

## EFFECT OF INOCULATION WITH *PIRIFORMOSPORA INDICA* AND *SEBACINA VERMIFERA* ON GROWTH OF SELECTED BRASSICACEAE PLANTS UNDER GREENHOUSE CONDITIONS

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### ABSTRACT

The effect of inoculation of two root-colonizing basidiomycete fungi *Piriformospora indica* and *Sebacina vermifera* on the host plants from the family Brassicaceae (*Brassica oleracea*, *B. napus*, *B. nigra*, *Lepidium sativum*, *Descurainia sophia* and *Matthiola incana*) was studied in a pot experiment. Both fungi were able to colonize all plant species tested but with different efficiency. Colonized plants reacted with increase of fitness, bigger leaves and more side shoots. Plant heights of five inoculated species were higher than of uninoculated controls. Five species had higher dry weight of shoots and four had higher dry weights of roots. The highest stimulation was stated for *B. oleracea* and the lowest for *L. sativum*. It can be connected with the intensity of root colonization, which was greater in *B. oleracea*. In most cases both fungi influenced growth and dry weight of plants similarly but *B. nigra* and *D. sophia* were stimulated more by *P. indica* and *B. napus* by *S. vermifera*.

**Key words:** Brassicaceae, *Piriformospora indica*, *Sebacina vermifera*, plant height, dry weight

### INTRODUCTION

The family Brassicaceae comprises approximately 338 genera and 3709 species (Warwick & Al-Shehbaz 2006), and includes several important economic crops (e.g., *Brassica* spp., *Lepidium sativum*, *Matthiola* spp., etc.) used as a source of oil, mustard condiments, vegetables, forage and fodder. The Brassicaceae are usually found in temperate regions of the northern hemisphere, especially Mediterranean regions, and colonize a great variety of habitats including the arctic and alpine regions and some of the most climatically inhospitable deserts, though it is poorly represented in the tropics (Hedge 1976).

*Piriformospora indica* (Verma et al. 1998) and *Sebacina vermifera* (Warcup 1988) are filamentous fungi belonging to the order Sebaciniales. Weiss et al. (2004) suggest that Sebaciniales represent one of the earliest Basidiomycete lineages with mycorrhizal members, which can form ecto-, ericoid and orchid mycorrhiza. *P. indica* was first isolated from the rhizosphere of *Prosopis juliflora* and *Zizyphus nummularia* in Rajasthan, India, and form a mutualistic symbiosis with a broad spectrum of host plants (Verma & Arya 1998; Pham et al. 2004). In contrast to the arbuscular mycorrhizal fungi (AMF) of the obligate biotrophic nature, *P. indica* and *S. vermifera* can be easily cultured on synthetic media (Varma et al. 2001; Kost & Rexer 2013). These fungi colonize roots; form pear-shaped spores within the cortex, and does not enter vascular tissue and stems or leaves of plants. The ability of *P. indica* and *S. vermifera* in improving the growth rate of various host plants is well documented. Endophytic root colonization by this fungus enhances growth of the host plants (Sahay & Varma 1999; Varma et al. 1999; Rai et al. 2001; Kumari et al. 2003; Peskan-Berghoefer et al. 2004; Waller et al. 2005; Druege et al. 2007; Das et al. 2012; Franken

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2012; Varma et al. 2012; Unnikumar et al. 2013). *S. vermifera* increased plant growth in *Nicotiana attenuata* (Barazani et al. 2005) and plant cell growth in suspension cultures of *Linum album* (Baldi et al. 2008). Beyond stimulating effects in biomass production, *P. indica* apparently supports its host by protecting it from pathogenic fungi (Waller et al. 2005). Ghahfarokhi and Goltapeh (2010) demonstrated that *P. indica* and *S. vermifera* were the most potent agents for the biocontrol of root plant pathogen *Gaeumannomyces graminis* var. *tritici*. In this study, we examined whether *P. indica* and *S. vermifera* influence the growth and development of some members of the family Brassicaceae.

## MATERIALS AND METHODS

### Fungal solid culture of *P. indica* and *S. vermifera*

Endophytic fungi (*P. indica*, *S. vermifera*) were obtained from Department of Plant Pathology, Tarbiat Modares University, Tehran, Iran (Prof. Mohammadi Goltapeh). These fungi were maintained on Kaefer's medium (Kaefer 1977). The plates were inoculated with actively growing mycelium and then incubated at 25 °C for a week in the dark and stored at 4 °C for further use.

### Fungal liquid culture of *P. indica* and *S. vermifera*

Mycelial liquid cultures were prepared in 500 ml flasks containing 200 ml of autoclaved Kaefer's liquid medium and inoculated with four 10 mm mycelia discs cut from 10-day-old solid cultures of *P. indica* or *S. vermifera*. Culture flasks were kept on a shaker (2.42 Hz) and incubated for 15 days at room temperature (25 ± 1 °C) until a dense mycelial suspension was generated. Then, the cultures were stored at 4 °C until they were used for the pot culture experiment.

### Pot culture experiment

*P. indica* and *S. vermifera* were investigated for their ability to grow and develop symbiosis with *Brassica oleracea* (cabbage), *Brassica napus* (rape-seed), *Brassica nigra* (black mustard), *Lepidium sativum* (Persian broadleaf cress), *Matthiola incana* (hoary stock 'Cheerful White') and *Descurainia so-*

*phia* (flixweed). Pot culture experiment was conducted using a completely randomized design in four replications (eight plants for each species). Seeds of species were surface-sterilized by soaking in 1% sodium hypochlorite for 1 min, rinsed thrice in sterile distilled water, and placed in perlite for germination. After 4-10 days when the plumules and radicles appeared, two germinated seeds were transferred to each pot. Pots were filled with a substrate containing 2:1:1 sterile mixture of sand, peat and perlite of the following chemical properties: pH 7.12, EC 0.9 dS/m, organic carbon 0.7%, total N 0.3%, available phosphorus 8.2 mg/kg and potassium 285 mg/kg. Pots were placed in a greenhouse at 18 °C, and a photoperiod of 16/8 h light/dark.

Fungal mycelia from liquid culture were washed with an excess of distilled water to completely remove medium and then mycelium was crushed in a blender for 10 s. For inoculation, 1 g of crushed mycelia was added to germlings 24 h after potting. Efforts were made to keep the root system in direct contact with the fungal inocula (Kumari et al. 2003; Varma & Schuepp 1995). Plant morphology and height as well as shoot and root dry weights were analyzed after 75 days.

For microscopic observations, the roots were washed thoroughly under running tap water and cut into 1 cm pieces. Segments were stained following the techniques described by Phillip and Hayman (1970) and Dickson et al. (1998). The root-pieces were examined under a microscope at a magnification of 10-60 X.

The percentage of root colonization was determined with the method proposed by Giovannetti and Mosse (1980). For each plant species, 10 roots were selected randomly from the stained roots, and then each selected root was divided into 10 pieces. The percentage of colonization was calculated by the presence of chlamydospores in each piece as follows (Rai et al. 2001):

$$\text{Percent colonization} = \frac{\text{No. of root segments colonized}}{\text{Total no. of segments observed}} \times 100$$

### Statistical analysis

The collected data were statistically computed using the software SAS 6.12. Data were subjected to analyses of variance and treatment means were

compared by approximate Duncan's multiple-range tests at  $p \leq 0.05$ .

## RESULTS

Microscopic inspection of the roots inoculated with *P. indica* and *S. vermifera* showed a high number of chlamydospores in root cells (Fig. 1). The percentage of colonized roots differed among plant

species (Table 1). The highest colonization values – 68 and 63% were found in *B. oleracea* inoculated with *P. indica* and *S. vermifera*, respectively, followed by *P. indica* (49%) and *B. nigra*. A much lower percentage of colonized roots were found in *L. sativum*, *D. sophia* and *M. incana* (30-15%). The percent of colonization was mostly similar for two fungi; only *B. oleracea* and *D. sophia* were colonized more intensively by *P. indica*.

Table 1. Effect of roots inoculation with *P. indica* and *S. vermifera* on plant height (cm), shoot and root dry weight (g) and root colonization (%) in six species of family Brassicaceae in pot culture experiment. Percentages connected with plant height, shoot and root dry weights were calculated taking controls as 100%. In parentheses is the percentage increase in value relative to the control

Plant species	Parameters	<i>Piriformospora indica</i>	<i>Sebacina vermifera</i>	Control
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
<i>Brassica oleracea</i>	Plant height	26.63 $\pm$ 2.11 <sup>a</sup> (17%)	29.08 $\pm$ 2.81 <sup>a</sup> (27%)	22.85 $\pm$ 2.09 <sup>b</sup>
	Shoot dry weight	2.94 $\pm$ 0.32 <sup>a</sup> (41%)	2.54 $\pm$ 0.32 <sup>a</sup> (22%)	2.09 $\pm$ 0.05 <sup>b</sup>
	Root dry weight	0.52 $\pm$ 0.12 <sup>a</sup> (41%)	0.51 $\pm$ 0.07 <sup>a</sup> (38%)	0.37 $\pm$ 0.02 <sup>b</sup>
	Root colonization	68 $\pm$ 3.27 <sup>a</sup>	63 $\pm$ 3.83 <sup>b</sup>	0 $\pm$ 0 <sup>c</sup>
<i>Brassica napus</i>	Plant height	38.20 $\pm$ 3.80 <sup>a</sup> (17%)	41.18 $\pm$ 4.21 <sup>a</sup> (26%)	32.58 $\pm$ 2.12 <sup>b</sup>
	Shoot dry weight	2.30 $\pm$ 0.10 <sup>b</sup> (17%)	2.62 $\pm$ 0.17 <sup>a</sup> (34%)	1.96 $\pm$ 0.18 <sup>c</sup>
	Root dry weight	0.40 $\pm$ 0.08 <sup>a</sup> (82%)	0.50 $\pm$ 0.08 <sup>a</sup> (127%)	0.22 $\pm$ 0.02 <sup>b</sup>
	Root colonization	39 $\pm$ 6.00 <sup>a</sup>	45 $\pm$ 3.83 <sup>a</sup>	0 $\pm$ 0 <sup>b</sup>
<i>Brassica nigra</i>	Plant height	59.00 $\pm$ 8.45 <sup>a</sup> (61%)	54.75 $\pm$ 13.40 <sup>a</sup> (49%)	36.75 $\pm$ 1.50 <sup>b</sup>
	Shoot dry weight	1.02 $\pm$ 0.11 <sup>a</sup> (76%)	0.75 $\pm$ 0.11 <sup>b</sup> (29%)	0.58 $\pm$ 0.07 <sup>c</sup>
	Root dry weight	0.22 $\pm$ 0.03 <sup>a</sup> (83%)	0.16 $\pm$ 0.02 <sup>b</sup> (33%)	0.12 $\pm$ 0.01 <sup>c</sup>
	Root colonization	49 $\pm$ 3.83 <sup>a</sup>	44 $\pm$ 5.66 <sup>a</sup>	0 $\pm$ 0 <sup>b</sup>
<i>Lepidium sativum</i>	Plant height	23.50 $\pm$ 3.51 <sup>a</sup> (15%)	21.25 $\pm$ 0.96 <sup>a</sup> (4%)	20.50 $\pm$ 0.58 <sup>a</sup>
	Shoot dry weight	0.85 $\pm$ 0.11 <sup>a</sup> (44%)	0.72 $\pm$ 0.10 <sup>ab</sup> (22%)	0.59 $\pm$ 0.06 <sup>b</sup>
	Root dry weight	0.11 $\pm$ 0.02 <sup>a</sup> (0)	0.12 $\pm$ 0.03 <sup>a</sup> (9%)	0.11 $\pm$ 0.02 <sup>a</sup>
	Root colonization	20 $\pm$ 3.27 <sup>a</sup>	15 $\pm$ 5.03 <sup>a</sup>	0 $\pm$ 0 <sup>b</sup>
<i>Descurainia sophia</i>	Plant height	15.00 $\pm$ 2.71 <sup>a</sup> (76%)	11.00 $\pm$ 1.41 <sup>b</sup> (29%)	8.50 $\pm$ 1.73 <sup>b</sup>
	Shoot dry weight	0.29 $\pm$ 0.07 <sup>a</sup> (71%)	0.19 $\pm$ 0.02 <sup>b</sup> (12%)	0.17 $\pm$ 0.03 <sup>b</sup>
	Root dry weight	0.15 $\pm$ 0.03 <sup>a</sup> (66%)	0.11 $\pm$ 0.01 <sup>b</sup> (22%)	0.09 $\pm$ 0.01 <sup>b</sup>
	Root colonization	29 $\pm$ 3.83 <sup>a</sup>	16 $\pm$ 3.27 <sup>b</sup>	0 $\pm$ 0 <sup>c</sup>
<i>Matthiola incana</i>	Plant height	26.53 $\pm$ 4.83 <sup>a</sup> (37%)	26.75 $\pm$ 1.73 <sup>a</sup> (38%)	19.35 $\pm$ 1.70 <sup>b</sup>
	Shoot dry weight	1.25 $\pm$ 0.33 <sup>a</sup> (23%)	1.38 $\pm$ 0.16 <sup>a</sup> (35%)	1.02 $\pm$ 0.13 <sup>a</sup>
	Root dry weight	0.32 $\pm$ 0.03 <sup>a</sup> (7%)	0.35 $\pm$ 0.03 <sup>a</sup> (17%)	0.30 $\pm$ 0.03 <sup>a</sup>
	Root colonization	28 $\pm$ 3.27 <sup>a</sup>	30 $\pm$ 2.31 <sup>a</sup>	0 $\pm$ 0 <sup>b</sup>

Values within a row followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test at the  $p \leq 0.05$  levels.

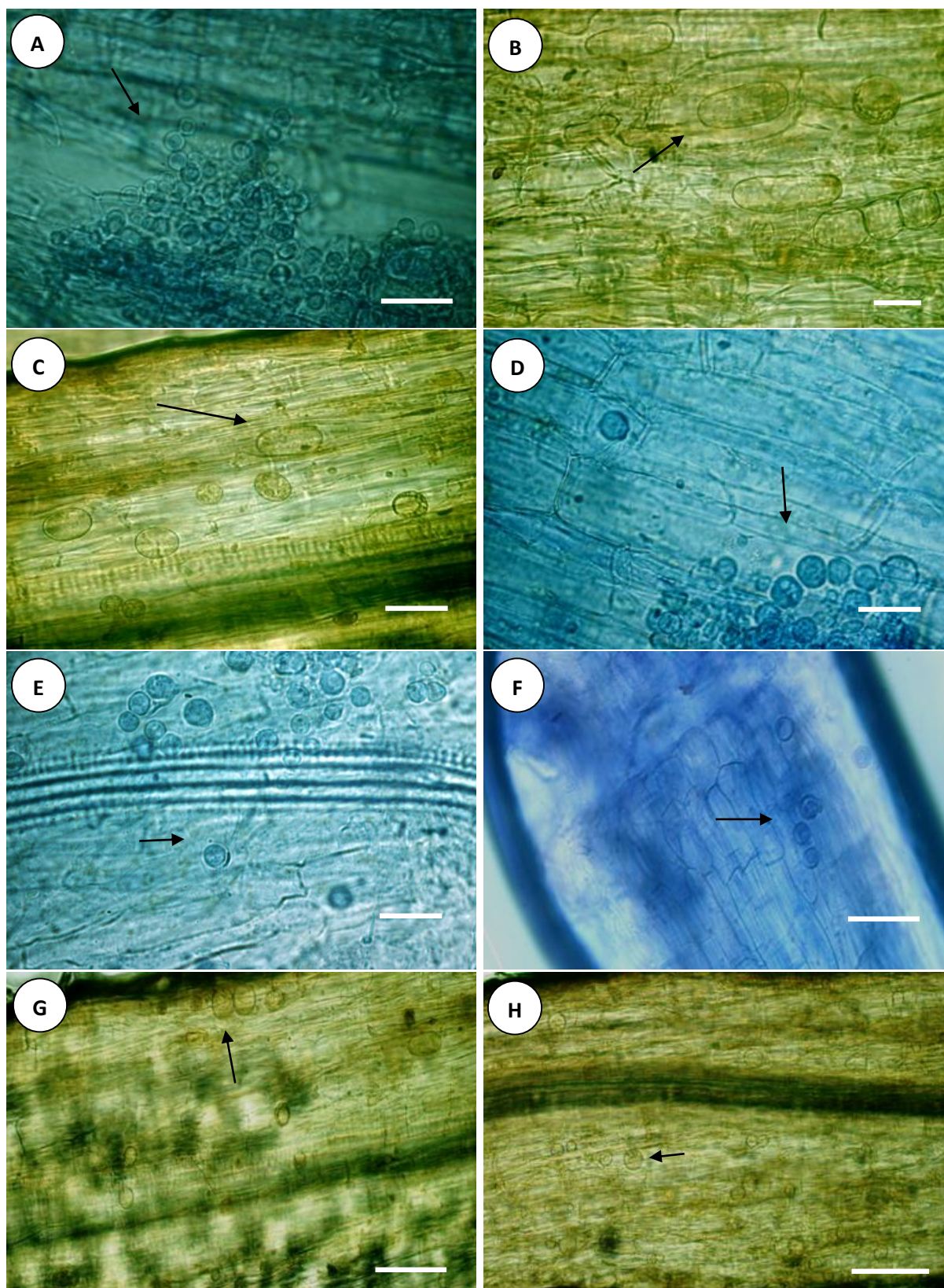


Fig. 1. Endophytic fungi in roots of Brassicaceae. A - *P. indica* in *B. oleracea* (Bar = 40  $\mu$ m). B, C - *S. vermifera* in *B. nigra* (Bars = 10  $\mu$ m, 20  $\mu$ m). D - *P. indica* in *B. napus* (Bar = 20  $\mu$ m). E - *P. indica* in *D. sophia* (Bar = 20  $\mu$ m). F - *P. indica* in *L. sativum* (Bar = 40  $\mu$ m). G, H - *S. vermifera* in *M. incana* (Bar = 40  $\mu$ m, 60  $\mu$ m). Black arrows show chlamydospores



Plants of all species inoculated with *P. indica* and *S. vermifera* presented better fitness with larger leaves and with more side shoots (Figs 2-7). Both the fungi significantly increased plant height of all species with the exception of *L. sativum*. Plant heights of *B. oleracea* and *B. napus* increased by 17-27%, depending on the fungus inoculated, in comparison to controls. Length of *B. nigra* and *D. sophia* was stimulated by *P. indica* by 61 and 76% in comparison to the control. Both fungi stimulated plants length similarly in *M. incana* (37-38%) (Table 1).

All plants, with the exception of *M. incana*, inoculated with the above fungi had higher shoot dry

weight in comparison to the control plants. The increase was highest in *B. nigra* and *D. sophia* inoculated with *P. indica* (by 76-71%). Shoot and root dry weights of inoculated plants of *B. oleracea* were higher in comparison to controls. *P. indica* and *S. vermifera* increased shoot dry weight by 41 and 22% and root dry weight by 41 and 38% (Table 1).

Root dry weight of *B. napus* was most affected by *S. vermifera* (127% increase) and *P. indica* (82% increase). Shoot and root dry weight of *B. nigra* was most affected by *P. indica* (76 and 83% increase, respectively) in comparison to controls (Table 1). Root dry weight of *L. sativum* and *M. incana* was not affected by fungal inoculation.

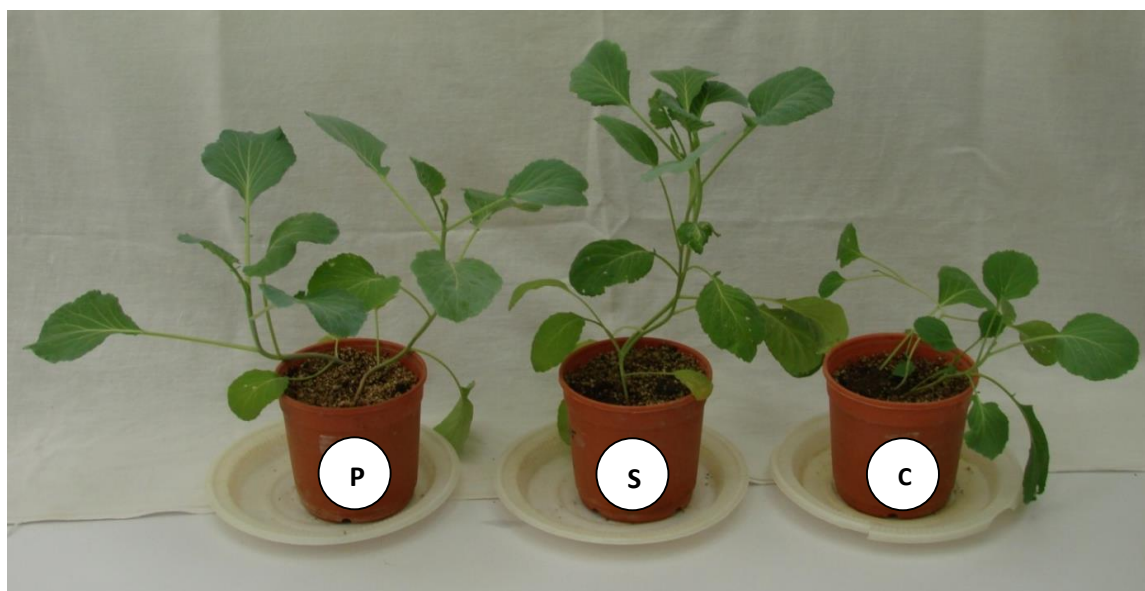


Fig. 2. Effect of *P. indica* and *S. vermifera* on growth of *B. oleracea*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

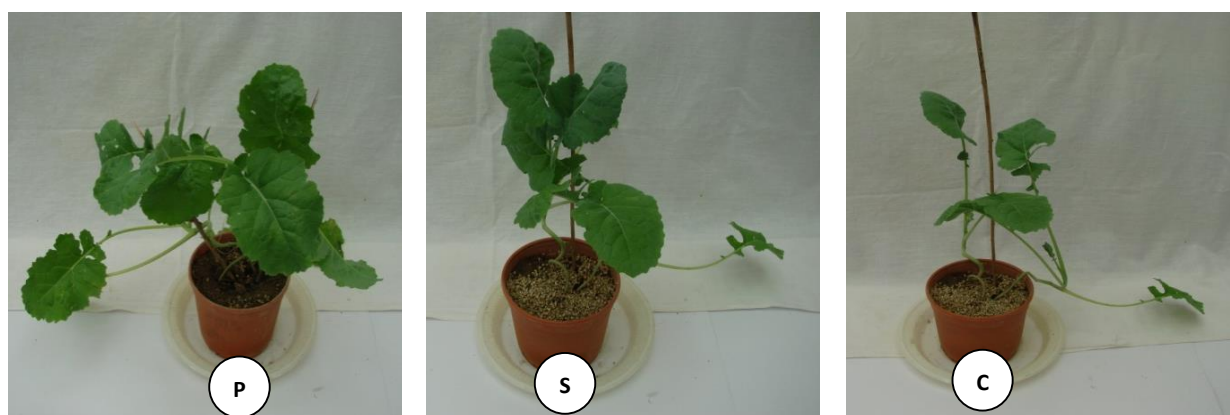


Fig. 3. Effect of *P. indica* and *S. vermifera* on growth of *B. napus*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

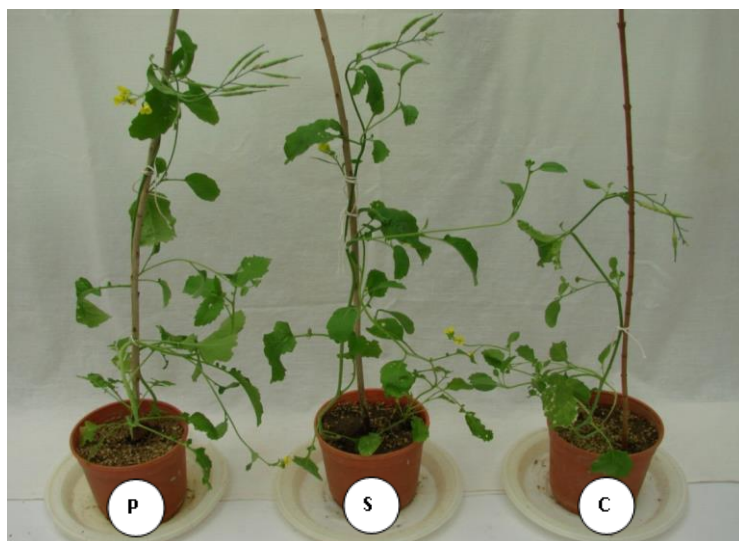


Fig. 4. Effect of *P. indica* and *S. vermifera* on growth of *B. nigra*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

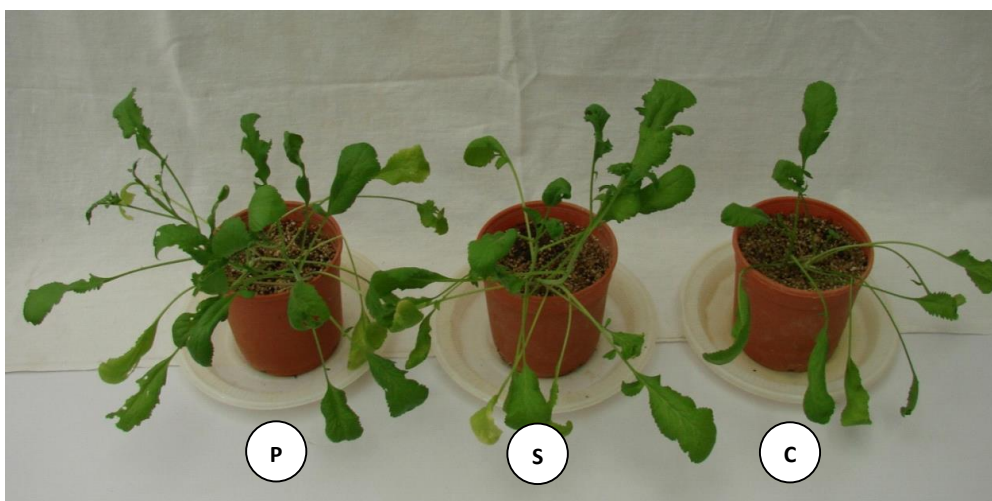


Fig. 5. Effect of *P. indica* and *S. vermifera* on growth of *L. sativum*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

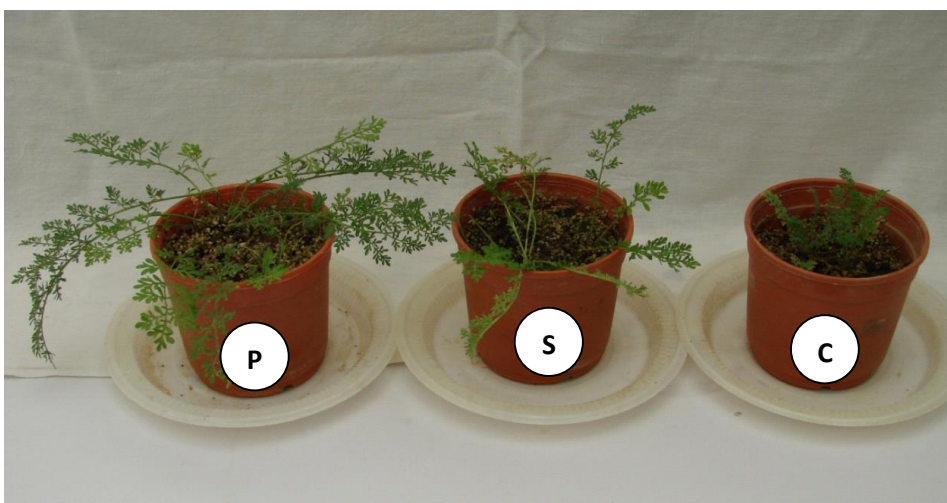


Fig. 6. Effect of *P. indica* and *S. vermifera* on growth of *D. sophia*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

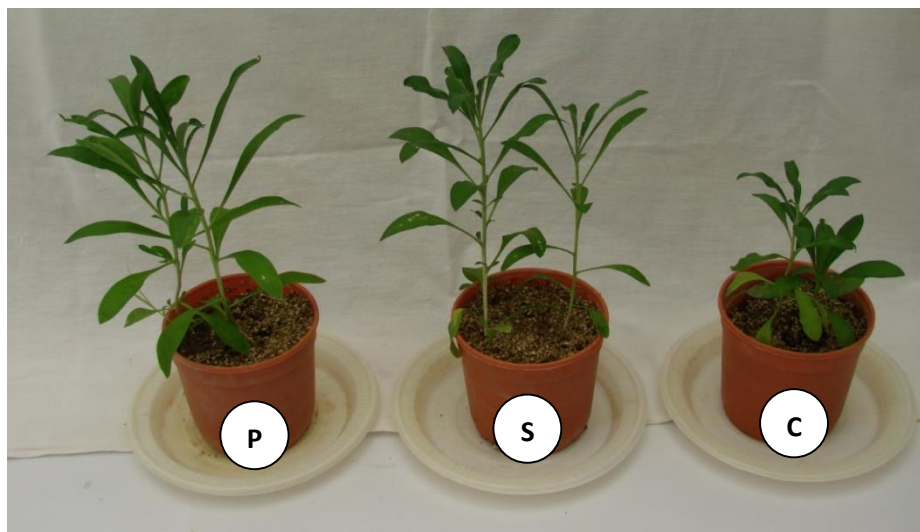


Fig. 7. Effect of *P. indica* and *S. vermifera* on growth of *M. incana*. P - *Piriformospora indica*, S - *Sebacina vermifera* and C - control

Taking into account plant morphology and shoot and root dry weight, *B. oleracea*, *B. nigra*, *L. sativum* and *D. sophia* were more stimulated by *P. indica* and *B. nigra*, and *M. incana* by *S. vermifera*. The weakest reaction was found in *L. sativum* where only shoot dry weight was influenced by *P. indica*. At the same time, roots of this plant were 3-4 times less colonized than roots of *B. oleracea*.

## DISCUSSION

Rhizosphere beneficial microorganisms increase the growth of plants through facilitation of the nutrient's uptake by either increasing the extent of roots or solubilizing macronutrients such as phosphorus or sulphur (López-Bucio et al. 2007). Various fungi colonizing roots have positive effects on host performance through the production of growth-promoting substances (Clay 1984; Jumpponen 2001).

The endophytic interaction of *P. indica* with plant roots is accompanied by an enormous acquisition of nitrogen and phosphorous from the environment. Experiments using  $^{32}\text{P}$  have shown that *P. indica* plays an important role in the acquisition of phosphorus by the roots especially in the arid and semi-arid regions (Yadav et al. 2010; Varma et al. 2012).

In this study, inoculation with *P. indica* and *S. vermifera* increased height of all plants, except of *L. sativum* and shoot and/or root dry weight except for *L. sativum* and *M. incana*. The more intense root proliferation in inoculated plants has been attributed to the synthesis of phytohormones (Singh et al. 2000; Varma et al. 2001). Sirrenberg et al. (2007) suggested that auxin produced by *P. indica* affects root growth and it is responsible for, or at least contributes to, the beneficial effect of fungus on its host plants. Vadassery et al. (2008) stated that *P. indica* produces low amounts of auxins and relatively high level of cytokinins, therefore cytokinin levels are higher in colonized roots of *Arabidopsis thaliana* compared with the uncolonized roots of controls. Our results are in agreement with those of Kumari et al. (2003), who showed that *B. oleracea* var. *capitata* (cabbage), *Spinacia oleracea* (spinach) and *B. juncea* (mustard) were colonized with *P. indica*. Members of the Brassicaceae (e.g. *A. thaliana*) and Chenopodiaceae are known to be non-host plants for mycorrhizal fungi, but they are able to interact with *P. indica* (Peskan-Berghoefer et al. 2004; Pham et al. 2004). The results of our experiments clearly demonstrate high effectiveness of *P. indica* and *S. vermifera* in increasing the crop growth. The shoots growth of *L. sativum* was not stimulated by fungi. Ghimire et al. (2009) reported that *S. ver-*



*mifera* increased plant height, root length, and biomass production of switchgrass (*Panicum virgatum* L.). Varma et al. (1999) reported that inoculation with *P. indica* promotes plant growth and biomass production in maize, tobacco, poplar, artemisia, parsley and *Bacopa*. In our experiment, species reacted differently for applied fungi. *B. oleracea*, *B. nigra*, *L. sativum* and *D. sophia* were under stronger influence of *P. indica* and *B. napus* and *M. incana* were more influenced by *S. vermifera*. The differences in plant reaction might be attributed to the level of root colonization by the two fungi. In Baldi's et al. (2008) publication, increase of cell growth in suspension cultures of *Linum album* was dependent on the concentration of fungal cells at co-cultivation cultures. Rai et al. (2001) reported that shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and flowers and seed production of *Spilanthes calva* and *Withania somnifera* were enhanced in the presence of *P. indica* in roots. *P. indica* increased disease resistance and yield of the monocotyledonous plant barley (Waller et al. 2005). Barazani et al. (2005) showed that colonization of *Nicotiana attenuata* with *S. vermifera* and *P. indica* stimulated seed germination and plant growth. Barazani et al. (2007) concluded that *S. vermifera* increased the growth and fitness of *N. attenuata* plants through downregulation of ethylene production.

In conclusion, *P. indica* and *S. vermifera* are able to colonize *B. oleracea*, *B. napus*, *B. nigra*, *L. sativum*, *D. sophia* and *M. incana* roots and to increase plant height, dry mass of shoots and roots in most plants. However, the impact of fungi depends on the plant species.

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