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Evaluation of *Trichoderma* spp. for the control of seedling rot disease of cardamom (*Elettaria cardamomum* Maton)

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Abstract

Trichoderma viride and T. harzianum were evaluated as seed dressing prior to sowing of seeds and as soil application in nursery beds at the time of transplanting cardamom (*Elettaria cardamomum*) seedlings for the control of seedling rot or damping off caused by *Pythium vexans*. Coating of seeds with *Trichoderma* spp. did not alter the percentage of germination but reduced the percentage of damping off in sick soil. Soil drenching of *Trichoderma* spp. mascerates 1 week prior to transplanting of seedlings in sick soils was effective in reducing seedling mortality compared to *Trichoderma* application after transplanting or as seedling dip.

Key words : cardamom, *Elettaria cardamomum, Pythium vexans,* seedling rot, *Trichoderma harzianum, Trichoderma viride.*

Introduction

Damping off or seedling rot disease caused by *Pythium vexans* de Bary is commonly observed in cardamom (*Elettaria cardamomum* Maton) nurseries in Kerala and Karnataka. Raising seedlings in formalin fumigated nursery beds (Pattanshetty *et al.* 1974) and using fungicides such as Emisan and Mancozeb (Thomas *et al.* 1988) were reported to be effective in controlling the disease. As disease management strategies are focussed on biocontrol agents in recent years, the efficacy of antagonistic fungi namely, *Trichoderma* spp. was evaluated for the control of nusery rot disease and raising of healthy seedlings of cardamom.

Materials and methods

The experiment was conducted in pot cultures under greenhouse conditions at Myladumpara (Kerala, India) in two phases. In the first phase, the effectiveness of *Trichoderma* spp. as a seed dressing agent was tested to study its efficacy in improving seed germination and preventing seedling rot incidence. Seeds of *vazhukka* variety were coated with sporulated suspension of *T. viride* and *T. harzianum* and were sown in 8"

x 12" size earthernware pots containing sterilized soil and *Pythium* sick soil. The control consisted of uncoated seeds sown in healthy and sick soils. For coating the seeds, *T. viride* and *T. harzianum* suspensions were prepared by mascerating fully sporulated 5 day old PDA cultures (1 plate culture per 10 ml water) and soaking the seeds in it overnight. Later the seeds were air dried and sown. Sick soil was made by artifically inoculating sterilized soils with *P. vexans* isolated from seedling rot affected seedlings. Fully grown 7 day old sporulated cultures of *P. vexans* were used for soil inoculation at the rate of 2 plates per 5 kg of soil per pot. The PDA cultures were mascerated in water and the suspension was mixed with soil. The pots were kept moist and after 14 days, the healthy and *Trichoderma* coated seeds were sown in these pots (Table 1).

In the second phase of the experiment, *Trichoderma* in suspension form was tested on seedlings at the time of transplanting. Healthy and *Pythium* sick soils in pots were prepared as described earlier and the pots were kept ready for transplanting. Pots containing sterile soils were inoculated with *P. vexans* 14 days prior to transplanting so that the pathogen could establish. Liquid formulations of *T. viride* and *T. harzianum* were prepared as described earlier. Disease free healthy seedlings obtained from *Trichoderma* coated and uncoated seeds sown in healthy soils in the first phase were used as the planting material in the second phase. The seedlings were of 3 to 4 leaf stage and about 3 months old. The experiment was laid out in pot cultures in a RBD with nine treatments (Table 2).

Three methods of *Trichoderma* application were followed at the time of transplanting. These were : seedling dip in *Trichoderma* suspension at the time of planting, drenching the suspension 1 week before planting in sick soils, and drenching the suspension 1 week after transplanting. Untreated seedlings planted in sick soil served as control. The inoculated seedlings were main-

Table 1. Effect of *Trichoderma* coating on seed germination and seedling rot incidence in cardamom in healthy and sick soils

Treatment	Seed germination (%)	Disease incidence (%)
Healthy soils		
T1 - *T. viride* coated seeds	48.05 (43.92)	6.02 (7.35)
T2 - *T. harzianum* coated seeds	50.20 (45.12)	4.47 (8.69)
T3 - Uncoated seeds	51.45 (46.21)	9.00 (15.66)
Sick soils		
T4 - *T. viride* coated seeds	51.40 (45.80)	1.36 (4.40)
T5 - *T. harzianum* coated seeds	39.10 (38.15)	11.73 (15.63)
T6 - Uncoated seeds	47.30 (43.08)	18.06 (18.38)
C D at 5%	NS	NS

Figures in parentheses are arcsine transformed values
NS = Not significant

Table 2. Incidence of seedling rot in *Trichoderma* treated cardamom seedlings planted in sick soils

Treatment	Disease incidence (%)
T1 - Seedlings raised from *Tv* coated seeds	70.00 (57.10)
T2 - Seedlings raised from *Th* coated seeds	40.00 (38.95)
T3 - Seedlings dipped in *Tv* suspension	66.88 (55.28)
T4 - Seedlings dipped in *Th* suspension	44.00 (40.86)
T5 - *Tv* drenched while transplanting	35.00 (36.06)
T6 - *Th* drenched while transplanting	50.00 (45.00)
T7 - *Tv* drenched 7 days before transplanting	8.30 (12.02)
T8 - *Th* drenched 7 days before transplanting	5.00 (6.64)
T9 - Untreated control	65.00 (53.94)
CD at 5%	17.10

Figures in parentheses are arcsine transformed values
Tv = Trichoderma viride ; Th = Trichoderma harzianum

tained in pots in the greenhouse and observations on disease incidence were recorded.

Results and discussion

Seed dressing with *Trichoderma* spp. did not alter the percentage of seed germination in healthy or in sick soils. Uncoated seeds sown in healthy and sick soils had higher disease incidence while *Trichoderma* coated seeds sown in healthy and sick soils had lesser percentage of disease incidence. However, this was statistically not significant (Table 1).

In the second experiment, where all the healthy seedlings were planted in sick soils, the effect of *Trichoderma* spp. on disease control varied in different treatments. In T1 and T2 where the transplanted seedlings were raised from *Trichoderma* coated seeds, there was a high disease incidence when planted in sick soil. The seedlings failed to survive in sick soils although they received *Trichoderma* 3 to 4 months before (at the seed stage) indicating that seed dressing does not give protection at a later stage. In treatments T3 and T4 where seedling dip in *Trichoderma* suspension was done at the time of transplanting, there was no reduction in disease incidence. Significant reduction in disease incidence was noticed in treatments T7 and T8 where *Trichoderma* was applied in sick soil 1 week before transplanting the seedlings. In these two cases, since application was done 1 week prior to planting of seedlings, *Trichoderma* would have had adequate time for multiplication and antagonism in the soil against *Pythium* resulting in lesser incidence of the disease in seedlings planted in these pots. In all other cases, the period for *Trichoderma* multiplication and its establishment in the soil was too short before initiation of infection by the already established *Pythium* in the soil.

Seed treatment with antagonists has been reported as an effective method for protection of young seedlings against soil pathogens (Mukhopadhyay *et al.* 1992). In our experiments, seed coating was only partially helpful in reducing the disease incidence. Pretreatment of

sick soils with *T. harzianum* has been reported to the advantageous in offering better protection to various crops (Ordentlich & Chet 1989). Greff & Duinevel (1992) have stressed the need for application of bioagents sufficiently in advance in sick soils prior to planting of tulips for controlling *Pythium* root rot. The quantity of inoculum of the antagonist to multiply and establish in the rhizosphere and its competancy to suppress the pathogen seem to contribute to the extent of protection from pathogens. Our results show that if *Trichoderma* spp. is applied in soil before the establishement of the pathogen, seedling rot incidence in cardamom nurseries can be greatly reduced. A better package would probably be seed coating with *Trichoderma* followed by repeated soil application of *Trichoderma* before initiation of infection. However, further studies are required to quantify the population levels and duration of antagonists in sick soils for ideal crop protection.

References

Greff R D E & Duinevel D T 1992 Towards biological control of *Pythium* root rot in forced tulips. Bulletin OILB/SROP 15 : 45-47.

Mukhopadhyay A N, Shrestha S M & Mukherjee P K 1992 Biological seed treatment for control of soil borne plant pathogens. FAO Plant Prot. Bull. 40 : 21-30.

Ordentlich A & Chet I 1989 Biological control of soil borne plant pathogenic fungi by antagonistic *Trichoderma* in Israel. Agre Serch 3 : 137-152.

Pattanshetty H V, Deshpande R S & Shivappa T G 1974 Effect of different fungicides on control of damping off of cardamom seedlings. J. Plantn. Crops 2 : 34.

Thomas J, Naidu R & Bhai R S 1988 Rhizome rot and root rot disease of cardamom - a review. J. Coffee Res. 18 (Suppl.) : 38-45.

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Greff R D E & Duinevel D T 1992 Towards biological control of *Pythium* root rot in forced tulips. Bulletin OILB/SROP 15 : 45-47.

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Sclerotium rot - a minor disease of vanilla (*Vanilla planifolia* Andrews)

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Abstract

During a survey in vanilla (*Vanilla planifolia*) growing tracts of Moovattupuzha in Ernakulam District (Kerala, India) a new type of bean rot affecting vanilla beans was observed. The disease was characterized by rotting of bean bunches and subsequent development of thick fungal mat over the bean surface. The causal organism was identified as *Sclerotium rolfsii*, the fungus was brought into pure culture and its pathogenicity proved.

Key words: anthracnose, *Sclerotium* rot, *Sclerotium rolfsii*, Vanilla, *Vanilla planifolia*.

Introduction

Natural vanillin is one of the world's most favoured spices, and is extracted from the cured fruits or beans of *Vanilla planifolia* Andrews. Vanilla is a perennial fleshy climbing orchid cultivated in several tropical countries. Vanilla is susceptible to a number of fungal diseases such as root rot and wilts caused by *Fusarium* spp., anthracnose by *Calospora* sp., leaf spot caused by *Colletotrichum* spp. (Purseglove *et al.* 1981) and a

few viral diseases (Pearson *et al* .1991) which cause considerable damage to the beans or to the whole plant resulting in heavy crop losses.

A survey was conducted during the south west monsoon season of 1999 in some of the vanilla growing areas near Moovattupuzha of Ernakulam District (Kerala, India) to study the occurrence of various diseases on vanilla. During the survey, a new type of disease affecting the beans was noticed from Ramamangalam . The disease appeared as rotting of a few or all the beans in a bunch. The rotting was initiated from the bean tips and advanced towards the stalk regions. The infected bunch was covered with a thick white feathery mycelial mat of the fungus especially on the distal portions of the beans. The infected beans showed rotting symptoms with deep sunken wound like areas, which appeared reddish brown in colour. Some of the beans were completely rotten. Running threads of fungal mat were also seen on the leaves and beans and rarely on stem also. Such plant parts also showed reddish brown sunken lesions.

Laboratory studies

The infected beans were brought to the laboratory and after surface sterilization, portions were plated in Potato Dextrose Agar Medium. The fungal growth on the infected specimens was also plated in PDA for isolation of the fungus in pure culture. In both cases a pure white-colored fungal growth was obtained which was very similar to the feathery white fungal strands observed on the infected beans in the field. Within 5 to 7 days, small creamy white sclerotial formations were noticed at the margins of the colony. On further incubation, creamy white sclerotia increased in size and changed its color to light brown and later to chocolate brown. The sclerotia measured 1-3 mm in diameter. More than 100-125 sclerotia were formed in a single PDA plate culture.

Pathogenicity studies

Pathogenicity of the fungus was tested on healthy beans using both the sclerotia and mycelial bits of the fungus obtained in the culture media. The sclerotia and 3 mm size culture discs of mycelium were inoculated separately to healthy beans, leaves and stems. The inoculated portions were kept moist by keeping a wet cotton pad, and covered with a polythene bag and incubated at 20-22° C. Three to four days after inoculation, fungal growth appeared on the beans and within 10 days the symptoms observed in the field developed. The symptoms observed include development of rotten patches with sunken surface areas and these extended to the whole-infected area. The formation of sclerotia were observed in later stages as rotting extended to other portions in inoculated beans.

Culture characteristics and identification

In culture media the colonies were fast growing, reaching about 9 cm diam. within 3 days after incubation at 23°C. The mycelium was white and thick with many hyphal strands arising along the sides of the culture flasks or petriplates. Sclerotia were formed superficially and these were produced near the margin of the colony. The sclerotia were globose, smooth and found sufficient to initiate infection as shown by the pathogenicity tests. The fungus was identified as *Sclerotium rolfsii* Sacc.(Teleomorph = *Athelia rolfsii* (Curzi)Tu & Kimbrough, Syn. *Corticium rolfsii* (curzi) (Domsch *et al* .1980). It is an important plant pathogenic state of Athelia species. It belongs to Deuteromycetes of order Sterile Mycelia (Ainsworth *et al.* 1973).

S. rolfsii Sacc. a soil borne pathogen causes blight and root and stem rot in tropical and subtropical countries on more than 500 species of plants in about 100 dicotyledonous families comprising mostly compositae and leguminosae (Aycock 1966). It thrives well

at 25-35°C with high moisture, and attacks crowded plants on shady habitats, while in dry soil the infection tends to occur below the soil surface. The principal propagules are the sclerotia produced by the fungus.

S. rolfsii has earlier been reported as a common pathogen in crops such as ginger and turmeric (Nair & Menon 1983). In ginger, the fungus causes thread blight disease while in turmeric it causes basal rot in the field and storage rot at the post harvest stage. A similar type of vanilla bean rot caused by *S. rolfsii* has been reported from China (Huang Quiping 1995). However this is the first report of the occurrence of this fungus on vanilla from India.

References

Ainsworth G. C, Frederic K. Sparrow & Alfred S. Sussman 1973 The Fungi – An Advanced Treatise Vol. IVB. Academic Press, INC

Aycock R 1966 Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C Agric. Expt.Stn. Tech. Bull.174(1).

Domsch K. H, Gams W & Traute-Heidi Anderson 1980 Compendium of Soil Fungi Vol.1. Academic Press London.

Huang Quiping 1995 Sclerotium rot infection on Vanilla fragrans and its control. Subtropical- plant –Research Communications (China) 24(1)

Nair M C & Menon M R 1983 Diseases of Crop Plants of Kerala. Kerala Agricultural University. Vellanikkara, Trichur, Kerala.

Pearson M N Jackson G V H, Zettler F W & Frison E A 1991 FAO/IBPGR Technical Guidelines for the safe movement of Vanilla Germplasm. Edited by M.N. Pearson , GVH Jackson, FW Zettler, & EA Frison .

Purseglove J W, Brown E G, Green C L and Robbins S. R. J 1981 Spices Volume 2 Longman Scientific and Technical, Tropical Agriculture Series (Eds.) G. Wrigley. AICTA.