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BIOLOGICAL CONTROL OF 'AZHUKAL' DISEASE OF SMALL CARDAMOM CAUSED *BY PHYTOPHTHORA MEADII* Mc Rae

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ABSTRACT

'Azhukal' or capsule rot of small cardamom (*Elettaria cardamomum* Maton) caused by *Phytophthora meadii* Mc Rae is the most serious fungal disease resulting in heavy crop loss. Control of this disease has been achieved by plant sanitation and routine application of fungicides. However, an attempt was initiated to manage this disease through non-chemical means by using fungi and bacteria antagonistic to *P. meadii*. The antagonistic activity of *Trichoderma viride, Trichoderma harzianum, Laetisaria arvalis* and *Bacillus subtilis* was evaluated against the pathogen both under *in vitro* and field conditions. The fungal antagonists tested under *in vitro* conditions interacted with the pathogens by inducing growth inhibition, anastomoses and hyphal lysis while the bacterial antagonist *B subtilis* suppressed the pathogen by growth inhibition. These fungal antagonists when applied in conducive soils reduced the disease potential index (DPI) which resulted in 'Azhukal' disease control of around 30 to 50 per cent.

INTRODUCTION

Capsule rot of small cardamom (*Elettaria cardamomum* Maton), popularly known as 'Azhukal' is the most important fungal disease caused by *Phytophthora meadii* Mc Rae. The disease occurs during South West monsoon season and lasts upto North East monsoon resulting in a crop loss of about 50%. Infection occurs on capsules and leaves of mature plants and in severe cases it extends to the whole plant parts. Since its first report (Menon *et al.* 1972), considerable work has been done on several aspects of the disease, particularly on fungicidal control (Menon *et. al.* 1973, Nambiar and Sarma, 1974, Kunhikrishnan Nair *et. al.* 1982). Plant sanitation coupled with timely application of fungicides has been found to give good control of 'Azhukal' (Joseph Thomas, *et. al.* 1989). Since, cardamom is an export oriented spice crop, fungicidal usage has to be restricted to minimize residual problems. The recent strategy in plant disease management is focussed on the use of various types of bio-agents which are antagonistic to pathogens. Antagonistic fungi such as species of *Trichoderma* are of wide use in controlling several plant diseases (Baker and Cook, 1974, Chet *et. al.* 1979). In the present investigation, attempts are made to study the mycoparasitism and bio-control potential of selected antagonists such as *Trichoderma viride, T. harzianum, Laetisaria arvalis* and *Bacillus subtilis* on *P. meadii*, the causal organism of 'Azhukal' disease.

MATERIALS AND METHODS

The studies were carried out both under laboratory and field conditions. The antagonism of bio-agents against *P. meadii* was studied by dual culture method in Potato dextrose agar (PDA) plates. The fungal antagonists viz., *T. viride*, Pers. Fr, *T. harzianum* Rifai and *L. arvalis* Burdsall obtained from Tamil Nadu Agricultural University, Coimbatore and the bacterial antagonist *B. subtilis* (Ehrenberg Conn) obtained from Kerala Agricultural University, Trichur were used for the studies.

Growth inhibition of *P. meadii* against the antagonists was studied in dual cultures, in petri plates. Five mm size discs of *P. meadii* and the fungal antagonists were inoculated in PDA plates at a distance of 5 cm from each other. The inoculated plates were incubated at 28° C for 10 days and the mean colony diameter of pathogen and antagonists were measured at 24h intervals. The growth rates of antagonists and the pathogen were determined individually in monocultures. The mean colony diameter of the pathogen in dual cultures were compared to that in single cultures and per cent growth inhibition was calculated. The bacterial antagonist subtilis

was streaked on both sides of discs of *P. meadii* at a distance of 2.5 cm and incubated for 8 days at 28°C. The mean breadth of *P. meadii* colony facing the bacterial colony was measured as against that in monocultures and the per cent growth inhibition was calculated. For studying the mycoparasitism, small portions of intermingling areas were randomly selected for microscopic observations.

Effect of antagonists on *P. meadii* infection on cardamom seedlings was studied in green house and on mature plants under field conditions. Three months old cardamom seedlings of cultivar Malabar, raised in earthen pots of 15 cm diameter were used for the study. Seven day old well sporulated cultures of the fungal antagonists thoroughly blended in sterile water and diluted to a concentration of 5×10^5 to 6×10^5 colony forming units (CFU) were used. Inoculation was carried out by drenching each pot with 100 ml of this suspension. *B subtilis* was grown on nutrient broth for 10 days and 100 ml of this at a concentration of 6.5×10^5 CFU was inoculated similarly. Inoculum of the pathogen also was prepared in a similar manner and the pots were inoculated 8 days after the application of the antagonists. Observations were recorded as percentage disease incidence and seedling mortality.

The bio-control efficacy of these antagonists were further evaluated under field conditions. For this, sick plots in two locations showing severe 'Azhukal' incidence due to *P. meadii* infection were selected. Four replicated plots containing 12 plants each were randomly selected for each of the antagonists. First application of the antagonists was carried out in May before the onset of South West monsoon rains. Thirty days later, a second application of antagonists was given. Inocula of antagonists were prepared as in the case of green house tests. Macerated cultures diluted in water to a concentration of 5×10^5 CFU were applied in the respective plots as soil drenching at the plant base @ 2l/plant. The control plots were kept uninoculated. Observations on mean disease incidence in each plot were recorded as percentage capsule infections at thirty days intervals.

The population levels of *P. meadii* in these plots were assessed by leaf baiting technique using *Delonix regia* (Tsao, 1960) before giving differential treatments and also three months after treatment. The fungal antagonists mass multiplied on well decomposed farm yard manure (FYM) was used as inocula during the second year of the field trials. About 500 gm of FYM containing antagonists were used per plant.

RESULTS AND DISCUSSION

In dual cultures where antagonistic fungi were plated against *P. meadii*, the former grew luxuriantly and much faster than the latter. Marked reduction in growth rate of *P. meadii* towards the side of the antagonist was evident even from third day onwards after plating. The growth rates of the pathogen against the fungal antagonists were presented in Fig 1. In paired cultures, *P. meadii* grew freely upto 48 hours until its edges touched the antagonists. The antagonists overgrew the pathogen and suppressed it from further growth. The growth inhibition caused by *Trichoderma* spp. and *L. arvalis* was almost comparable to each other. Similar overgrowth of *Trichoderma* Species over pathogen has been reported earlier Mukherjee *et al.* 1989).

Microscopic observations of intermingling areas of antagonistic fungi and *P. meadii* showed that the antagonist hyperparasitized the latter from 72h onwards after pairing. The mycoparasitism was in the form of hyphal coiling, anastamoses and hyphal lysis. Similar types of mycoparasitism has earlier been reported with *T. harzianum* on *Pythium* sp. (Bell *et. al* 1982). *T. viride* caused lysis of haphae of *P. meadii* in their intermingling areas. Elad *et al.* (1982, 1983) correlated such lysis with the secretion of wall dissolving enzymes by the antagonists.

The *in vitro* interaction of the bacterial antagonist *B. subtilis* on *P. meadii* was different from that of the fungal antagonists. The *P. meadii* colony grew in a linear fashion along side the bacterial streaking. Mycellial growth towards *B. subtilis* was arrested by clearly visible inhibition zones. The pathogen grew freely away from the bacterial side and produced more aerial hyphae than prostrate hyphae. In no case the fungal hyphae was found touching the bacterial colony. Similar inhibition of *Phytophthora* spp. caused by *B. subtilis* has been reported earlier (Podile

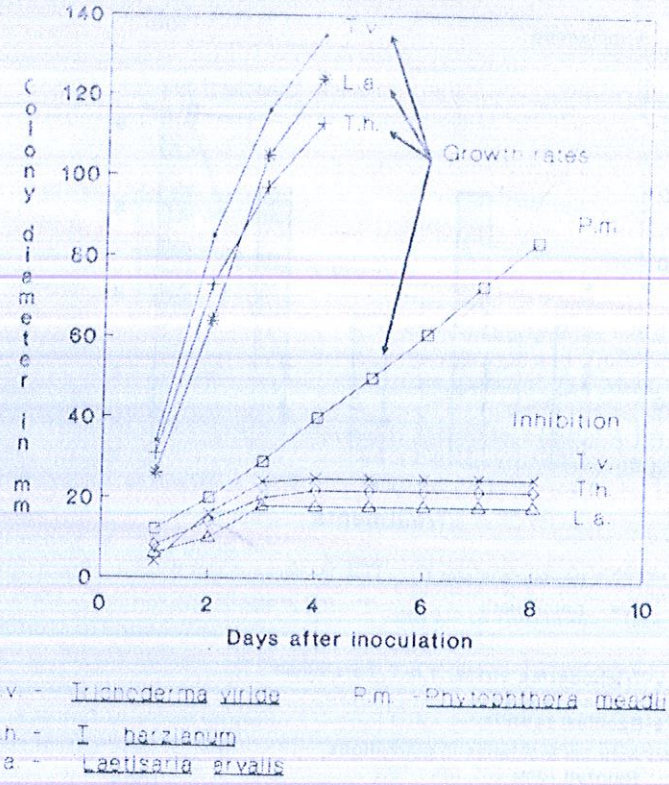


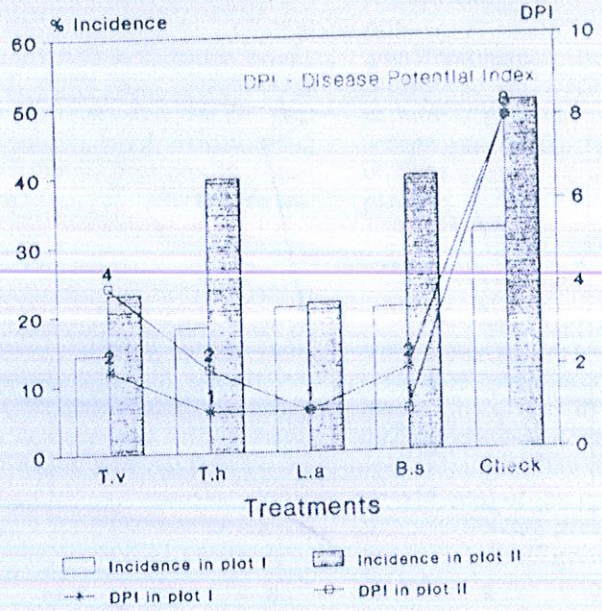
Fig.1 Effect of antagonists on the growth of *P. meadii*

and Dube, 1987) and such growth inhibition has been attributed to the antifungal activity of *B. subtilis* (Baker *et. al.* 1983; Singh and Deverall, 1984).

The bio-control effects of the antogonists on *P. meadii* infection in seedlings are presented in Table I. In control plots where the pathogen alone was inoculated without the antagonists, the infection and mortality rates were higher. Infection due to *P. meadii* was expressed in the form of lesions on leaves, drooping of leaves and wilting of seedlings. In severe cases, the collar and roots showed rotting resulting in the death of seedlings. Pots inoculated with the antagonists showed reduced infection and mortality rates as compared to control pots where no antagonist was applied. Among the antagonists, *T. viride* offered the maximum disease control followed by *L. arvalis*.

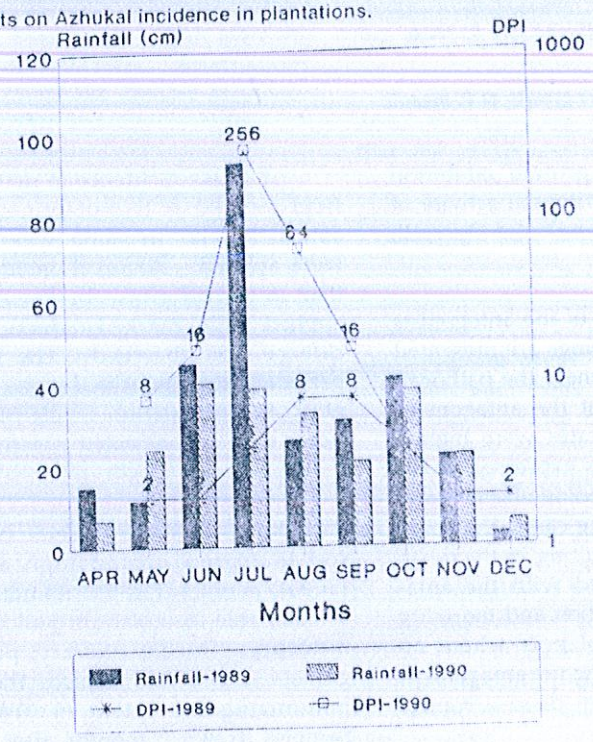
Disease incidence in mature plants in the field treated with antagonists also showed similar trends. In plants where no antagonists were applied, 'Azhukal' incidence was higher than in treated plots. In *T. viride* and *L. arvalis* applied plots, marked reduction in disease incidence was observed. The DPI determined periodically showed that in antagonists applied plots, the population levels of *P. meadii* were lesser than in untreated control plots (Fig. 2).

Observations recorded on rainfall and DPI data showed that the *P. meadii* propagules in the soil increased during high and continuous rainfall periods (Fig 3). Rainfall seems to play a major role in inoculum build up and disease incidence. On the contrary, presence of antagonists in the soil reduces the DPI there by minimizing the extent of disease incidence. Samples drawn 3 months after the application of antagonists showed that the population levels



T.v-Trichoderma viride; T.h-T. harzianum
L.a-Laetisaria arvalis;
B.s-Bacillus subtilis;

Fig. 2. Effect of antagonists on Azhukal incidence in plantations.



DPI - Disease potential index

Fig. 3. Effect of rainfall on DPI in sick soils.

Table I. Effect of antagonists on *P. meadii* infection in cardamom seedlings.

Treatments	Infection (%)	Mortality (%)	Disease control (%)
Trichoderma viride	36.70	24.20	54.76
T. harzianum	40.20	33.30	38.00
Laetisaria arvalis	33.45	28.50	46.70
Bacillus subtilis	46.25	42.50	20.56
Control *(P. meadii)* (No antagonist)	58.50	53.50	-

of *P. meadii* were not increased as compared to untreated control plants. Evidently, the application of antagonists reduced the extent of disease incidence. *Trichoderma* spp. has already been reported to be effective in managing rot diseases of several crop plants (Okamoto and Isaka, 1988; Tsao *et al.*, 1988). The results of our studies show that application of antigonists such as *Trichoderma* spp and *L. arvalis* in sick soils greatly reduced the incidence of 'Azhukal' disease of cardamom and also resulted in lower population levels of *P. meadii* in the soil.

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DISCUSSION

P.K. RAMESH : What is the quantity of isolates required for effective control of Azhukal on plant or area basis? Under normal shaded conditions in cardomom field, how is the control and multiplication of the isolates possible?

R. SUSEELA BHAI : Our studies show that applying 2 litres of culture suspension of antagonist at a concentration of 5×10^7 CFU / ml / plant twice is effective in controlling the disease. This is done under the normal cardamom growing condition.

B. CHANDRA MOULI : What is the survival rate of the *Trichoderma* under field conditions? If you are recommending biocontrol how many applications and at what intervals? Is one application sufficient?

R. SUSEELA BHAI : The extensive studies on these aspects are going on. The number of applications and its periodicity for large scale field control can be determined based on survival rate, which is being studied.

ROHINI IYER : Have you tried native isolates in comparison to Coimbatore isolates? Have you tried combination of antagonists? How do the antagonists behave under flooded conditions?

R. SUSEELA BHAI : Yes. This also gave similar results. In separate experiments the combination of antangonist was tried. As flooded condition is not a feature in high ranges, this aspect was not studied.

K.V. PETER : Can you list out antagonistic fungi recorded in the cardamom growing tract of high ranges? Why the natural antagonistic system gets disturbed in favour of pathogens?

R. SUSEELA BHAI : *Trichoderma herzianum* and *T. viride* are isolated from cardamom soils. As the natural antagonistic population is very low, we have to multiply them and apply in the field for effective biocontrol.

M.K. NAIR : Whether the fungal application is prophylatic or control measure? What will happen when already infected plant is given fungal application?

R. SUSEELA BHAI : The antagonist application should be prophylatic. Further infection can be checked.

P.K. KOSHY : Have you isolated all the four antagonists from infected cardamom capsules? If not, what is the source of antagonists?

R. SUSEELA BHAI : No, not from cardamom capsules. But we have isolated *Trichoderma* species from cardamom soils of different locations. The present *Trichoderma* isolates were obtained from TNAU, Coimbatore.