Schiffer, R.J., D.M. Elgerama and G.A. Strobel. 1989. *Pseudomonas* for biological control of Dutch elm disease. II . Further studies on the localization, persistence and ecology of *Pseudomonas* isolates injected into elms. Neth. J. Pl. Pathol. 95: 293-304.

Sivakumar, G. and N.T. Narayanaswamy. 1998. Biological control of sheath blight of rice with *Pseudomonas fluorescens*. Oryza 35: 57-60.

Utkhede, R.S. and J.E. Rahe. 1983. Interactions of antagonist and pathogen in biological control of onion white rot. J. Phytopathol. 142:131-140.

Vidhyasekaran, P. and M.Muthamilan. 1995. Development of formulation of *Pseudomonas fluorescens* for control of chick pea wilt. Pl. Dis. 79: 782-786.

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IN VITRO EFFECT OF PSEUDOMONAS FUORESCENS ON CAPSULE ROT OF CARDAMOM (*ELETTARIA CARDAMOMUM* MATON) CAUSED BY PHYTOPHTHORA MEADII

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ABSTRACT

Capsule rot disease of cardamom incited by *Phytophthora meadii* Mc Rae is a major threat in cardamom plantations during the monsoon period. Integrated management strategy has already been developed for the management of the disease at field level where *Trichoderma* species are being used as the biocontrol component. In the present study, in an attempt to isolate natural biocontrol agents for *Phytophthora* from cardamom capsules, a species of fluorescent pseudomonas was obtained in culture which is found to be inhibiting the growth of *Phytophthora* under *in vitro* conditions. In order to exploit the biocontrol potential of these bacteria for the management of capsule rot infection, a laboratory experiment was laid out using culture suspensions and filtrates. The experiment consisted of 10 treatments and 2 replications with different forms and periods of inoculation of *P.fluorescens* and *P.meadii* zoospores. The treatment includes spraying the panicles with *Pseudomonas* culture suspension and culture filtrate before and after infecting by spraying with zoospore suspension of *Phytophthora meadii*, dipping the culture suspension in culture filtrate for 24 hours and then spraying with zoospore suspension. The result clearly indicated that spraying 48hr old culture suspension on panicles prior to inoculation with zoospore suspension of *Phytophthora* and spraying with culture filtrate from 20 day old culture are effective in checking the infection. Hence this can be exploited as a management strategy for the field management of capsule rot infection, where either the fresh bacterial culture can be sprayed before the onset of monsoon or spray culture filtrates from stored cultures, after getting infected which could prevent or reduce the infection of capsules by the pathogens.

INTRODUCTION

Capsule rot of cardamom caused by *P. meadii* is one of the serious diseases of cardamom. The disease is of soil borne in nature. The disease makes it s appearance during the monsoon season on panicles and capsules leading to direct crop loss. The pathogen propagules emerge from the dormant stage and infect the capsules through rain splash. The present system of managing the disease is through integrated approach. Integrated disease management strategies like phytosanitation, cultural practices, and fungicidal application together with bioagents like *Trichoderma harzianum* can be able to control the disease. Present practice is to spray fungicides such as 1% Bordeaux mixture or 0.35 POTASSIUM PHOSPHONATE and soil application of biocontrol agent *Trichoderma* soon after the initiation of the monsoon showers to resist the infection on the capsules and to reduce the soil borne inoculum. The study for exploiting the potential of *Pseudomonas fluoresens* was based on the reports of the potentiality of *Pseudomonas fluoresens* in controlling soil borne diseases.

MATERIALS AND METHODS

Bacterial culture: 'Fluorescent pseudomonas' culture was obtained, as a secondary growth inhibiting *Phytophthora* culture in petri dishes, during the course of investigations of *Phytophthora* sp. isolated from infected cardamom capsule (as part of Ph.D work) . The bacteria grows well in King'B medium emitting fluorescence under UV lamp. After 24hr of inoculation into culture media , the center of the culture showed reddish tinch with slight green colouration in the media. Later on the intensity of green colour intensified. The culture having fluorescence under UV light of wave lenth 26nm with excretion of duffusible yellow-green pigment is identified as *Pseudomonas fluorescens*

For the present study, 'Fluorescent pseudomonas' culture was grown in King'B broth of 48hrs and used as the culture suspension. The same culture suspension filtered through millipore filter for cell free culture filtrate. The culture grown for 20 days in King'B broth as such was used as the 'old culture suspension'and filtered one was used as the 20 d old culture filtrate.

Phytophthora meadii isolated from freshly infected capsules and grown in carrot agar was used as the pathogen inoculum. Zoospores released from the sporulated culture discs and filtered through sterile muslin cloth for zoospore suspension

Panicles of average 30cm length having 20-25 capsules were taken for inoculation studies.

In vitro assay : Effect of *Pseudomonas fluorescens* on mycelial growth of P. meadii

Live bacteria in dual culture

Carrot agar plates containing 15 ml of the medium was centrally inoculated with 5mm discs of 72hr old culture of *P. meadii*. Bacterial culture was streaked on either side of *Phytophthora* disc at a distance of 3 cm apart and incubated in the dark

for 96hrs at 22-24°C. The distance of inhibition zone between soil bacteria and the pathogen was recorded (Jin and Hee 1989)

Culture filtrate

Secondly the culture filtrate was incorporated into the CA medium @ of 1ml and 2ml respectively in 50ml of the media and poured into petridishes. These dishes were inoculated with *Phytophthora* culture discs as above and incubated. The growth rate was measured after 72hrs and calculated the growth inhibition. Effect on sporulation was tested by keeping *Phytophthora* culture discs taken from the periphery of 72 hr old CA culture in culture filtrate as well as in sterile distilled water for 24-48hrs. Zoospore germination was also tested by incubating the zoospores in culture filtrate as above at 22- 24°C

Culture suspension and cell free culture filtrate on panicle infection

i. Panicles kept in Culture suspension , Culture filtrate, old culture filtrate and old culture suspension respectively in trays @ 500ml of 48 hr old culture grown in King'B broth /tray, for 24 hours and then transferred to Zoospore suspension (10^8 zoospores/ml) for 24 hrs and then incubated under humid conditions for 96hrs.

ii. Panicles kept in zoospore suspension (10^8 zoospores/ml) in trays for 24 hours and then transferred to culture suspension, culture filtrate and old culture filtrate respectively for 24 hrs. Panicles were then removed and incubated under humid conditions for 96hrs

iii. Similarly the panicle was dipped in culture filtrate for 24 hrs and then sprayed with zoospore suspension . Obsevations were taken on the number of capsules infected and calculated the % inhibition.

RESULTS AND DISCUSSION

In vitro assay

Effect of *Pseudomonas fluorescens* on mycelial growth of *P. meadii:* In dual culture with live bacteria there was a reduction in the mycelial growth towards the growth of bacteria. The percent reduction in growth when compared to control ranges from 62.96-72.22%.

Mycelial inhibtion was also noticed when cell free culture filtrate was incorporated into the medium @ of 1ml and 2ml /50ml of the medium. The percentage inhibtion was 48.5% and 60.4% respectively. There was absolutely no sporulation when culture discs were incubated in culture filtrate when compared to control which formed > 195 sporangia /microscopic field. In case of zoospore germination, the zoospores were found lysed in the culture filtrate whereas in control there was 42.21% germination (Table 2).

Treatment of panicles with Culture suspension, Culture filtrate and old culture filtrate and culture suspension prior to infesting with zoospore suspension showed zero infection in both culture suspensions and around 15.84 and 6.94 respectively in culture filtrates. But when zoospores are infested prior to treatment with bacterial suspensions and fltrates, the percent infection ranges from 34-65%. When dipping the

panicles in culture suspension for 24 hrs prior to zoospore spray resulted in less infection percentage (22.26%) when compared to control where zoospore alone was infested (71.62%) (Table 3).

Table 1. Treatment details: In vitro effect of *Pseudomonas fluorescens* on Panicle infection by *Phytophthora meadii*

Pre- treatment	Post- treatment
1.Culture suspension	2.Culture suspension
3.Culture suspension (20 days old)	4. Culture suspension (20 days old)
5. Culture filtrate	6. Culture filtrate
7. Culture filtrate(20 days old)	8. Culture filtrate(20 days old
9. Dip in culture filtrate	10. Control

Table 2. *In vitro* effect of *Pseudomonas fluorescens* on *Phytophthora meadii*

Pseudomonas fluorescens	%Mycelial inhibition	%Sporangial production	Zoospore germination
1.Live bacteria (Dual culture)	69.59	-	Zoospore lysis
2.Cell free culture filtrate @1ml/50ml	48.50	Nil	Zoospore lysis
2.Cell free culture filtrate @2ml/50ml	60.40	Nil	Zoospore lysis
3. Control	0.0	195/microscopic field	42.21%

Table 3. In vitro effect of *Pseudomonas fluorescens* on Panicle infection by *Phytophthora meadii*

Pseudomonas fluorescens	% infection		% infection	% disease reduction	
Treatments	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment
1.Culture suspension	0.0	34.38		100	52.00
2. Culture suspension (20 days old)	0.0	62.95		100	12.11
3. Culture filtrate	15.84	53.856		77.88	24.80
4. Culture filtrate(20 days old)	6.94	35.51		90.31	50.42
5. Dip in culture filtrate	22.255			68.92	
6. Control			71.62		
Lsd at 0.05 alpha level =	8.991				

The experimental results are highly significant. The results of the present study very clearly indicated the pre-treatment effect of *Pseudomonas fluorescens* in inhibiting the growth of *P. meadii* as well as in preventing the infection. It is inhibitory to all stages of growth of the fungus. Fresh culture suspension or old culture filtrates are found to be superior to old culture suspension and fresh culture filtrates. So it can be advisable to use *Pseudomonas fluorescens* culture in 48hrs or to use the culture filtrates after 2o days of storage. In the first case the surface colonization of the bacteria encounter the pathogen attack where as in the second

case, the inhibitory principle is the antibiotic produced by the bacteria which is acting as the curative agent. In case of cardamom, as indicated from the studies, panicle application by spraying culture suspension on the panicles could be effective in reducing the infection as well as the spread of the disease. Both cell free culture filtrate, culture suspension are effective in controlling infections. Culture filtrate should be taken only after incubating the cultures for at least 20 days as indicated from the experiments. There are also reports of foliar application of *Pseudomonas fluoresens* against leaf spot and rust disease of ground nut (Subramanian *et al* 1980). As it is known, rhizosphere colonization by the bacteria, it can also colonize the panicles and prevent infection or cause lysis of the hyphae or zoospores. It also supported the statement of Howie (1985) that spread of bacteria introduced into seed and their multiplication in the rhizosphere would be better. In recent years many investigators reported that seed coating method was effective to control soil borne pathogens (Baker 1968, Chang *et al* 1968). Here also instead of seed coating panicle coating prior to infestation is found practicable. Further studies are warranted for exploiting the potential of *Pseudomonas fluorescence* for field application for effective biological control of capsule rot disease of cardamom.

Literature cited

Baker ,R 1968 Mechanism of Biological control of soil borne plant pathogens Ann Rev. *Phytopathol* 6: 263-294

Chang, I-Pin and Kommedahl, T 1968 biological control of seedling blight of corn by coating kernels with antagonistic microorganisms. *Phytopathology* 58: 1395-1401

Howie, W.J.(1985) Factors affecting colonization of wheat roots and suppression of take-all by Pseudomonads antagonistic to Gaeumannomyces graminis var.tritici. *Ph.D Thesis* , Washington state university, Pullman 82pp.

Jin Hee Park and Hee Kyu Kim 1989 Biological control of Phytophthora crown rot and root rot of green house pepper with Trichoderma harzianum and Enerobacter agglomerans by improved method of application. *Korean J. Plant pathol.*5(1): 1-2

Subramanian P, Mehan V.K, D.J. Nevvil and Mc Donald 1980 Research on fungal disease of Ground nut. In ICRISAT proceedings of International Workshop on ground nut. Patencheru, India Pp 193-198.