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WOOD CHARCOAL POWDER, A CARRIER OF PAECILOMYCES LILACINUS SPORES

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Summary. The suitability of wood charcoal powder as a carrier of *Paecilomyces lilacinus* for field application was studied *in vitro*. This carrier in low-density-polyethylene pouches could support up to 1 x 10^6 spores per g material for at least six months. The storage of charcoal packets at constant (28 ± 1 °C) / ambient (14-39 °C) temperature or under aerated/non-aerated conditions did not influence the fungal spore viability.

The discovery of *Paecilomyces lilacinus* (Thom) Samson as a potential biocontrol agent of phytoparasitic nematodes (Jatala *et al.*, 1979) stimulated continuing research in this area (Jatala, 1986). The evaluation of this oviparasitic fungus at different centres has yielded positive results in most cases (Davide and Zorilla, 1983; Villanueva and Davide, 1984; Roman and Rodriguez-Macrano, 1985; Dube and Smart, 1987; Reddy and Khan, 1988, 1989; Cabanillas and Barker, 1989; Cabanillas *et al.*, 1989). However, the efficacy of the organism has understandably been found to be variable, due mainly to the different agroclimatic conditions in which applications were made (Jatala, 1986; Cabanillas and Barker, 1989).

As a consequence of these developments, efforts have been made to mass propagate *P. lilacinus* for field application, using low-cost agricultural or industrial by-products to minimize costs (Villanueva and Davide, 1984; Sharma and Trivedi, 1987; Bansal *et al.*, 1988). Although such substrates have given promising results (Walia *et al.*, unpubl.), most of them are carriers with a nutrient base and because they are biodegradable, there is the risk of contamination by undesirable micro-organisms. Moreover, the quantities of these substrates required for successful nematode control are usually in excess of the desirable limits and are impractical.

Recently, Cabanillas *et al.* (1989) prepared and tested five formulations (alginate pellets, diatomaceous earth granules, wheat grains, soil, and soil plus chitin) of *P. lilacinus* against *Meloidogyne incognita* on tomato. Most fungus-infected eggmasses (32%) occurred in plots treated with pellets. Among the other possible fungal carriers, wood charcoal powder was considered by us as a better alternative since it is chemically inert and is not susceptible to contamination by other micro-organisms. It can retain water for long periods, is of fine and uniform texture, and can be used for field application by seed coating or soil application. Also, it is non-hazardous, cheap, readily available, and is easy to handle and process. Considering these merits, the influence of wood charcoal powder and related abiotic conditions of storage on the survival of *P. lilacinus* spores was studied.

Materials and methods

Two laboratory experiments of 6 months duration each were conducted to determine the suitability of charcoal powder as a carrier for *P. lilacinus* spores. In the first experiment, the survival of *P. lilacinus* spores at three different initial densities, i.e., 1 x 10^4, 1 x 10^5 and 1 x 10^6 per g charcoal powder and at a constant temperature (28 ± 1 °C) under aerated (packets punctured) conditions, was monitored by monthly observations. In the second experiment, the effects of ambient and constant temperature and aeration were studied on the spore viability.

The isolate of *P. lilacinus* used in the experiments was obtained from the International Potato Center, Lima (Peru) and the fungus was maintained on potato dextrose agar (PDA) for further use.

Paecilomyces lilacinus spores were harvested after ten days growth on PDA by placing 10 ml sterile water on the plate and scratching the surface of the fungal mat. The spore suspensions so collected were pooled together and one drop of Tween-80 was added. The spore count was ascertained using a haemacytometer. The desired dilutions were prepared by adding sterile distilled water.

Wood charcoal powder was prepared by milling the charcoal and sieving through a 200 mesh screen. The required quantity of tap water was added to achieve 20% (w/w) moisture level. The moist charcoal powder was

then autoclaved at 15 psi (121 °C) for 30 min., cooled to ambient temperature and 50 g samples were aseptically transferred to low density polyethylene pouches (10 x 20 cm) which were then sealed. Five ml of the respective spore suspension were injected into each packet using a sterilised syringe and needle, and the holes were closed with cellophane tape. Except for one treatment in experiment II, the packets were punctured at five places with a needle, to facilitate gaseous exchange (aeration) during storage. The contents were mixed thoroughly and the packets were stored in an incubator (28 ± 1 °C) or in the laboratory (14-39 °C).

The contents of the packets were assayed for fungal spore viability at the start of the experiments, monthly intervals in experiment I, and after six months in experiment II.

At each observation, four packets were randomly selected, their contents mixed thoroughly, and one g sample from each packet was used for assay by a dilution-plating method. Serial dilutions were prepared in sterile water until an estimated spore concentration of 1 x 10^3 was reached, and 0.1 ml sample was spread on PDA in each petri plate. The plates were incubated in an incubator at 28 ± 1 °C for seven days. The number of colony forming units (CFU) per g dry wt. of carrier was calculated. The initial and subsequent moisture level of charcoal powder was also assessed at each observation by hot air drying 10 g sample from the same packets.

Results and discussion

In the first experiment (Table I), irrespective of the time period, the fungal spore load tended to increase over the initial spore load, the rate of increase being 1.06, 1.15 and 1.42 times when the initial spore loads were 1 x 10^6, 1 x 10^5 and 1 x 10^4 per g charcoal, respectively. Thus, maximum increase in the number of spores was observed at the lowest initial level. Although the rate of increase was lowest at 1 x 10^6 initial level, the final spore population in this case was the highest when compared with the other two treatments. The time period also influenced the number of spores, irrespective of the initial spore load. A steep increase in the spore count was observed after the first month. However, the spore count reached the maximum by the fifth month. A significant interaction between initial spore load and time period was observed. An increasing trend was found at 1 x 10^4 initial level with the passage of time. When the initial number of spores was 1 x 10^6, the spore number reached peak population after one month, but then gradually declined and after six months reached almost the initial level. The trend, however, was not clear with 1 x 10^5 spores, where the final population also reached 1 x 10^6 after five months.

In the second experiment, the effect of temperature and aeration on the spore viability was studied. The data in Table II show that the storage of charcoal packets at constant (28 ± 1 °C) or ambient (14-39 °C) temperature had no significant effect on the number of viable spores after six months and the initial spore load of 1 x 10^6 per g of charcoal was maintained throughout. Similarly, aeration did not influence the spore numbers significantly.

It can be concluded from these experiments that *P. lilacinus* spores can survive in wood charcoal powder for at least six months. The maximum spore load that charcoal powder can sustain over a period of six omonths is roughly 1 x 10^6 per g carrier. Even if the spore load is less, it tends to increase and stabilize around 1 x 10^6 with time. The temperature regime [fluctuating (14-39 °C) / constant (28 ± 1 °C)] or provision of aeration by puncturing the packets did not alter the viability of the fungus.

Considering the practical method of application for delivering the fungus into the soil, the choice and amount of carrier required, and the number of fungal spores to be introduced into the soil, wood charcoal powder provides a better alternative than other materials reported so far. The merits of this carrier material have already been stated. Further, charcoal carrying the desired spore load can be coated on to seeds and thus the fungal spores can be introduced uniformly into a field using a minimum quantity of the carrier material.

In microplot studies, the quantities of two effective levels, 10 and 20 g of *P. lilacinus* infected wheat grains, was 0.208 and 0.416 ton per ha, as row treatment (Cabanillas and Barker, 1989); or 0.375 ton per ha when applied 1.5 kg per 40 m^2 (Jatala, 1986). However, Backman and Rodriguez-Kabana (1975) suggested that considering the costs of carrier material, storage space and delivery at an economical level, applications of biocontrol agents in excess of 100-200 kg per ha are uneconomic. Preliminary trials in our laboratory have shown that seed coating in case of okra (seed rate 20 kg per ha), required only 2 kg of charcoal powder. Each seed can deliver approximately 5 x 10^3 spores if the carrier contains 1 x 10^6 spores per g. Furthermore, even though the total number of spores may be less than required for optimal nematode control, the fungal spores introduced by this method will be concentrated in the rhizosphere, at least for the first crop. The spore intensity can also be augmented by maintaining a higher spore load in the carrier or by a higher seed rate.

Thus overall, wood charcoal powder provides an ideal medium for field application of *P. lilacinus*. However, the practical aspects on the extent of nematode control achieved by this method remains to be seen. Further work on these lines is in progress.

TABLE I - *Viability of* Paecilomyces lilacinus *spores in wood charcoal powder over a period of six months (means of 4 replicates)*

Initial spore load (log_{10}) per g wood charcoal powder	Number of viable spores (log_{10}) per g wood charcoal powder at different time intervals						
	1 month	2 months	3 months	4 months	5 months	6 months	Mean
6.000	6.690	6.490	6.019	6.400	6.528	6.129	6.376
5.000	5.649	5.479	5.314	5.778	6.144	6.270	5.772
4.000	5.330	5.389	5.423	5.737	6.184	6.188	5.708
Mean	5.890	5.786	5.585	5.972	6.285	6.196	

Temperature 28 ± 1 °C — L.S.D. (1%) for fungus spore load: 0.128
Packets punctured — L.S.D. (1%) for time period: 0.182
L.S.D. (1%) for fungus x time: 0.315

TABLE II - *Effect of temperature and aeration on the viability of* Paecilomyces lilacinus *spores in wood charcoal powder after six months (means of 4 replicates)*

Temperature	Number of viable spores (log_{10}) per g carrier after 6 months		Mean
	Packets punctured	Packets unpunctured	
Constant 28 ± 1° C)	6.228	6.039	6.113
Ambient (14-39° C)	6.134	5.881	6.007
Mean	6.181	5.960	

Initial spore load (log_{10}): 6.000
L.S.D. (1%) for temperature: N.S.
L.S.D. (1%) for aeration: N.S.
L.S.D. (1%) for temperature x aeration: N.S.

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