Nematol. medit. (1991), *19*: 211-212

Department of Nematology, Harayana Agricultural University, Hisar — 125004, India

EFFECT OF CULTURE MEDIA ON SPORULATION OF *PAECILOMYCES LILACINUS* AND ITS EFFICACY AGAINST *MELOIDOGYNE JAVANICA* IN TOMATO

by
F.A. Zaki[1] and D.S. Bhatti

Summary. Evaluation of different whole grain substrates for the sporulation of *Paecilomyces lilacinus* revealed that the fungus sporulated profusely on maize, gram, oats, rice and wheat. Maximum numbers of spores/g were observed on rice (52.8×10^8) followed by gram (24.5×10^7). *P. lilacinus* cultured on gram seed was the most effective in reducing the gall index (78.6%) and second-stage juveniles of *Meloidogyne javanica* (94.2%) and increasing plant growth. Percentage of eggs destroyed by the fungus was maximum (58.6%) with the fungus cultured in gram.

Paecilomyces lilacinus (Thom) Samson is able to parasitize eggs of *Meloidogyne* spp. The fungus has been used for the biocontrol of root-knot and cyst nematodes (Jatala, 1986; Davide and Zorilla, 1983; Dube and Smart, 1987), but has failed in certain instances (Dickson and Mitchell, 1985). The fungus has been cultured successfully on many substrates. However, their effect on the efficacy of the fungus for the control of nematodes has not been investigated. In this study various seeds were tested for the sporulation of *P. lilacinus* and their effect on its efficacy against *Meloidogyne javanica* (Treub) Chitw. infecting the tomato plant.

Materials and methods

The culture of *Paecilomyces lilacinus* (Peruvian) was obtained from Dr. J.N. Sasser, North Carolina State University, U.S.A. Whole seeds of wheat, maize, rice, oats and gram (*Cicer arietinum* L.) were soaked in water for 12-18 hours and then blot dried. Each batch of seeds was placed in a 500 c.c. Erlenmeyer flask, plugged with cotton and autoclaved at 1.0546 Kg/cm^2 pressure for 50 minutes. After 12 h, each batch was inoculated with 1 ml of spore suspension from 10-days-old *P. lilacinus* culture on PDA. The flasks were manteined at $28 \pm 1C°$ in a Biological Oxygen Demand incubator and tumbled daily to ensure a uniform fungal growth. The spore count after 10 days from initial inoculation was estimated by the method of Zaki and Bhatti (1988) and the fungus medium was used immediately.

Clay pots (15cm-diam) were filled with 1 Kg autoclaved sandy soil and waterered with a solution of fertilizers (NPK 10:5:5 and $Znso_4$). Ten eggs masses (about 4,000 eggs) of *M. javanica* and 5 g of seed fungus inoculum were added to the pots and a 35 day old tomato (cv. HS-101) seedling planted in each. There were four replications of each treatment and the experiment was arranged in a completely randomized design. Data were recorded on plant height, fresh shoot and root weight, root gall index, soil population of second-stage juveniles, number of eggs/egg mass, percentage of egg masses infected and eggs destroyed 40 days after transplanting. The data were analysed by the standard 'Analysis of Variance' method.

The plants were removed from the pots and the roots gently freed of soil. Second-stage juveniles were extracted from the soil by Cobb's sieving and modified Baermaum funnel method. The nematode suspension collected after 48 h was transferred to a measuring cylinder and after vigorous bubbling five 2 ml aliquots were pippetted and the number of juveniles counted with the aid of a stereoscopic microscope. Total juveniles per root were calculated.

The percentage of root galling was visually estimated and converted to 0-10 scale (Barker, 1985).

Five egg masses were randomly collected from infected roots and dissolved in sodium hypochlorite. All the contents were transferred to a graduated cylinder containing water and the numbers of eggs in five 2 ml aliquots were counted from which the total in the five egg masses was calculated.

Ten egg masses randomly collected from infected roots were treated with sodium hypochlorite solution for 2-3 minutes. Each mass was transferred on to a glass slide and crushed under a cover slip. The presence of fungal mycelium in the egg masses was assumed to indicate infection. The identification of the fungus was confirmed by colony characteristic of lilac colour by inoculating surface sterilized egg masses on potato dextrose agar.

[1] Associate Professor, Division of Entomology, S.K. University of Agricultural Sciences & Technology Shalimar, Srinagar — 191 121 — Kashmir.

The presence of fungal hyphae and spores inside the egg and the vacuolation of the aborted embryos was taken as indicative of egg destruction. Apparently healthy eggs were also counted. Empty shells of eggs were presumed to be hatched out, and therefore, counted as healthy eggs for the purpose of computing percent eggs destroyed.

Resulta and discussion

The fungus grew profusely on all of the grain media tested. The highest spore count was on rice (52.8×10^8/g), followed by gram (24.5×10^7/g), wheat (42.4×10^5/g), maize (12.5×10^6/g) and oats (44.7×10^5/g). Differences among substrates were significant. The higher count in rice may be attributed to the relatively larger surface area of the smaller seeds compared with the others, as well as its richer carbohydrate (78.2%) content.

Data on the effect of these substances colonized with *P. lilacinus* on tomato infected with *M. javanica*, showed maximum improvement in growth parameters in the treatments receiving the fungus cultured on gram seed followed by rice- fungus (Table I). Considering the nutritional aspect, gram in a rich source of N (3.33%), K (808mg/100g) and P (340mg/100g), which might be a contributing factor to the maximum shoot and root growth.

Gall index was significantly reduced in the treatments with the fungus cultured on gram, rice and maize (Table I) with the maximum reduction in gram-fungus; reduction in second-stage juveniles occurred in all of the treatments with the greatest in rice and gram colonized fungus. Since the egg masses were used as inoculum, it was expected that the fungus would infect egg masses and eggs at the pre-penetration stage and the egg laying stage of mature females later. The pre-penetration egg destruction could reduce the primary source of inoculum, which is important to mitigate crop damage. This seemed to hold good in the treatments with fungus colonized on gram and rice which had lower gall indices, probably because of the higher spore counts and presumably greater virulence of the fungus on gram and rice than other substrates.

Egg masses collected from the nematode-infested plants showed the highest per cent infection (post-penetration infection) in treatments with fungus cultured on rice and gram which is commensurate with the observation on population reduction. However, per cent eggs destroyed in infected egg-masses did not differ significantly among the various treatments. By and large, the fungus cultured on gram seeds was the best in improving plant growth, causing optimum egg mass infection and reducing the nematode population, despite the fact that gram without fungus could reduce the population of *M. javanica* on tomato (unpublished data). However, thorough, intensive and controlled experimentation is needed to finally prove that the method of production influences the efficacy of the fungus.

TABLE I - *Effect of different substrates on the efficacy of* Paecilomyces lilacinus *against* Meloidogyne javanica *and the growth of tomato plants.*

Fungus-Medium	Plant Height (cm)	Fresh shoot weight (g)	Fresh Root weight (g)	Gall Index	Juveniles/ pot	Eggs/ egg-mass	Egg-mass Infected (%)	Eggs Destroyed (%)
Maize	11.8^{d}	2.5^{d}	2.8^{d}	6.37^{b}	255^{bcd}	336^{a}	49.5^{bc}	46.5^{ab}
Gram	23.5^{a}	10.0^{a}	6.0^{a}	2.07^{d}	70^{de}	121^{b}	87.1^{a}	58.6^{a}
Oat	8.8^{e}	1.0^{e}	1.0^{d}	9.50^{a}	246^{bc}	406^{a}	35.3^{c}	24.6^{c}
Rice	15.9^{c}	4.5^{c}	3.1^{bc}	4.25^{c}	22^{e}	125^{b}	88.5^{a}	49.2^{ab}
Wheat	14.9^{c}	2.5^{d}	3.3^{bc}	9.30^{a}	266^{b}	303^{a}	54.3^{b}	35.7^{bc}
Control (Inoc.)	11.5^{d}	1.7^{de}	2.5^{c}	9.70^{a}	1250^{a}	314^{a}	—	—
Control (non-inoc.)	19.8^{b}	6.3^{b}	3.9^{b}	—	—	—	—	—

Figures in a column followed by the same letter do not differ significantly for P = 0.05.

Literature cited

BARKER K.R., 1985 — Nematode extraction and bioassays. *In*: (Eds K.R. Barker, C.C. Carter and J.N. Sasser). An Advanced Treatise on *Meloidogyne*, vol. II, Methodology. Plant Pathology N.C. State Univ. and USAID, North Carolina, pp. 19-35.

DAVIDE R.G. and ZORILLA R.A., 1983 — Evaluation of a fungus, *Paecilomyces lilacinus* (Thom) Samson for the biological control of the potato cyst nematode *Globodera rostochiensis* Woll. as compared with nematicides. *Phillipp. Agric.*, *66*: 397-404.

DICKSON D.V. and MITCHELL D.J., 1985 —Evaluation of *Paecilomyces lilacinus* as a biocontrol agent of *Meloidogyne javanica* on tobacco. *J. Nematol.*, *17*: 519. (Abstract.)

DUBE B. and SMART G.C.JR., 1987 — Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. *J. Nematol.*, *19*: 222-227.

JATALA P., 1986 — Biological control of plant-parasitic nematodes. *Annu. Rev. Phytopath.*, *24*: 453-89.

ZAKI F.A. and BHATTI D.S., 1988 — Economical method for mass culturing of *Paecilomyces lilacinus* (Thom) Samson. *Curr. Sci.*, *57*: 153.

Accepted for publication on 16 April 1991.