

## Use of Antagonists to Control the Oospore Infection in Pearl Millet Downy Mildew

S. SHISHUPALA and H.S. SHETTY

Department of Studies in Applied Botany, University of Mysore, Manasagangotri, Mysore-570 006

### ABSTRACT

Four fungi viz., *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *Chaetomium globosum* and one bacterium *Bacillus subtilis* were evaluated for their efficacy in reducing downy mildew incidence in pearl millet due to oospore infection under green house conditions. All the antagonists applied to the soil along with wheat bran decreased the downy mildew incidence and increased the plant stand and growth of pearl millet seedlings. The possibility of using these antagonists to reduce the downy mildew disease due to oospore inoculum has been discussed.

Key words : Pearl millet, downy mildew, Oospore infection, Antagonists, Soil-amendment, *Trichoderma* spp., *Bacillus subtilis*

*Sclerospora graminicola* (Sacc.) Schroet., an oomycetous fungus, is a serious threat to pearl millet cultivation. Since the pathogen causes downy mildew systemically, earlier workers used different fungicides to control the disease. Though metalaxyl is effective in combating this disease, it is not readily available and not economical. Further, several pathogens have developed resistance to this chemical (Reuveni *et al.*, 1980; Bruin and Edgington, 1981; Cohen and Samoucha, 1984; Wiglesworth *et al.*, 1988). Moreover, the resistance to metalaxyl in some of the pathogens also makes them more tolerant to other N-Phenylamide systemic fungicides, thus narrowing the choice of effective fungicidal strategies for control. This prompted us to look for antagonists for disease management.

The oospores of the pathogen not only infest the soil but stick on to the surface of the seeds serving as primary inoculum. Large amounts of oospores are produced in downy mildew-infected plants which get incorporated in the soil during natural shredding of the crop or during harvesting and these oospores can survive in the soil up to 10 years (Safeulla, 1976). Hence the present investigation was aimed at reducing the soil borne inoculum by soil amendment with the antagonists so that the primary downy mildew incidence can be reduced.

### MATERIALS AND METHODS

The fungal antagonists like *Trichoderma harzianum* Rifai., *T. viride* Pers. ex Gray, *Aspergillus niger* van Tiegh. and *Chaetomium globosum* Kunze ex Steud. were isolated from soil where pearl millet was grown regularly. The bacterial an-

tagonists *Bacillus subtilis* (Cohn) Prazmowski was obtained from the Microbiology Discipline, CFTRI, Mysore. They were multiplied on wheat bran water in the ratio of 1:2 (w/v) in one liter Erlenmeyer flasks. The 15-day-old culture of each antagonist was added to the unsterilized soil at the rate of 2% (w/w). About 4 Kg of soil amended with antagonist culture was taken in 24 cm diameter earthen pots. Leaf debris containing oospores collected in the previous year was added to each pot at the rate of one g/Kg of soil. The inoculum load of fungal antagonists was calculated by taking 0.1 g of wheat bran culture of each antagonist into 10 ml of sterile water and spore count was made using a haemocytometer. Inoculum load of *B. subtilis* was determined by taking one gram of wheat bran culture into 100 ml of sterile water and serial dilution method was followed. Colony counts were taken from each dilution after three days of incubation and inoculum load was represented as cfu (colony forming units). Downy mildew susceptible pearl millet hybrid HB-3 seeds were sown in the pots and watered regularly. Soil with only oospore inoculum served as control. For each treatment, five replicates were maintained. Downy mildew incidence was recorded at regular intervals upto 60 days and the percentage of disease incidence was calculated in each treatment. The infected seedlings were removed as soon as the downy mildew symptom was observed to prevent the secondary infection due to sporangial inoculum. The data were subjected to analysis of variance and Duncan's multiple range test.

### RESULTS AND DISCUSSION

*Trichoderma harzianum*, *T. viride* and *A. niger* showed profuse mycelial growth and sporulated very well in the wheat bran medium whereas, in

Table 1. Efficacy of antagonists against pearl millet downy mildew due to soil-borne oospores

Treatment	Antagonist population (10 <sup>8</sup> spores/Kg soil)	Mean downy mildew incidence (%)
<i>Trichoderma harzianum</i>	33	6.3 <sup>b</sup>
<i>T. viride</i>	20	0.8 <sup>c</sup>
<i>Aspergillus niger</i>	101	1.0 <sup>c</sup>
<i>Chaetomium globosum</i>	(-)*	2.6 <sup>bc</sup>
<i>Bacillus subtilis</i>	5.9**	4.8 <sup>bc</sup>
Control	0	23.2 <sup>a</sup>

Numbers in column followed by the same letter are not significantly different (P = 0.05) according to DMRT

\* The soil was amended with 2% mycelial mat

\*\* Represents the colony forming units (cfu)

case of *C. globosum*, only mycelial growth was observed without any sporulation even after 15 days. In *B. subtilis*, rotting was observed in wheat bran due to bacterial growth.

All the antagonists applied to the soil increased the plant stand and growth of the pearl millet seedlings. Early emergence of the seedlings from the soil was also noticed in antagonists treatment. Among the antagonists used, *T. viride* gave the maximum increase in the plant stand. Wheat bran-amended soil slightly influenced the growth of the seedlings, but not as much as in antagonists-amended soil.

The seedlings grown in soil amended with only oospore inoculum showed maximum incidence of downy mildew (23.2%) (Table 1). *T. harzianum* amendment of the soil significantly reduced the disease incidence from 23.2% to 6.3%. The lowest incidence of downy mildew occurred in *T. viride*-amended soil recording only 0.8% disease incidence. *A. niger* also reduced the downy mildew incidence significantly compared to control. Its effectiveness was as good as *T. viride*. Both *C. globosum* and *B. subtilis* reduced the disease incidence significantly when compared to control. Thus all the antagonists decreased the downy mildew incidence. No extensive work has been done in controlling obligate parasites like downy mildews using biocontrol agents. De Diaz and Polanco (1984) reported that *Rhizidiomycopsis* sp. and *Phylyctochytrium* sp. decreased the oospore inoculum of *Peronosclerospora sorghi* in the soil under immersion. *Humicola fuscoatra* and *Actinoplanes missouriensis* were reported as hyperparasites on oospores of some Peronosporalean members (Humble and Lockwood, 1981; Sutherland and Lockwood, 1984; Lockwood, 1985). All the antagonists used in the present study reduced the downy mildew incidence significantly indicat-

ing their efficacy to survive in the soil and their antagonism towards soil-borne oospores of *S. graminicola*. The exact mechanism of antagonism is not known. In field conditions, oospore inoculum acts as the primary source of inoculum which leads to systemic infection. The present finding indicates the potential of biocontrol for the downy mildew disease which causes heavy loss to pearl millet.

#### ACKNOWLEDGEMENT

The senior author is grateful to University of Mysore, for providing financial assistance.

#### REFERENCES

- BRUIN, G.C.A. and EDGINGTON, L.V. 1981. Adaptive resistance in Peronosporales to metalaxyl. *Can. J. Plant Pathol.*, 3, 201-206.
- COHEN, Y. and SAMOUCOA, Y. 1984. Cross-resistance to four systemic fungicides in metalaxyl resistant strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*. *Plant Dis.*, 68, 137-139.
- DE DIAZ, G.S. and POLANCO, D.C. 1984. Fungi parasitic on oospores of *Peronosclerospora sorghi* under immersion. *Agron. Trop. (Maracay)*, 34, 87-94.
- HUMBLE, S.J. and LOCKWOOD, J.L. 1981. Hyperparasitism of oospores of *Phytophthora megasperma* var. *sojae*. *Soil Biol. Biochem.*, 13, 355-360.
- LOCKWOOD, J.L. 1985. Approaches to biological control of soybean root diseases. *Plant Prot. Bull. (Taiwan)*, 27, 279-293.
- REUVENI, M., BYAL, H. and COHEN, Y. 1980. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Dis.*, 64, 1108-1109.
- SAFEULLA, K.M. 1976. *Biology and control of the downy mildews of pearl millet, sorghum and finger millet*. Wesley Press, Mysore, India, 304 pp.
- SUTHERLAND, E.D. and LOCKWOOD, J.L. 1984. Hyperparasitism of oospores of some Peronosporales by *Actinoplanes missouriensis* and *Humicola fuscoatra* and other actinomycetes and fungi. *Can. J. Plant Pathol.*, 6, 139-145.
- WIGELSWORTH, M.D., REUVENI, M., NESMITH, W.C., SIEGEL, M.R., KUC, J. and JUAREZ, J. 1988. Resistance of *Peronospora tabaciana* to metalaxyl in Texas and Mexico. *Plant Dis.*, 72, 963-967.