resulted in higher root infection than that of uninoculated control and seed inoculation with *A. brasilense* (table 1). Considerable variation existed among the three different VAM fungi and their combination with *A. brasilense* as far as the VAM colonization was concerned. Soil inoculation with *G. fasciculatum*, in general, resulted in maximum root infection.

One application of superphosphate or rock phosphate to soil at the rate of 50 kg P_2O_5/ha resulted in higher dry matter content and total phosphorus uptake in plants than that of the control (no P application). The preformance of plant, in general, was better with single super phosphate than that of rock phosphate (table 1). Soil inoculation with different VAM fungi in the presence of either single superphosphate or rock phosphate produced more dry matter production and greater phosphorus uptake than their corresponding controls. Dual inoculation with both the endosymbionts namely, *A. brasilense* and VAM fungi in the presence of superphosphate or rock phosphate also resulted generally in increased dry matter production and total P uptake in plants. Among the three VAM fungi tested, *G. fasciculatum* either singly or in combination with *A. brasilense* resulted in a better performance than that of the other mycorrhizae (table 1).

Azospirillum inoculation has been shown to increase the root biomass[6] and therefore, the significant increase in dry matter production and phosphorus uptake in plants inoculated with *A. brasilense* and different VAM fungi when tested singly or in combination with super phosphate or rock phosphate could be attributed to an increased phosphate transport by mycorrhizae aided by an increased root biomass.

The authors thank Dr N. S. Subba Rao for encouragement, and Dr K. R. Krishna, ICRISAT, Hyderabad for supplying the VAM cultures.

29 December 1986; Revised 21 September 1987

1. Daft, M. J. and Nicholson, T. H., *New Phytol.*, 1966, **65**, 343.
2. Mosse, B., Powell, C. L. and Haymann, D. S., *New Phytol.*, 1976, **76**, 331.
3. Murdoch, C. L., Jackobs, J. A. and Geardemann, J. W., *Plant Soil*, 1967, **27**, 329.
4. Jackson, M. L., *Soil chemical analysis*, Prentice Hall, New Delhi, 1971.
5. Phillips, J. M. and Hayman, D. S., *Trans. Br. Mycol. Soc.*, 1970, **55**, 158.
6. Dewan, G. I. and Subba Rao, N. S., *Plant Soil*, 1979, **53**, 295.

A NEW AND CHEAPER TECHNIQUE FOR RAPID MULTIPLICATION OF *ARTHROBOTRYS CONOIDES* AND ITS POTENTIAL AS A BOI-NEMATICIDE IN MUSHROOM CULTURE

P. S. GREWAL and H. S. SOHI
National Centre for Mushroom Research & Training, Chambaghat, Solan 173 213, India.

BIOLOGICAL control of pests seems to be a suitable alternative to avoid the hazards associated with pesticide use in mushroom culture. However, lack of simple and cost-effective techniques for mass production of the bio-control agents and their establishment in the new environment are the two major problems hindering their commercial use. In order to achieve these objectives, an effort was made to isolate the nematophagous fungi naturally occurring in synthetic compost during cropping. Two species of *Arthrobotrys* viz *A. conoides* and *A. oligospora* were isolated and studied for bio-management of *Aphelenchoides composticola* infesting *Agaricus bisporus*.

In the present study a simple technique for quick multiplication of *A. conoides* was developed. Both the species were isolated on malt extract agar (MEA) medium which supported good mycelial growth along with profuse sporulation. The technique used in the present study involved the use of wheat grain. The grains were soaked in water for 20 min followed by 10–15 min boiling. After draining excess water, these were dried overnight. Chalk ($CaCO_3$) and gypsum ($CaSO_4$) were mixed thoroughly with the grain at 2 and 6%. The mixture was filled in milk or glucose bottles and sterilized at 15 pounds pressure for one hour. The bottles were inoculated with culture bits under aseptic conditions. These were then incubated at 25 ± 1°C. The substrate was covered completely within 12 days. Profuse sporulation was noticed subsequently as pinkish coloration.

Since a number of pesticides are used in mushroom cultivation to control various pests and diseases, the effect of commonly used ones on the mycelial growth of both the *Arthrobotrys* species was studied using food poison technique. In general, *A. conoides* was less sensitive to different pesticides as compared to *A. oligospora*. The mycelial growth was inhibited by bavistin in both the species at

50 ppm and above. BHC also showed 30–56% inhibition in *A. conoides* and 25–75% in *A. oligospora* at 25 to 250 ppm. Carbofuran was highly toxic to both the species from 100 to 800 ppm. Diazinon and dichlorvos were comparatively less toxic and showed no significant inhibition in both the species up to 40 ppm. However, at 60 and 80 ppm both the insecticides had significant inhibitory effect.

In a field trial, the fungal culture multiplied on wheat grain was added to the mushroom compost heavily infested with *A. composticola* and *Rhabditis* spp. Isolation made from the compost after 25 days of inoculation revealed the production of three-dimensional rings by the test fungus (figure 1). It also captured the nematodes until they were strangled (figure 2). The population analysis after 25 days of inoculation showed a 42.5% decrease in total population of both the nematode species in bags where the nematophagous fungus was mixed thoroughly with the compost. However, within this period the fungus could not colonize the compost at lower depths when the inoculum was put only at the top of the bags. Earlier it was found that the incorporation of dried plant materials to the compost resulted in increased population of some nematophagous fungi[1]. *A. conoides* grew rapidly in compost and unlike some other nematode trapping species did not inhibit the growth of *A. bisporus*[2,3].

Table 1 *Effect of cultural metabolites of A. conoides on spawn run of A. bisporus and population of A. composticola*

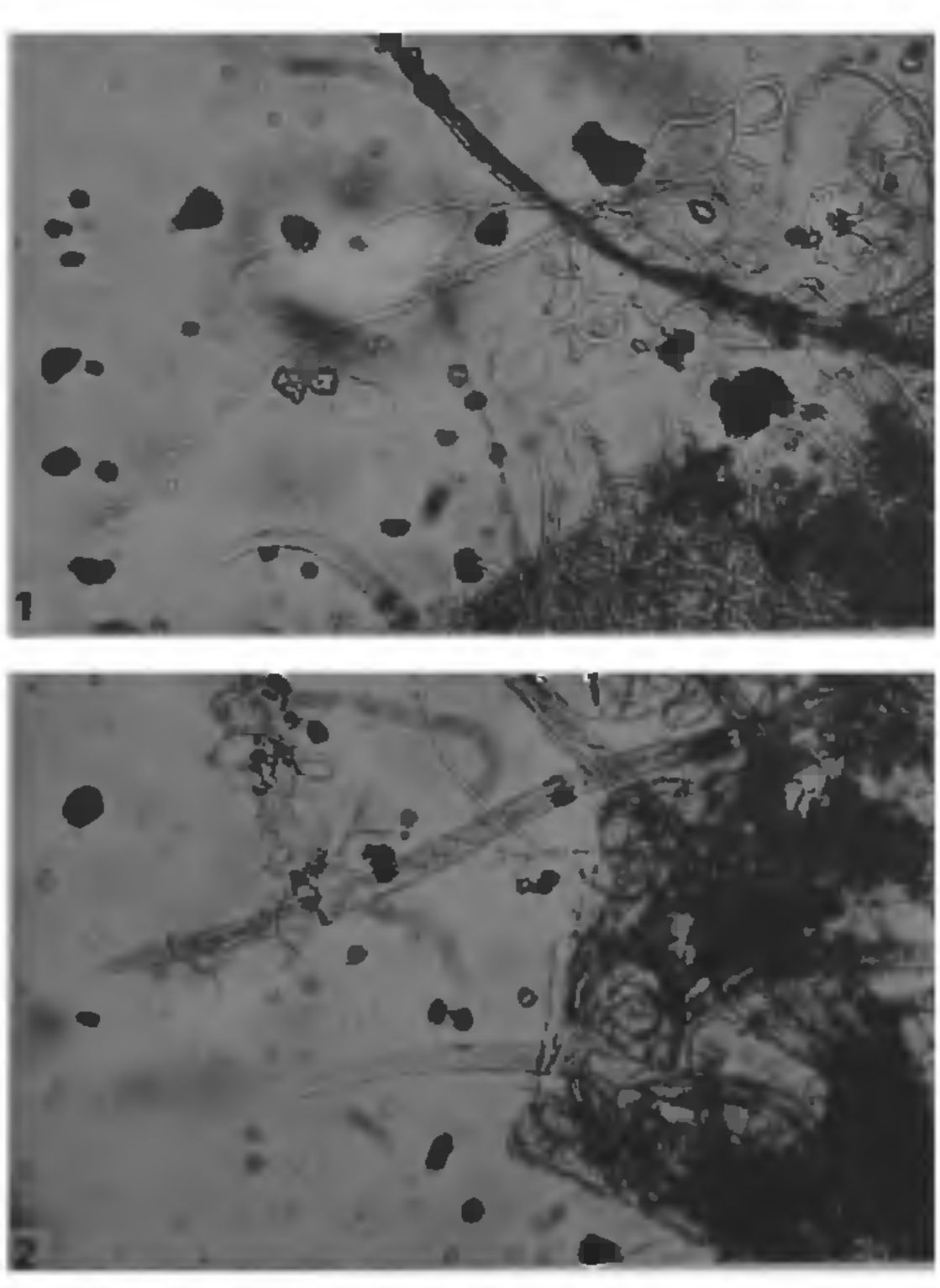
Treatments		Spawn run (visual)	Nematode population/ 100 g compost 20 days after treatment
Spawn+cultural filtrate	(10%)	Good	–
–do–	(5%)	Very Good	–
–do–	(2%)	Very Good	–
Spawn+nematode+ cultural filtrate	(10%)	Good	23.5
–do–	(5%)	Moderate	69.6
–do–	(2%)	Moderate	120.3
Spawn+nematode		Poor	1134.5
Spawn alone		Very Good	–

– Nematodes were inoculated at 50 individuals per 200 g compost.

In a separate study carried out by the authors, fresh and autoclaved cultural metabolites of *A. conoides* were found highly toxic to *A. composticola* under *in vitro* conditions. Effect of fungal metabolites on spawn run and nematode population in compost were also studied under *in vivo* conditions. For this, the pasteurized compost was filled in polythene bags (200 g in each). Details of different treatments are given in table 1. No inhibition was observed in spawn run of *A. bisporus* at 2 and 5% concentration of the cultural metabolite. However, there was moderate inhibition at 10% concentration. Drastic reduction in nematode population was observed in all the treatments. There are reports in literature about the nematicidal properties of different fungal metabolites[4,5].

The results clearly indicate the possibility of bio-control of mushroom infesting nematodes by the incorporation of *A. conoides* multiplied on wheat grain, in synthetic compost.

27 July 1987; Revised 22 September 1987



Figures 1 and 2. 1. Three-dimensional rings produced by *A. conoides*, and **2.** Captured and strangled nematode in the rings produced by *A. conoides*.

1. Sohi, H. S., Grewal, P. S. and Seth, A., *Cultivating edible fungi*, Elsevier, Amsterdam, 1987, p. 121.

2. Cayrol, J. C., Frankowski, J. P., Laniece, A., D'Hardemare, G. and Talon, J. P., *Pepinier. Hortic. Marazchers*, 1978, **184**, 23.
3. Choleva, B., *Gradinar. Hazar. Nauka*, 1973, **10**, 133.
4. Kerry, B. R., *Helminth. Abstr.*, 1984, **B53**, 1.
5. Krizokova, L., Balan, J., Nemac, P. and Kolezesevary, A., *Folia Microbiol.*, 1976, 493.

PROLINE ACCUMULATION IN RICE LEAVES AS A CONSEQUENCE OF SENESCENCE

S. K. MOHANTY, TARULATA PARIDA and R. SRIDHAR
Department of Plant Pathology, Central Rice Research Institute, Cuttack 753 006, India.

ACCUMULATION of proline in plants subjected to abiotic stress conditions is a general phenomenon[1,2]. The physiological significance of proline accumulation in plant tissues during stress is not clear. Despite its possible role at high concentrations as a storage compound of reducing equivalent and nitrogen for rapid growth after the stress[3,4], an osmoticum[5,6], a protective agent of enzymes and cellular structures[7] and as an organic solute[8] has been speculated, these proposals have not been convincingly proved. On the other hand, Hanson *et al*[9] considered proline accumulation as a pathological consequence in water-stressed barley leaves. Nevertheless, this view has been questioned[8]. Studies from this laboratory have shown that proline accumulates in tungro (a virus complex)-diseased rice leaves[10]. We demonstrate in this study that proline accumulates as a consequence of leaf senescence in rice.

Five cm apical segments of fully expanded third leaf blades from 20-day-old rice seedlings (cv. Taichung Native 1) were floated with their adaxial side up in groups of five on 20 ml of either distilled water or 2×10^{-5} M kinetin (Loba Chemie, Austria), gibberellic acid (GA_3; Polfa-Kunto, Poland) or abscisic acid (ABA, Sigma Chemical Co., USA) in 10 cm petri dishes. The petri dishes were incubated in the dark at 28 ± 2°C for three days. Duplicate samples, each replicate consisting of five leaf blade segments totalling approximately 75 mg, were collected at 24 hr intervals for 3 days. The concentrations of total chlorophyll pigments extracted from these segments in 80% (v/v) ethanol and of free proline extracted from identical and parallel samples in 3% (w/v) aqueous sulphosalicylic acid were determined spectrophotometrically[11].

As a measure of leaf senescence, the total chlorophyll content of senescing and detached leaf blade segments was determined. During the three days of incubation of the detached leaf blade segments in darkness, the leaf blade segments

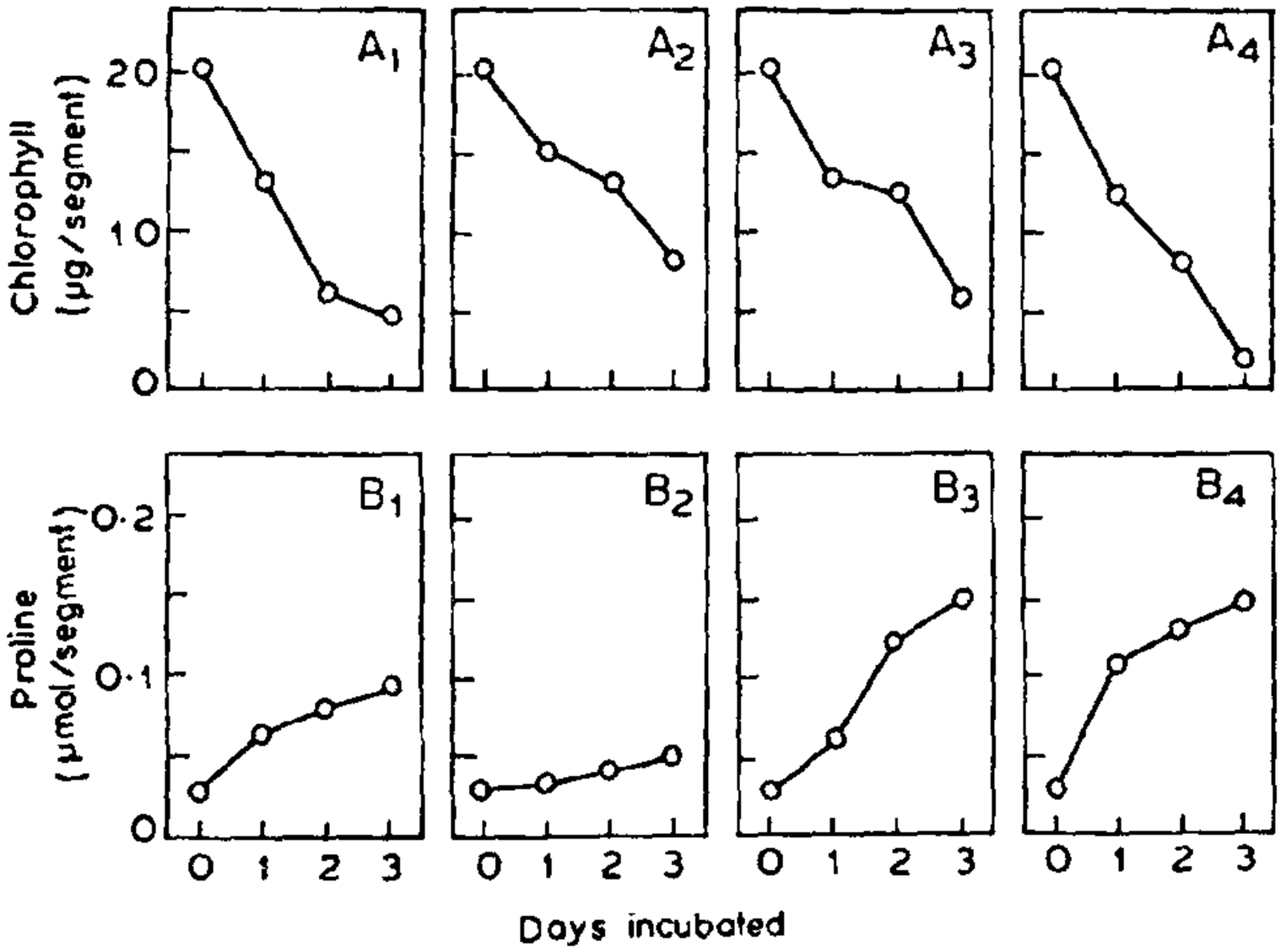


Figure 1. Time course of total chlorophyll (A) and free proline (B) contents of leaf blade segments (cv. Taichung Native 1) floated on water (1) and on 2×10^{-5} M kinetin (2), GA_3 (3) and ABA (4) solutions in the dark. The individual values were always within ±5% of the mean.