

## **Impact of olive pomace wastes and fungicide treatment on indigenous arbuscular mycorrhizal fungi associated with chickpea (*Cicer arietinum* L.) under field conditions**

**<sup>1\*</sup>Assaf TA, <sup>2</sup>Turk MA and <sup>2</sup>Hameed KM**

<sup>1\*</sup> *Department of Grassland Science, Georg August University, Germany*

<sup>2</sup> *Department of Plant Production, Jordan University of Science and Technology, Jordan*

*\*Corresponding author: tassaf@gwdg.de*

### **Abstract**

The effect of soil amendment with olive pomace wastes (OPW) on population and root infection of indigenous arbuscular mycorrhizal (AM) fungi associated with chickpea has been studied under field conditions. Yield components, phosphorus (P) percentage, AM spore density and root length colonization of AM fungi were recorded. We found that OPW increased biomass, seed yield, and shoot phosphorus concentration of chickpea plants. Our results also indicated that AM fungal spore population and colonization levels were substantially enhanced by the application of OPW. Fungicide applications significantly decreased both spore population and colonization length of AM fungi. We concluded that organic matter amendments potentially increased AM fungal association and hence might increase growth and yield of chickpea plants in the field.

**Key words:** Microorganisms; Organic matter; Olive pomace; Soil amendment; Spore populations.

**Abbreviations:** AM fungi- arbuscular mycorrhizal fungi; OPW- olive pomace wastes.

### **Introduction**

Most legumes are symbiotically associated with Arbuscular mycorrhizal (AM) fungi and rhizobia. The beneficial effect of these symbiotic relationships on plant growth is well documented. However, in cultivated soils, the populations as well as the infectivity of AM fungi may be affected by cultural practices such as soil amendment with organic matter. It was reported that AM fungi usually increase as a result of soil amendment with organic matter which leads often to the benefit of plant from this relationship (Ryan et al., 1994; Douds et al., 1997). This result might be explained by the effect of organic matter on soil structure, water retention capacity, microbial activity, or chemicals released from organic matter (Ryan et al., 1994).

Olive pomace wastes (OPW) are produced in large quantities in many Mediterranean countries during November-February (Mechri et al., 2008). Once the olives have been ground into a paste, the paste is separated into three components; vegetable water, oil, and the husk. The husk (pomace) contains the skins, pulp and pit fragments. Olive pomace could be applied to the soil, either intentionally as soil amendment or unintentionally as a disposal method. Al-Sakit and Al-Momani (1989) found that OPW treatment improved the growth of olive seedlings when applied as a fertilizer. However, it has been found that the yields of wheat and barley were decreased as a response to soil amendment with OPW and this was attributed to the presence of phytotoxic

**Table 1.** Plant height (cm) and number of leaves and branches per plant of chickpea as affected by different levels of olive pomace wastes (OPW) and soil treatments.

| Soil treatments        | OPW % | Stem height | Branches No. | Leaves No. |
|------------------------|-------|-------------|--------------|------------|
| Fungicide              | 0%    | 41.7        | 3.0          | 102.7      |
|                        | 10%   | 45.3        | 3.3          | 121.7      |
|                        | 20%   | 54.7        | 3.7          | 151.3      |
|                        | 30%   | 46.0        | 3.7          | 175.0      |
| Control                | 0%    | 42.7        | 3.7          | 136.3      |
|                        | 10%   | 62.7        | 3.7          | 157.7      |
|                        | 20%   | 54.0        | 3.0          | 198.7      |
|                        | 30%   | 57.3        | 4.3          | 207.7      |
| Significance (P value) |       |             |              |            |
| Soil treatment         |       | 0.0383      | 0.4754       | <.0001     |
| OPW                    |       | <.0001      | 0.3036       | <.0001     |
| Soil treatment x OPW   |       | 0.0003      | 0.2525       | <.0001     |

compounds, Phenols (Aqeel, 2001). On the other hand, differential effects of OPW on different crops were also recorded (Aqeel, 2001; Al-Hassan, 2000). Previous investigations on the effects of OPW on plant growth and their association with AM fungi were done mostly on pot experiments with selected species and strains of AM fungi (Al-Sakit and Al-Momani, 1989; Al-Hassan, 2000). Therefore, investigating the influence of OPW amendments on plant growth and AM fungi under field conditions is still lacking.

The objective of the present experiment was to investigate the effect of soil amendment with OPW on indigenous AM fungi in terms of spore population and root infection. Another objective was to study the effect of OPW on the growth of chickpea (*Cicer arietinum* L.) under field conditions.

## Materials and methods

### Study area

Field plots were located at the research station of Jordan University of Science and Technology located in the northern part of Jordan [32° 30' N latitude, 35° 59' E longitude, and 520 m altitude). The soil was sampled for analysis of physico-chemical properties

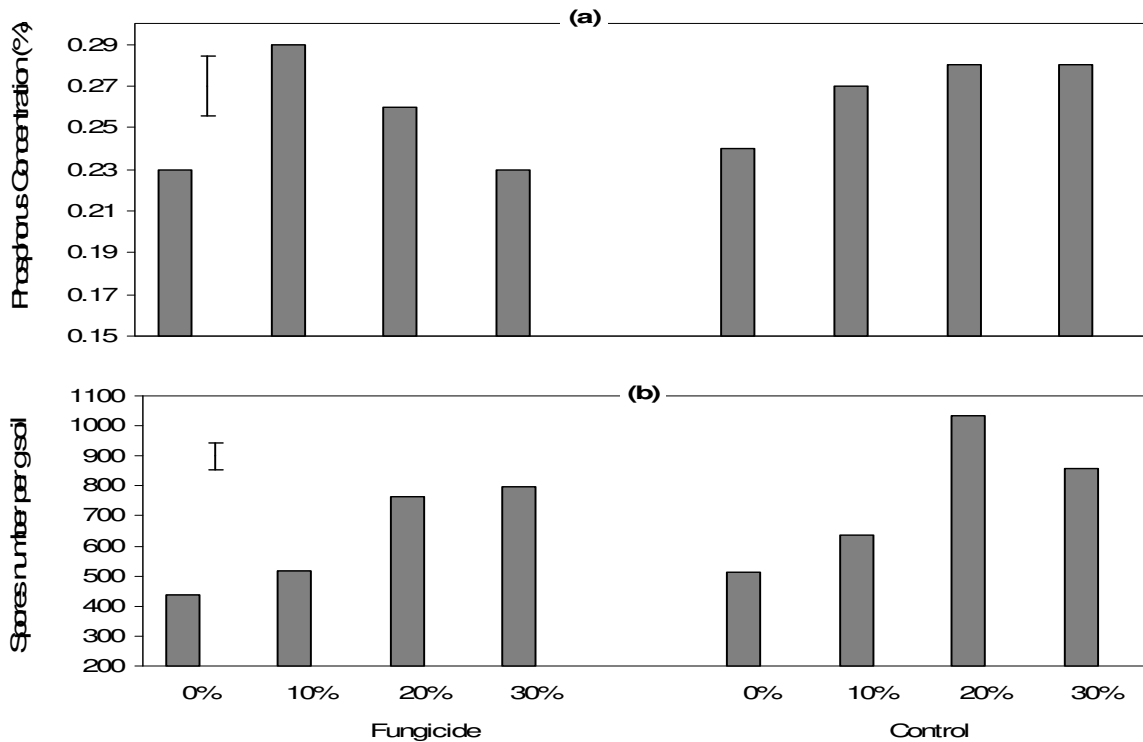
and AM fungal population shortly prior to the initiation of the experiment. The soil was a silty clay loam with a pH 8.4 (1: 1; soil:water). Soil nutrient determinations included 0.24% total N, 9.9 ppm available P and 0.93% organic matter. The total indigenous AM fungal spore density prior to the start of the experiment was 223 spores 10g<sup>-1</sup> soil.

### Land Preparation and Application of OPW

Soil was prepared by plowing twice using disk plow followed by disk harrowing to mix and level soil surface. Experimental plots (2 x 1 m<sup>2</sup>) consisted of five rows of 2 m long, 20 cm apart and 50 cm space between the plots. Soil was mixed with fresh OPW at four different levels: 0%, 10%, 20%, and 30%, on volume basis. OPW was surface applied and incorporated to 15 cm into the soil. Those levels were attained by adding 0 m<sup>3</sup>, 0.015 m<sup>3</sup>, 0.030 m<sup>3</sup>, and 0.045 m<sup>3</sup> of OPW per 1m<sup>2</sup> of soil, respectively.

### Soil Treatments and planting

After OPW incorporation, experimental plots were either treated with a systematic fungicide (Metalaxyl-50 g Kg<sup>-1</sup>) or left untreated as control. Dry seeds chickpea were planted by hand at rate of 30 plants m<sup>-2</sup>.



**Fig 1.** Effect of soil amendments with different levels of olive pomace wastes (OPW) and soil treatments on: (a) shoot phosphorus concentration ( $F=4.00$ ,  $P= 0.0074$  and (b) spore density ( $F=7.97$ ,  $P=0.0001$ ). Vertical bars represent LSD values at  $P \leq 0.05$ .

### **Harvest and measurements**

Yield per unit area were obtained by harvesting the three central rows (150 cm x 60 cm = 0.9 m<sup>2</sup>). Biomass was determined by weighing the total dry matter of the above-ground parts while seed yield was measured by weighting the seeds. In addition, five plants from each plot were dug out. Aboveground parts were used to measure plant height, number of leaves and number of branches; the average values were used for statistical analysis. Root system and surrounding soil were stored in cool chamber (4°C) for mycorrhization assessments.

Phosphorus concentrations were determined for the harvested plants. Dried plant materials (only shoots) were finely ground using a scientific mill and analyzed for phosphorus concentrations using the method of Olsen (1954).

### **AM colonization and spore density**

Roots were cleared with 10% KOH followed by acidification in HCl, and stained with 0.05 % Trypan Blue using the procedure of Phillips and Hayman (1970). The incidence of mycorrhizal colonization was estimated as the percentage of the length of each segment which is colonized according to the method of Bierman and Linderman (1981). Spores were extracted from 10g soil samples using the floatation-adhesive technique (Sutton and Barron, 1972). Number of spores was counted under a compound microscope.

### **Experimental design and Statistical Analysis**

Field plots were laid out in split plot design. Soil treatments (fungicide and control) were occupied the main plots and OPW levels occupied the subplots. This resulted in twelve treatment combinations; each

**Table 2.** The main effects of soil treatments and olive pomace wastes (OPW) amendments on biomass, seed yield, and harvest index of chickpea.

| Treatments                    | Biomass yield<br>(kg ha <sup>-1</sup> ) | Seed yield<br>(kg ha <sup>-1</sup> ) | Harvest index<br>(%) |
|-------------------------------|-----------------------------------------|--------------------------------------|----------------------|
| <b>Soil Treatments</b>        |                                         |                                      |                      |
| Fungicide                     | 5162                                    | 2260                                 | 43.9                 |
| Control                       | 5707                                    | 2295                                 | 40.8                 |
| <b>OPW %</b>                  |                                         |                                      |                      |
| 0%                            | 4800                                    | 1988                                 | 41.4                 |
| 10%                           | 4858                                    | 2065                                 | 42.8                 |
| 20%                           | 5614                                    | 2349                                 | 42.0                 |
| 30%                           | 5838                                    | 2379                                 | 41.0                 |
| <b>Significance (P value)</b> |                                         |                                      |                      |
| Soil treatment                | 0.0135                                  | 0.0118                               | 0.3408               |
| OPW                           | <.0001                                  | <.0001                               | 0.6584               |
| Soil treatment x<br>OPW       | 0.0998                                  | 0.0907                               | 0.0904               |

treatment was replicated three times. Considering the split-plot structure of the trial, data were analyzed with mixed model using the procedure MIXED in SAS (SAS Institute, 1996). The following model was fitted (same symbols as above):  $C_{ijm} = \mu + F_i + S_j + (FS)_{ij} + R_m + (FR)_{im} + e_{ijm}$ ; where  $\mu$  is general mean;  $F_i$  is soil treatments ( $i = 1$  or  $2$ );  $S_j$  is OPW levels ( $j = 1 \dots 4$ );  $(FS)_{ij}$  is the interaction between both treatments;  $R_m$  is block effect ( $m = 1 \dots 3$ );  $(FR)$  is the interaction between soil treatment and block; and  $e$  is the residual term. Data presented are LSMEANS in the analysis.

## Results

### Plant characters

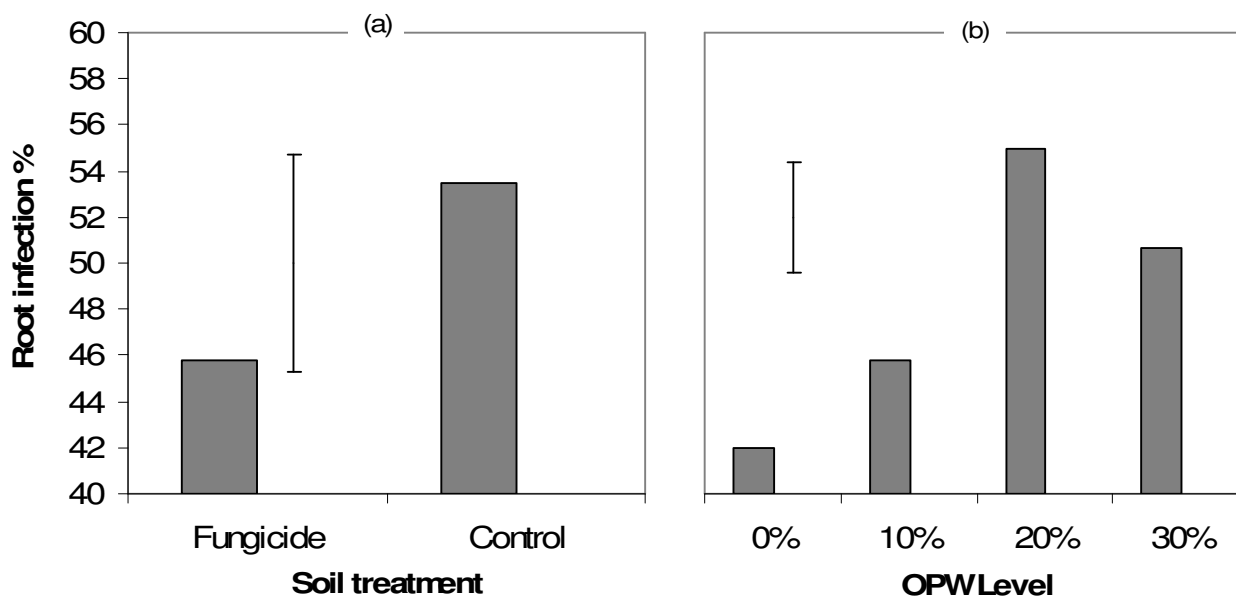
Chickpea phenology was significantly affected by OPW manuring and soil treatments (Table 1). Application of OPW at all rates significantly increased plant height and leaves number compared to 0% OPW (Table 1). However, application of OPW at the rate of 30% produced lower plant height compared to no OPW application for fungicide treatments, but not for the control treatment.

Although higher number of branches was recorded with OPW application, treatment effects were not significantly different.

### Yield and Phosphorus concentration

For biomass, seed yield, and harvest index, statistical analysis showed no significant interaction between soil treatments and OPW amendments; hence the results were shown separately for the two factors. Generally, yields were enhanced by OPW amendments (Table 2). For plants grown in soil amended with 30% OPW there were 20% and 22% increase in biomass and seed yield, respectively, than in 0% OPW. Soil fungicide treatment significantly decreased biomass as well as seed yield compared to the control treatment. On the other hand no significant differences were detected for harvest index in response to the applied treatments.

Chickpea plants grown in soils amended with OPW had slightly higher phosphorus concentrations compared to 0% OPW level (Fig. 1a). However, lower concentrations were associated with fungicide treatment than with control treatment.



**Fig 2.** Length percentages of chickpea roots infected with arbuscular mycorrhizal (AM) fungi as affected by: (a) soil treatments ( $F=3.65$ ,  $P=0.1335$ ) and (b) olive pomace wastes (OPW) amendments at different levels ( $F=12.37$ ,  $P=0.0001$ ). Vertical bars represent LSD values at  $P\leq 0.05$ .

### ***AM colonization and spore populations***

There were significant differences in AM infection percentages and spores density as a response to soil treatments and OPW amendments (Fig. 1 and 2). Spore density was positively correlated with OPW amendments, though this effect is less noticeable for the fungicide treatment than for the control treatment. On the other hand, significantly higher spores density was observed for the control treatment compared to the fungicide treatment.

Soil fungicide treatment slightly decreased root infection percentages with AM fungi compared to control treatments, although the differences between soil treatments were not significantly different. Regarding the main effects of OPW amendments, percent infection was significantly higher in soil amended with OPW at the rates of 10% and 20% compared to 0% level, and significant effect was not detected for the higher rate of OPW (30%) compared to 10% and 20% levels.

### **Discussion**

Our results confirm previous work on plant growth response to organic amendments (Al-Abudlsalam and Garawani, 1998; Mandimba, 1998). It is generally recognized that the benefits of organic matter are not only due to supply of the nutrient elements, but also to the improvement of soil physical characters (McCoy, 1998). Our results showed that an enhanced mycorrhizal infection may account for enhanced plant growth through improved nutrient uptake (Muthukumar and Udaiyan, 2000).

Organic manuring tends to maintain soil structure, is less disruptive to the soil environment, encourages populations of beneficial soil microbes, facilitates crop rooting, improves water retention capacity and results in a more even distribution of nutrients in the soil profile (Arden-Clarke and Hodges, 1988). Groaker and Sreenivasa (1994) also reported an enhancement of growth, yield and root colonization in wheat as a result of organic amendment.

Soil amendment with OPW at the rate of 30% had negative effects on AM infection as well as spore density under control treatment. Two mechanisms could explain that response. First, OPW amendments might cause a reduction in plant growth, as a result of phenolic compounds, and hence might reduce soluble carbohydrates in the root system. Muthukumar and Udaiyan (2000) indicated that high carbohydrate concentrations in roots coincide with increased colonization levels. Second, the chemical composition and/or the decomposition products of the OPW amendment may have discourage mycorrhizal development in chickpea plants. However, this finding could be also attributed to the enhancement of soil microorganisms as a result of high percentage of organic matter, these microorganisms may compete or even parasitize mycorrhizal fungi (Sreenivasa and Bagyaraj, 1989).

The observed reduction in chickpea growth in response to high rates of OPW amendment was consistent with previous findings, indicated that fresh OPW contains high levels of phenolic compounds which might affect plant growth adversely (Aqeel, 2001; Al-Hassan, 2000). Pages et al. (1985) also indicated that OPW contains high levels of phytotoxic compounds, which may inhibit seed germination or reduce plant growth.

The higher shoot P concentrations found in chickpea grown on OPW amended soil is probably due to more efficient uptake of available P from the soil and manure and possibly to mineralization of organic phosphorus (Jayachandran et al., 1992) or due to the advantageous effect of the higher AM colonization (Tarafdar and Marschner, 1994; Verma and Arya, 1998).

AM soil infectivity fluctuates in relation to agricultural practices (Plenchette, 1989), amongst which soil sterilization and fungicide application are often considered to be the most detrimental, leading to a decrease in indigenous fungal populations (Trappe et al., 1984). Our results indicated that soil treatment with fungicide decreased AM fungi population and infection percentages, although the differences among infection percentages were not significantly different. Consequently, fungicide treatment might have weakened AM fungus propagules in treated soil and reduced their survival (Schreiner et al., 2001). However, soil fungicide treatment in our experiment did not completely eliminate AM fungi propagules. An explanation for this result could be that soil fungicide treatments

under field conditions may adversely affect AM propagules in the surface soil (Menge, 1982), but not the deep soil layer.

We concluded that AM fungal spore population, colonization levels and the mycorrhizal response can be substantially altered by soil treatments and applications of different levels of OPW. Our results clearly showed that soil amendments with OPW significantly enhance number of AM spores as well as colonization percentages. OPW might have an indirect effect on AM fungi, through the effect of OPW on plant growth, or directly, through the effect of chemical compounds present in OPW on the growth of AM fungi itself. Future experiments should focus on the role of the chemical compounds contained in OPW on spore germination as well as the growth of AM hyphae to test if those compounds have direct effects on population and growth of AM fungi.

## References

- Al-Abudlsalam MA, Garawany MM (1998) Barley response to soil type, organic manure and sulphur rates. Arab Univ J Agri Sci 6:135-149.
- Al-Hassan I (2000) Evaluation of several methods to control weed in faba bean. M.Sc. thesis, Jordan University of Science and Technology, Irbid, Jordan.
- Al-Sakit I, Al-momani A (1989) Effect of vesicular arbuscular mycorrhizal fungi of olive seedling absorbtion of minerals from solid olive mill by-products. Dirasat 15:85-95.
- Aqeel A (2001) Nutritional status of several legumes and Gramineae plants grown in soil supplemented with olive mill by-products. M.Sc. thesis, Jordan University of Science and Technology, Irbid, Jordan.
- Arden-Clarke C, Hodges RD (1988) The environmental effects of conventional and organic/biological farming systems. II. Soil ecology, soil fertility and nutrient cycles. Biol Agric Hort 5:223-287.
- Bierman B, Linderman RG (1981) Quantifying vesicular-arbuscular mycorrhizae: a proposed method towards standardization. New Phytol 87:63-67.
- Douds DD, Galvez L, Franke-Snyder M, Reider C, Drinkwater LE (1997) Effect of compost addition and crop rotation point upon VAM fungi. Agric Ecosyst Environ 65: 257-266.
- Groaker SBN, Sreenivasa MN (1994) Effects of inoculation with *Glomus fasciculatum* in

- conjunction with different organic amendments on growth and grain yield of wheat (*Triticum aestivum* L.). *Microbiol Res* 149:419-423.
- Jayachandran K, Schwab AP, Hetrick BAB (1992) Mineralization of organic phosphorus by vesicular-arbuscular mycorrhizal fungi. *Soil Biol Biochem* 24:897-903.
- Mandimba GR (1998) Response of soyabean crops to *Mucuna pruriens* green manure in symbiosis with cowpea *Bradyrhizobia* in Congo. *Biol Agric Hort* 16:15-23.
- McCoy EL (1998) Sand and organic amendment influences on soil physical properties related to turf establishment. *Agron J* 90:411-419.
- Mechria BB, Mariemb BF, Bahamb M, Elhadji SB, Hammamia M (2008) Change in soil properties and the soil microbial community following land spreading of olive mill wastewater affects olive trees key physiological parameters and the abundance of arbuscular mycorrhizal fungi. *Soil Biol Biochem* 40:152-161.
- Menge JA (1982) Effect of soil fumigants and fungicides on vesicular-arbuscular fungi. *Phytopathology* 72:1125-1132.
- Muthukumar T, Udaiyan K (2000) Influence of organic manures on arbuscular mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp. in relation to tissue nutrients and soluble carbohydrate in roots under field conditions. *Biol Fertil Soils* 31:114-120.
- Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soil by extraction with sodium bicarbonate. US Dep of Agric Rep, Circular no. 939.
- Pages M, Estain V, and V Calvet V (1985) Physical and chemical properties of olive marc compost. *Acta horticult* 172:271-280.
- Phillips JM, Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55:158-161.
- Plenchette C (1989) Mycorrhizal soil infectivity of Deherain's plots. *C R Acad Agric Fr* 75:21-27.
- Ryan MH, Chilvers GA, Dumaresq DC (1994) Colonization of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in organic manure than on a conventional neighbour. *Plant Soil* 160:33-40.
- SAS Institute (1996) SAS user's guide: basics. SAS Institute, Cary, NC.
- Schreiner PR, Ivors KL, Pinkerton JN (2001) Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. *Mycorrhiza* 11:273-277.
- Sreenivasa MN, Bagyaraj DJ (1989) Use of pesticides for mass production of vesicular-arbuscular mycorrhizal inoculum. *Plant Soil* 119:127-132.
- Sutton JC, Barron GL (1972) Population dynamics of *Endogone* spores in soil. *Can J Bot* 50:1909-1914.
- Tarafdar JC, Marschner H (1994) Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with organic phosphorus. *Soil Biol Biochem* 26:387-395.
- Trappe JM, Molina R, Castellano M (1984) Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. *Annu Rev Phytopathol* 22:331-359.
- Verma RK, Arya ID (1998) Effect of arbuscular mycorrhizal fungal isolates and organic manure on growth and mycorrhization of micropropagated *Dendrocalamus asper* plantlets and on spore production in their rhizosphere. *Mycorrhiza* 8:113-116.