rot was prevalent. For the isolation of antagonistic fungi precolonised plate method was adopted (Krauss *et al.*, 1998). According to this method, the pathogen *P. meadii*

was first allowed to cover the carrot agar medium plates completely. Five bits of the field inoculum were inoculated in circular manner over the mycelial mat of the pathogen. All the samples were air-dried overnight to avoid bacterial contamination. Mycoparasites observed if any, were isolated and maintained, whereas in the case of bacterial antagonists, serial dilution method was adopted.

As a preliminary screening, dual culture method was adapted as described by Skidmore (1976). Simultaneous inoculation of pathogen and opposing antagonistic microorganism was done and the plates were incubated at 25 ± 1°C for four days. The plates inoculated with the *P. meadii* alone served as control. Growth of the *P. meadii* was measured after the incubation period in all the cases. The per cent inhibition of *P. meadii* by mycoparasites was calculated by using the following formula suggested by Skidmore (1976):

$$I = \frac{(C-C1)}{C} \times 100$$

where I is the percentage of inhibition, C, the fungal growth (in mm) from the point of inoculation to the colony margins in control and C_1, the growth (in mm) of the pathogen towards the antagonist.

Efficacy of the selected antagonistic fungi was further evaluated using a bioassay experiment described by Krauss (1996) with arecanut discs. Five to six months-old arecanuts were selected and discs (12 mm) were cored out. The discs were treated with 10 ml of a mixture of pathogen (10^5 zoospores) and mycoparasite (10^5 spores). The mixing of both the microorganisms was done just before the inoculation. The discs were then placed in the bottom half of the Petri plate with the outside layer facing upwards. To maintain sufficient humidity, a thin layer of sterilized moist cotton and filter paper

Table 1. Inhibition of the growth of *P. meadii* by different mycoparasites

Mycoparasite	Growth towards mycoparasite	Inhibition* (%)
Aspergillus niger (A14)	16.3	47.4 (43.5)
Aspergillus sp. (A8)	15.7	49.4 (44.7)
Fusarium oxysporum (F5)	15.0	51.6 (46.0)
Fusarium oxysporum (F7)	16.6	46.5 (43.0)
Penicillium sp. (P9)	17.7	42.9 (40.9)
Penicillium sp.(P10)	19.7	36.5 (37.2)
Trichoderma viride (TV1)	11.6	62.6 (52.2)
Trichoderma viride (TV3)	12.6	59.4 (50.4)
Trichoderma viride (TV6)	12.0	61.3 (51.6)
Trichoderma viride (TV11)	14.7	52.7 (46.5)
Trichoderma sp. (T2)	15.6	49.7 (44.8)
Trichoderma sp. (T4)	13.6	56.0 (48.5)
Control	31.0	
CD (P=0.05)		4.08

Figures in parentheses are arcsine-transformed values.

Table 2. Inhibition of the growth of *P. meadii* by different bacterial isolates

Bacterial antagonist	Growth towards test bacterium	% Inhibition
Bacillus sp. (B1)	18.8	50.5 (45.3)
Bacillus sp. (B2)	15.8	58.4 (49.8)
Bacillus sp. (B3)	21.7	42.9 (41.0)
B. polymyxa (B4)	15.5	59.2 (50.3)
B. cereus (B5)	15.2	60.0 (50.8)
Bacillus sp. (B6)	18.3	51.8 (46.0)
Bacillus sp. (B7)	19.8	47.9 (43.8)
Bacillus sp. (B8)	19.8	47.9 (43.8)
B. licheniformis (B9)	15.8	58.4 (49.8)
Bacillus sp. (B10)	22.7	40.3 (39.4)
Bacillus sp. (B11)	21.0	44.7 (42.0)
Bacillus sp. (B12)	21.5	43.4 (41.2)
B. laterosporous (B13)	21.3	43.9 (41.5)
Bacillus sp. (B14)	22.7	40.3 (39.4)
Bacillus sp. (B15)	21.0	44.7 (42.0)
Bacillus sp. (B16)	18.7	50.8 (45.5)
Bacillus sp. (B17)	21.2	44.2 (41.7)

CD(P=0.05)= 6.7
Figures in parentheses are arcsine-transformed values.
Note: Growth of *P. meadii* in Control: 38 mm

were placed under the lid of each plate. Care was taken that the discs did not touch each other and also the walls of the Petri-plate. The plates were bagged in a polythene cover and incubated at 24 ± 1°C for 10 days.

Rot severity on disc was recorded by measuring the percentage of discoloured surface area in the discs. Sterile distilled water and Bordeux mixture (1%) served as negative and positive controls, respectively. Further, reisolation of the pathogen was tried in all the discs to study whether the mycoparasite masked the growth or completely killed the pathogen. The percentage data obtained from dual culture and bioassay methods were subjected to analysis of variance (ANOVA).

Screening of mycoparasites was done with the help of dual culture technique. Among the 14 isolates screened, *Trichoderma viride* (TV1 and TV6) showed numerically maximum inhibition (52.2% and 51.6%, respectively), followed by *Trichoderma viride* (TV3) (50.4%) and *Trichoderma* sp. (T4) (48.5%) (Table 1). However, all the four isolates were on par with each other. Rest of the mycoparasites showed intermediate inhibition except *Penicillium* sp. (P9 and P10), which recorded lowest values (40.9% and 37.2%). Srinivasulu *et al.* (2004) studied the effectiveness of different species of *Trichoderma* (*T. viride*, *T. harzianum* and *T. hamatum*) against basal stem rot pathogen (*Ganoderma lucidum* and *G. applanatum*) of

Table 3. Rot values of areca discs and recovery of *P. meadii* from arecanut discs treated with mycoparasites

Treatment	Rot values over control (%)	Recovery of *P. meadii* *	
		No. of discs	Recovery (%)
Aspergillus niger (A14)	2.6 (9.2)	9	45
Aspergillus sp. (A8)	100.0 (90.0)	17	85
Fusarium oxysporum (F5)	3.2 (10.3)	6	30
Fusarium oxysporum (F7)	99.7 (88.1)	10	50
Penicillium sp. (P9)	62.8 (52.4)	20	75
Penicillium sp. (P10)	100.0 (90.0)	15	100
Trichoderma viride (TV1)	2.1 (8.2)	-	0
Trichoderma viride (TV3)	4.2 (11.8)	-	0
Trichoderma viride (TV6)	4.1 (11.6)	-	0
Trichoderma viride (TV11)	2.8 (9.6)	-	0
Trichoderma sp. (T2)	39.2 (38.8)	7	35
Trichoderma sp. (T4)	3.1 (10.1)	-	0
Bordeaux mixture	4.8 (12.7)	9	45
Sterile dist. Water	100.0 (90.0)	20	100

CD(p=0.05) = 2.04, *No. of discs kept in each treatment: 20

Figures in parentheses are arcsine-transformed values.

coconut and maximum suppression was found with *T. harzianum* over the control. Similar results were obtained by Rudresh *et al.* (2005), when they screened *Trichoderma* sp. against *Fusarium oxysporum f.* sp. *ciceri*, *Rhizoctonia solani* and *Sclerotium rolfsii*, the causative agents of chickpea wilt.

The extent of inhibition of *P. meadii* in different bacterial isolates is presented in Table 2. The bacterial isolates namely, *Bacillus polymyxa* (B4) and *Bacillus cereus* (B5) showed numerically slightly higher inhibition (50.3% and 50.8%, respectively), but statistically these are on par with the isolates *Bacillus* sp. (B1), *Bacillus* sp. (B2), *Bacillus* sp. (B6), *Bacillus licheniformis* (B9) and *Bacillus* sp. (B16). Guetsky *et al.* (2002) reported suppression of *Botrytis cinerea* on strawberry leaves by bacterial antagonists (*Bacillus mycoides*) and by yeast *Pichia guilermondii*.

In this screening method, *Aspergillus* sp. (A8) (90%) and *Penicillium* sp. (P9) (90%) showed maximum rot value and did not differ from control (water). The fungus *Fusarium oxysporum* (F7) (88.1%), as well as *Penicillium* sp. (P10) (52.4%) showed higher rot values than Bordeaux mixture.

Three out of 14 isolates namely, *Trichoderma viride* (TV1) (8.2%) and TV11 (9.6%) and *Aspergillus niger* (A14) (9.2%) were significantly superior to Bordeaux mixture (2.7%). The isolates *Trichoderma viride* TV6, *Trichoderma* sp. (T4), and *Fusarium* sp. (F5)) did not differ significantly from control (Bordeaux mixture) (Table 3). Based on this bioassay *Aspergillus niger* (A14), *Fusarium*

Table 4. Rot values of areca discs and recovery of *P. meadii* from areca discs treated with bacterial isolates

Treatment	(%) Rot values over control*	Recovery of *P. meadii* **	
		No. of discs	% Recovery
Bacillus sp. (B1)	17.7 (24.9)	13	86
Bacillus sp. (B2)	5.7 (13.8)	5	33
Bacillus sp. (B3)	18.2 (25.1)	12	80
B. polymyxa (B4)	5.3 (13.2)	5	33
B. cereus (B5)	3.4 (10.5)	4	26
Bacillus sp. (B6)	13.7 (21.7)	10	66
Bacillus sp. (B7)	16.6 (23.9)	12	80
Bacillus sp. (B8)	18.5 (25.5)	13	86
B. licheniformis (B9)	3.6 (10.9)	3	20
Bacillus sp. (B10)	7.8 (16.1)	12	80
Bacillus sp. (B11)	6.2 (14.4)	9	60
Bacillus sp. (B12)	7.3 (15.6)	10	66
B. laterosporous (B13)	9.3 (17.7)	13	66
Bacillus sp. (B14)	18.8 (25.7)	13	86
Bacillus sp. (B15)	13.2 (21.3)	12	80
Bacillus sp. (B16)	13.8 (21.8)	12	80
Bacillus sp. (B17)	18.1 (25.1)	13	86
Bordeaux mixture	4.4 (12.1)	9	60
Sterile distilled water	100 (90.0)	15	100
CD=(p=0.05)	3.14		

** No. of discs per treatment =15

Figures in parentheses are arcsine-transformed values.

oxysporum (F5), *Trichoderma viride* (TV1, TV3, TV6 and TV11) and *Trichoderma* sp. (T4), were selected as promising mycoparasites. Similar results were obtained by Krauss (1996) and Krauss *et. al.* (1998) while screening biocontrol agents against crown rot of banana.

Reisolation of the pathogen was tried in all the above treatments. It was positive in the case of *Aspergillus* sp. (A8), *Penicillium* sp. (P9 and P10), and *Trichoderma* sp. (T2). The per cent recovery of *P. meadii* (pathogen) from discs treated with different mycoparasites and Bordeaux mixture is given in Table 3. Bordeaux treated discs also showed positive results in a considerable number of cases.

All the bacterial antagonists tested previously in dual culture method were screened by areca disc method. The Table 4 shows the extent of rotting over negative control (water). The

minimum rot area values were recorded in the isolates *Bacillus* sp. (B2) (13.8%), *B. polymyxa* (B4) (13.2%), *B. cereus* (B5) (10.5%), *B. licheniformis* (B9) (10.5%) and *Bacillus* sp. (B11) (14.4%) and these were on par with Bordeaux mixture (12.1%). Though *Bacillus* sp. (B2) and *B. polymyxa* (B4) recorded slightly higher values (13.8% and 13.5%, respectively) than Bordeaux mixture, all of them were on par. The *Bacillus* sp. (B6) showed reasonably good inhibition (46.0%) in the previous technique i.e. dual culture method, but failed to control the rotting effectively in this experiment (rot value is 21.7%). In this experiment *Bacillus* sp. (B2), *B. polymyxa* (B4), *B. licheniformis* (B5) and B. *cereus* (B9) emerged as promising antagonists and hence selected for further study as their performance in the dual culture was also satisfactory.

Reisolation of the pathogen was done from the discs used in bioassay experiment. Unlike mycoparasites, discs treated with all the bacterial isolates yielded *P. meadii* irrespective of disease incidence. Though *Bacillus* sp. (B2), *B. polymyxa* (B4), *B. cereus* (B5) and *B. licheniformis* (B9) showed lower rot incidence, isolation of *P. meadii* is positive. However, the recovery was low compared to Bordeaux mixture. The recovery of *P. meadii* is 60 per cent in Bordeaux treated discs, whereas it was less in the isolates i.e. *Bacillus* sp. (B2) (33.3%), *B. polymyxa* (B4) (33.3%), *B. cereus* (B5) (26.7%) and *B. licheniformis* (B9) (20%).

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