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# Growth of *Paecilomyces lilacinus* on Natural Substrates and Its Efficacy Against Citrus Nematode, *Tylenchulus semipenetrans*

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ABSTRACT

**Certain seeds, tubers and roots of plants were evaluated as substrates for mass production of the nematophagous fungus, *Paecilomyces lilacinus* (Thom.) Samson. Growth of *P. lilacinus* as measured by diameter of the colony, spore production, weight of mycelial mat and rate of growth indicated that wheat, bajra, jowar, and rice were suitable substrates for the multiplication of *P. lilacinus*. The population of *Tylenchulus semipenetrans* Cobb decreased markedly with increase in the inoculum level of *P. lilacinus*. However, application of *P. lilacinus* in the form of spore suspension was more effective than infected wheat grains in controlling *T. semipenetrans*.**

KEY WORDS: *Paecilomyces lilacinus*, substrates, biocontrol, *Tylenchulus semipenetrans*

The nematophagous fungus *Paecilomyces lilacinus* (Thom.) Samson has been reported to be effective against the citrus nematode *Tylenchulus semipenetrans* (Jatala, 1985). Investigations carried out for evaluation of certain substrates of plant origin for mass multiplication of this nematophagous fungus, and to test the efficacy of the fungus for the control of *T. semipenetrans* are reported in this paper.

## MATERIALS AND METHODS

### Effect of substrates on grwoth of *P. lilacinus*

Culture of *P. lilacinus* was maintained in slants and Petri dishes containing sterile oat meal agar medium prepared with oat meal at 25 g per 100 ml water and agar at 2 per cent. The suitability of certain substrates of plant origin for mass multiplication of *P. lilacinus* was evaluated by estimating the rate of fungal grwoth on agar and liquid media of the substrates as well as on sterilized substrates.

200 g of coarsely powdered grain or tuber cut into small pieces of 5 $mm^3$ was boiled with 500 ml of distilled water for 30 minutes. The substrate was strained through a muslin cloth and the volume made upto 1000 ml with distilled water. After adding agar at 2 per cent, the media were autoclaved (1.05 $kg/cm^2$) for 30 minutes and aseptically introduced into 10 cm dia sterile Petri dishes. The Petri dishes were inoculated with an 8 mm disc of freshly cut oat meal medium containing *P. lilacinus* mycelial growth on the top. Plain 2.0 per cent agar served as a standard of reference. Three replications were included for each substrate and all the Petri dishes were maintained in an incubator at 30 ± 1°C for 15 days. The fungal colony diameter was measured in opposite directions perpendicular to each other and the mean was calculated. Suspensions were prepared from 8 mm discs of fungal culture cut from each plate. The suspensions were serially diluted with sterile distilled water and spore density was estimated using a haemocytometer.

Substrate liquid media were prepared as mentioned earlier and apportioned in 250 ml Erlenmeyer flasks @ 80 ml per flask. These flasks were inoculated each with an 8 mm disc of fungal culture as described earlier. Flasks containing only sterile tap water served as standard and there were three replications for each treatment. The flasks were maintained in an incubator at 30 ± 1°C for 21 days and wet weight of mycelial mats were recorded after blotting out excess moisture.

Seventy g of each substrate was prepared as mentioned earlier and apportioned in 250 ml Erlenmeyer flasks. Sufficient quantity of sterile distilled water was added into the flasks and the substrates were allowed to soak for 60 min. The flasks were then sterilized and inoculated with 5 ml spore suspension of *P. lilacinus* and mixed well. Three replications were included for each substrate and the flasks were maintained in an incubator at 30 ± 1°C. The contents of the flasks were thoroughly shaken at regular intervals to promote uniform fungal growth. Twenty-one days after inoculation, growth of the fungus was rated

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**TABLE 1 Effect of certain substrates on growth parameters of Paecilomyces lilacinus**

| Name of substrates | | | Mycelial colony diameter (cm)[xz] | Spore density ($10^4$ spores/ml)[xz] | Mycelial wet weight (g)[yz] | Mean growth index[yz] |
|---|---|---|---|---|---|---|
| Scientific name | Common name | Part used | | | | |
| *Amorphophallus Campanulatus* Blume ex Dcne | Yam | Tuber | 5.8[bcd] | 32.0de | 3.23[c] | 2.7 |
| *Arachis hypogaea* L. | Groundnut | Shell | 3.6[a] | 4.6[a] | 2.14[b] | 1.0 |
| *Daucus carota* L. | Carrot | Root | 4.4[a] | 16.3[b] | 2.60[bc] | 1.7 |
| *Eleusine coracana* (L.) Gaentn | Ragi | Grain | 6.5[cd] | 17.0[b] | 3.10[c] | 3.7 |
| *Ipomoea batatas* (L.) Poir | Sweet potato | Tuberous root | 4.4[ab] | 19.0[bc] | 3.11[c] | 2.0 |
| *Oryza satiya* L. | Rice | Grain | 7.2[cd] | 26.6[cd] | 4.13[d] | 5.0 |
| *Pennisetum typhoides* Bunm | Bajra | Grain | 5.5[bc] | 23.0[bc] | 4.32[de] | 4.3 |
| *Solanum tuberosum* L. | Potato | Tuber | 5.6[bcd] | 25.0[cd] | 3.24[c] | 4.3 |
| *Sorghum vulgare* Pers. | Jowar | Grain | 7.5[d] | 35.3[e] | 4.74[e] | 4.7 |
| *Triticum aestivum* L. | Wheat | Grain | 6.4[cd] | 38.0[e] | 4.47[de] | 4.7 |
| *Control* | - | - | 3.5[a] | 3.6[a] | 1.33[a] | - |

x Recorded after 15 days of inoculation; mean of three replications.

y Recorded after 21 days of inoculation; mean of three replications.

z Data in columns followed by a common letter were not statistically different (P = 0.01) according to DMRT.

using a visual index of 1-5 scale (1 = No growth; 2 = Poor growth; 3= Moderate growth; 4 = Abundant growth; 5 = Very abundant growth).

### Efficacy of *P. lilacinus* in the control of *T. semi penetrans*

Six months old kagzi lime *(Citrus aurantifolia* (Christm.) Swingle seedilings grown under glass house conditions were transplanted singly in 25 cm dia earthen pots containing 5.0 kg of steam sterilized soil. Roots were inoculated with *T. semi-penetrans* @ 5000 freshly extracted second-stage juveniles per seedling. One month after inoculation, the seedlings were removed and *P. lilacinus* from wheat grain culture containing a spore load of 22.5 x $10^7$/g was completely incorporated into the pots at different concentrations (Table 2). Carbofuran was applied at the rate of 0.7 g a.i. per kg of soil. The seedlings were replanted in the treated pots and watered to saturate the soil. A control was maintained in which depotting and replanting procedures were followed without adding the nematicide or the fungus. All treatments were replicated seven times.

In another experiment, kagzi lime seedlings were inoculated with *T. semipenetrans* @ 5000 second-stage juveniles per plant. After one month, the fungal inoculum was applied at the same levels as in the first experiment. But, the inoculum was applied in the form of spore suspension prepared by agitating the infected grains with 500 ml water. Care was taken to add only 500 ml of spore suspension in all the treatments each containing 45 x $10^7$, 90 x $10^7$, 135 x $10^7$ and 180 x $10^7$ spores respectively. Water was added in case of control and all the treatments were replicated five times. The nematode population in soil and roots were estimated six months after fungal inoculation in both the experiments. The data were analysed statistically and differences between means were evaluated as per procedures given by Steel and Torrie (1980).

## RESULTS AND DISCUSSION

The fungal growth was generally greater on grain agar media than on tuber agar media. The maximum colony dia of 7.5 cm was recorded on jowar agar medium (Table-1). Wheat agar medium supported the highest spore density of 38 x $10^4$/ml followed by jowar and yam agar media with 35.3 and 32 x $10^4$ spores/ml. Rice and potato supported moderate spore densities of 26.6 and 25.0 x $10^4$ spores/ml respectively. When *P. lilacinus* was

**TABLE 2** **Effect of methods of application of *Paecilomyces lilacinus* on the population development of *Tylenchulus semipenetrans***

| Treatments (spores/kg soil) | Fungal ingected grain | | Fungal spore suspension | |
|---|---|---|---|---|
| | Total nematode population* (soil + root) | % reduction over untreated control | Total nematode populatiron * (soil + root) | % reduction over untreated control |
| $45 \times 10^7$ | $23047^e$ | 30.1 | $13553^c$ | 49.1 |
| $90 \times 10^7$ | $17679^d$ | 46.4 | $8883^b$ | 66.6 |
| $135 \times 10^7$ | $11683^c$ | 64.6 | $1157^a$ | 95.7 |
| $180 \times 10^7$ | $9350^b$ | 71.7 | $585^a$ | 97.8 |
| Carbofuran | $3800^a$ | 88.5 | x | - |
| Control (untreated) | $32990^f$ | - | $26620^c$ | - |
| Control (uninfected grain @ 8g/kg) | $32343^f$ | 2.0 | x | - |

x Not included

* Data in columns followed by a common letter were not statistically different (P = 0.01) according to DMRT.

grown on substrate liquid media, the highest mycelial wet weights were recorded in substrates from jowar, wheat and bajra. The growth of *P. lilacinus* was very abundant on sterilized rice and abundant on jowar, wheat, bajra and potato. Sharma and Trivedi (1986) also reported good growth of the fungus on rice.

The results of the experiment on the control of *T. penetrans* with *P. lilacinus* revealed that there was a significant decrease in the total nematode population with increasing levels of fungal inoculum (Table-2). When *P. lilacinus* was applied in the form of infected wheat grain @ $180 \times 10^7$ spores per kg soil, the final population of *T. semipenetrans* was reduced by 72 per cent. However, application of *P. lilacinus* in the form of spore suspension was more effective than the application of fungus-infected grains in controlling *T. semipenetrans*. There was 49.1 per cent reduction in nematode population even at the lowest inoculum level of $45 \times 10^7$ spores whereas 97.8 per cent reduction was recorded at the highest inoculum level $180 \times 10^7$ spores. Similarly Jatala (1985) reported that *P.lilacinus* controlled *T. semipenetrans* more effectively than carbofuran and increased the diameter of orange fruits.

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