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EVALUATION OF SOME AGRO-INDUSTRIAL WASTES FOR MASS PROPAGATION OF THE NEMATODE PARASITIC FUNGUS *PAECILOMYCES LILACINUS*

by
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The discovery of the fungus *Paecilomyces lilacinus* (Thom.) Samson as a promising biocontrol agent of phytonematodes (Jatala *et al.*, 1979) has renewed interest in biological control of nematodes. There is now considerable experimental evidence that confirms that *P. lilacinus* effectively controls root-knot and cyst nematodes (Jatala *et al.*, 1979; Strattner, 1979; Davide and Zorilla, 1983; Morgan-Jones and Rodriguez-Kabana, 1984; Villanueva and Davide, 1984). For experimental field applications, Jatala (1983) has suggested that the fungus should be cultured on cereal grains. However, this may not be economically viable for mass production of fungus inoculum aimed at general field application and hence certain low cost and abundantly available agricultural and industrial wastes were tested for their suitability as growth media for mass production.

Materials and Methods

Sugarcane molasses is a rich source of carbohydrates, as well as other nutrients. Two dilutions, i.e., 5% and 10% of molasses were prepared using distilled water. These were amended with nitrogen ($NaNO_3$ 0.2%) and/or phosphorus (K_2HPO_4 0.1%). One hundred ml of each molasses medium was poured in 250 ml Erlenmeyer flasks. After sterilization at 15 psi for 20 min, each flask was inoculated with a loopful of spore suspension from 10-day-old fungus culture grown on Potato Dextrose Agar slopes. The flasks were incubated at 30 ± 1°C. The mycelium was filtered after one week and oven dried at 60°C.

In another experiment, agricultural wastes such as gram husk, wheat bran, rice bran and rice husk were tested. Ten g of each of these substrates were placed in a flask to which 20 ml of 5% molasses (amended with N and P) or distilled water was added. After sterilization and inoculation as described earlier, the flasks were thoroughly shaken daily and kept in a Biological Oxygen Demand incubator at 30 ± 1°C. Rice grains alone and gram grains alone served as controls. After 10 days incubation, 100 ml sterile water and 0.1 ml Tween-80 (surfactant) were added to each flask and the contents were thoroughly shaken to disperse the spores uniformly. The number of spores was counted using a haemacytometer, by drawing a representative sample of the spore suspension from each flask.

Results and Discussion

In a preliminary experiment, various dilutions, viz., 5, 10, 15 and 20% of raw molasses were tested as media for the fungus. Since 15 and 20% molasses supported very poor growth compared with 5 and 10% dilutions, the latter two dilutions were further tried after amendment with N and P.

Addition of a nitrogen source did not produce significant improvement in fungal growth compared with unamended molasses at 5% dilution. However, the addition of both N and P significantly enhanced fungal growth at both 5 and 10% dilutions, and it was at par with potato dextrose broth (Table I). Hence 5% molasses amended with N and P was selected for further experimentation.

Molasses (5%), when used in conjunction with other substrates, enhanced sporulation only in the case of rice husk and rice bran. However, gram husk and wheat bran did not significantly improve sporulation. Rice husk produced the least number of spores. Maximum sporulation occurred on wheat bran (with or without molasses) which was comparable with rice grains (control) (Table II). Wheat bran should thus provide a suitable and economically viable substitute for rice grains as a growth medium for mass propagation of *P. lilacinus*.

Villanueva and Davide (1984) have used corn grit, rice hull, chopped water lily, mashed potato and ipil-ipil leaves for the mass production of *P. lilacinus*, but our findings could not be compared with theirs in view of the different techniques adopted for evaluation of the substrates.

TABLE I - *Effect of molasses alone and in combination with N and P on the growth of* Paecilomyces lilacinus *(mean of four replicates)*

Medium	Dry mycelial wt. (mg)
5% molasses alone[1]	175.50
5% molasses + N ($NaNO_3$ 0.2%)	165.75
5% molasses + P (K_2HPO_4 0.1%)	296.00
5% molasses + N + P	624.00
10% molasses alone	344.75
10% molasses + N	247.00
10% molasses + P	241.00
10% molasses + N + P	627.50
Potato Dextrose Broth (control)	684.25
Czapek Dox (control)	389.25
C.D. 1%	90.33

[1] Dilutions prepared on w/v basis

TABLE II - *Sporulation of* Paecilomyces lilacinus *on certain agricultural and industrial wastes (mean of three replicates)*

Substrate	Log. no. of spores/ flask
Gram husk + molasses[1]	10.36
Gram husk + distilled water	10.22
Rice husk + molasses	9.97
Rice husk + distilled water	9.29
Rice bran + molasses	10.57
Rice bran + distilled water	10.29
Wheat bran + molasses	10.91
Wheat bran + distilled water	10.73
Gram grains + distilled water (control)	9.92
Rice grains + distilled water (control)	10.87
C.D. 1%	0.23

[1] 5% molasses amended with N and P

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