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Evaluation of Plant Leaves, Oil Cakes and Agro-industrial Wastes as Substrates for Mass Multiplication of the Nematophagous Fungus, *Paecilomyces lilacinus*

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ABSTRACT

The suitability of leaves of certain plant species, oil cakes, as well as waste products of sago industry were evaluated as substrates for mass multiplication of the nematophagous fungus, *Paecilomyces lilacinus* (Thom.) Samson. Leaves of subabool and neem were found to be suitable substrates and supported a higher spore load than tanners cassia, *Pongamia* and *Glyricidia*. Tapioca tuber and its peels also supported a moderately high spore load. Untreated thippi supported the least spore load. However, the spore load of *P. lilacinus* was significantly increased when thippi was soaked in KH_2Po_4 and $NaNo_3$ at 0.75% concentration. The oilcakes favoured the growth of *P. lilacinus*.

KEY WORDS: *Paecilomyces lilacinus*, substrates, plant leaves, agro-industrial wastes, oil cakes

In recent years, there is a renewed interest in the use of biocontrol agents in nematode management due to the high cost of nematicides and their risk to human beings and the environment (Jatala, 1985). The nematophagous fungus, *Paecilomyces lilacinus* (Thom.) Samson has been identified as an effective biocontrol agent of *Meloidogyne* spp. and *Globodera* spp. (Jatala *et al.*, 1979) and *Tylenchulus semipenetrans* Cobb (Anonymous, 1987). Certain cereals, millets, roots and tubers were identified as ideal substrates for mass multiplication of *P. lilacinus* (Ramakrishnamurthy, 1987). But, their economic importance as sources of food supply as well as the high cost involved in establishing the fungus in the field with infected grains, limit their commercial use. Hence, an attempt was made during the present investigations to evaluate certain commonly available plant leaves, oil cakes and agro-industrial waste products for their suitability as substrates for mass multiplication of *P. lilacinus*.

MATERIALS AND METHODS

Fresh leaves of five plant species *viz.*, *Glyricidia maculata* H.B. & K., *Azadirachta indica* Juss., *Pongamia glabra* Vent., *Leucaena leucocephala* (Lam.) de wit and *Cassia auriculata* L. were collected, washed with water and chopped into small pieces. The chopped leaves were taken in 250 ml Erlenmeyer flasks @ 20 g per flask. Broken sorghum and wheat grains, soaked in water for 30 minutes, were included for comparison and three replications were maintained for each treatment. The flasks were sterilized and inoculated with 2 ml of spore suspension of *P. lilacinus* and mixed well. The flasks were incubated at 30 ± 1°C in a B.O.D. incubator and the contents of the flasks were shaken periodically to promote uniform fungal growth. Ten days after inocubation, the spore load was estimated by vigorously shaking one gram of the substrate in 10 ml of sterile distilled water with two drops of Tween-20 using a haemocytometer.

The waste products of sago industry *viz.*, the sub- standard tapioca tubers, peels of tapioca tubers and thippi (the waste roughage obtained after the extraction of starch from tapioca tubers) were tested. Tapioca tubers and peels were cut into small pieces and half-cooked in boiling water for 15-20 minutes. Thippi was soaked in water or chemical solutions at 0.75% for 30 minutes and squeezed with muslin cloth. Twenty g of substrate and 1g of powdered rice bran were taken in a conical flask, sterilized, inoculated with 2ml of spore suspension and incubated at 30 ± 1°C. Three replications were maintained for each treatment.

The effect of four oil cakes *viz.*, castor, gingelly, groundnut and neem was tested on agar media. After powdering, 50 g was soaked in 200 ml of distilled water for 2 h with constant stirring. The suspension was filtered through Whatman No.1 filter paper. Agar was added to the extract @ 2%, sterilized and poured into 10 cm dia sterile Petri dishes. The Petri dishes were inoculated with spores of *P. lilacinus*. Plain agar and potato dextrose agar media served as standards. Three

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replicates were maintained in an incubator at 30 ± 1°C for 21 days. The diameter of the fungal colony was measured.

TABLE 1. Sporulation of *P. lilacinus* on leaves of certain plants

Substrate	Spore load/g* (x 10^7)
Sorghum grain	27.4^{c}
Wheat grain	22.5^{c}
Glyricidia leaf	5.3^{a}
Neem leaf	12.2^{b}
Pongamia leaf	8.8^{ab}
Leucaena leaf	13.0^{b}
Cassia leaf	9.2^{ab}

* Data in column followed by a common letter were not statistically different (P = 0.01) by DMRT

RESULTS AND DISCUSSION

Sorghum and wheat grains yielded the maximum spore production *viz.*, 27.4 and 22.5 x 10^7 spores/g respectively (Table- 1). Leaves of

TABLE 2. Sporulatiron of *P. lilacinus* on waste products of sago industry

Substrates	Spore load/g (x 10^7)
Tapioca tuber	10.9^{e}
Tapioca tuber peels	8.1^{cde}
Tapioca thippi	1.9^{a}
Thippi + KH_2PO_4	6.2^{bcd}
Thippi + $NaNO_2$	5.4^{abcd}
Thippi + $NaNO_3$	8.9^{de}
Thippi + $(NH_4)_2$ HPO_4	5.1^{abc}
Thippi + $(NH_4)_2$ SO_4	5.4^{abcd}
Thippi + urea	3.3^{ab}

Data in column followed by common letter were not statistically different (P = 0.05) by DMRT

subabool and neem yielded a moderately high spore production of 13.0 and 12.2 x 10^7 spores/g respectively. Production of spores on leaves of *Cassia* and *Pongamia* were on par with them. Mass multiplication of *P. lilacinus* on leaves has certain advantages over grains. Leaf sustrates are very cheap and easily available. Further, the degraded products of the leaves will also be effective against nematodes resulting in additive or synergistic reduction in nematode population. Earlier studies revealed that leaves of neem, *Pongamia* and subabool exhibited nematicidal properties against *T. semipenetrans* and *M. incognita* (Mani *et al.*, 1986; Venkata Rao *et al.*, *1986).*

The results (Table-2) revealed that tapioca tubers as well as peels yielded a good spore production of 10.9 and 8.1 x 10^7/g respectively. Thippi gave the least spore production. But soaking of thippi in various chemical solutions significantly enhanced the sporulation of *P. lilacinus*. $NaNo_3$ increased the spore load to the maximum of 8.9 x 10 7/g. It was followed by KH_2PO_4, $NaNo_2$ and $(NH_4)_2$ So_4 which were on par.

As oil cakes are commonly applied to fruit crops like citrus, their effect on the mycelial growth of *P. lilacinus* was studied. Results revealed that the mycelial growth of *P.lilacinus* on the oil cake agar media was better than in plain agar and PDA media (Table-3). Mycelial growth

TABLE 3 Growth of *P. lilacinus* on oilcake agar media

media	Mycelial colony dia (cm)
Plain agar	3.5^{a}
PFD	6.5^{b}
Castor cake agar	7.9^{b}
Gingelly cake agar	7.6^{b}
Groundnut cake agar	7.3^{b}
Neem cake agar	6.9^{b}

Data in column followed by a common letter were not statistically different (P = 0.01)by DMRT

of the fungus was maximum on castor cake agar medium followed by gingelly cake, groundnut cake and neem cake agar media which recorded 7.9, 7.6, 7.3 and 6.9 cm. respectively. The present studies clearly suggested that the oil cakes favoured the fungal growth. Thus, the present investigations suggested that *P. lilacinus* can be mass cultured on commonly available and cheap substrates such as leaves of subabool, neem, *Cassia* and *Pongamia*, waste tapioca tubers and their peels and thippi soaked in various chemical solutions.

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