Piriformospora indica inoculants enhance flowering, yield, and physiological characteristics of tomato (*Solanum lycopersicum*) in different growth phases

Esmaeel Kaboosi[1], Mehdi Ghabooli[1*], Rouhollah Karimi[2]

1. Department of Plant Production and Genetics, Faculty of Agriculture, Malayer University, Malayer, Iran
2. Department of Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Iran

---

Abstract

Piriformospora indica is an endophytic fungus with plant-promoting properties in a wide range of host plants. This study aimed to assess and compare the effects of different *P. indica* inoculants through morphological and physiological analysis at different times (4, 8, and 12 weeks) after inoculation. The study was conducted in a completely randomized design with three levels of fungus inoculation (non-inoculated and inoculated with *P. indica* spore and mycelium). The results showed that both *P. indica* inoculants had a positive effect on the measured traits at different times after inoculation. Root and shoot dry weights increased significantly 4, 8, and 12 weeks after inoculation. *P. indica* improved the reproductive phase of tomato resulting in the increased dry weight of fruits by up to 51%. Most importantly, the endophyte enhanced tomato fruit yield by up to 73%. Based on the experimental data, *P. indica* increased total chlorophyll (25%), protein (143%), and carbohydrate (44%) contents in inoculated plants compared to non-inoculated plants. Besides, *P. indica* promoted the antioxidant capacity of the inoculated plants by increasing CAT and APX activity. In our study, plant inoculation with *P. indica* also remarkably led to an increase in K (172%) and P (41%) contents. Our data showed that both *P. indica* spore and mycelium have a long-term effect on tomato growth. The application of fungus inoculants promotes plant growth and yield. Hence, *P. indica* represents a suitable plant-stimulating biofertilizer for tomato in sustainable agriculture.

Keywords: Biofertilizer, biomass, fruit yield, growth performance, phosphorus

Kaboosi, E., M. Ghabooli, R. Karimi . 2022. *Piriformospora indica* inoculants enhance flowering, yield, and physiological characteristics of tomato (*Solanum lycopersicum*) in different growth phases*'. Iranian Journal of Plant Physiology 12* (3), 4183-4194.

Introduction

Tomato (*Solanum lycopersicum*) is one of the main vegetable crops worldwide due to its extensive consumption. This plant is a valuable food in human diet and is known as a protective food because of its unique nutritive value since it is a source of vitamins, carotenoids, lycopene, and antioxidant compounds (Santos-Sánchez et al., 2013). One of the factors affecting the productivity of tomato is production of good quality and vigorous seedlings. The well-grown seedlings with well-established root systems help to avoid seedling transplant shock (Vavrina, 1998).

* *Corresponding Author*
E-mail Address: m.ghabooli@malayeru.ac.ir
Received: February, 2021
Accepted: September, 2021

The use of beneficial microbes like endophytic fungi as biological agents at the vegetative stage can have advantageous effects. These effects include promotion of plant growth, biological control of diseases, increases in crop yield, and quality improvement. Plant growth-promoting fungi (PGPF) promote growth through the production of enzymes, phosphate solubilization (Malla et al., 2004; Wakelin et al., 2004), siderophore production (Costa and Loper, 1994) and, antagonism to phytopathogens (Ramamoorthy et al., 2001). They positively affect plant growth and lead to improved yields (Haas and Défago, 2005; Kloepper et al., 1980). The use of PGPF inoculants as biological agents for the production of vegetable seedlings has been reported by researchers (Kokalis–Burelle et al., 2002; Russo, 2006; Russo and Perkins-Veazie, 2010).

Piriformospora indica, belonging to the Sebacinales in the Basidiomycota was discovered in Thar desert, India and displays an endophytic lifestyle (Verma et al., 1998). *P. indica* is phylogenetically close to mycorrhizal endosymbionts of orchid and ericoid roots and can colonize the root cells of a broad range of host plants.

Inoculation of plant roots with *P. indica* can improve plant growth and yield in a broad range of the host (Baltruschat et al., 2008; Ghabooli et al., 2020; Lahrmann and Zuccaro, 2012; Sherameti et al., 2005; Waller et al., 2005; Yadav et al., 2010). This endophyte fungus also enhances the tolerance of colonized plants against abiotic and biotic stresses, promotes nutrient uptake, and increases seed production (Justice et al., 2018; Krishnaveni et al., 2014; Nouh et al., 2020; Oelmüller et al., 2009; Xu et al., 2018). *P. indica* enhances the establishment of micro-propagated plants (Sahay and Varma, 1999), adventitious root formation in cuttings, and lignan production of hairy root cultures; it also promotes flowering, and increases yield (Waller et al., 2005).

P. indica tremendously increases the growth and overall biomass of host plants including, rice (Kord et al., 2019), and barley (Ghabooli, 2014). There are some reports on tomato colonization with *P. indica*. Recent studies have shown that tomato root colonization with *P. indica* resulted in decreased disease severity caused by early blight (Terhonen et al., 2019). In tomatoes infected with *Verticillium dahliae*, *P. indica* increased leaf and fruit biomass and decreased disease severity. Also, *P. indica* reduced the concentration of Pepino mosaic virus in tomato shoots (Fakhro et al., 2010). Anith et al. (2015) showed that inoculation with the co-culture or a mixture of *P. indica* and *B. pumilus* significantly promoted tomato seedling growth (Anith et al., 2015). Finally, *P. indica* has been reported to act as a growth promoter for tomato and seems to alter Na^+/K^+ homeostasis and antioxidant enzymes of greenhouse tomatoes grown under salt stress (Abdelaziz et al., 2019).

Based on previous results, tomato is one of the most important hosts of *P. indica*, and symbiosis relationship between plant-fungus has positive effects on its growth and development. The bulk of former research focused on a single life phase of tomato. Hence, the purpose of this study was to study the long-term effect of *P. indica* on tomato from seedling phase to flowering stage and yield.

Materials and Methods

Fungus culture, plant material, and P. indica inoculation

This study was conducted in a completely randomized design in three levels of fungus inoculation (non-inoculated and inoculated with *P. indica* spore and mycelium) with three replications. *P. indica* was cultured according to the method of Ghabooli et al. (2014). After collection of fungal spores, their numbers were counted and adjusted to 5×10^5 spores per mL. To prepare the fungal mycelium, the active discs were placed in a liquid medium and then incubated in a shaker incubator at 28 °C and 150 rpm for 7-10 days. Next, mycelium was filtered and washed several times with distilled water to remove the medium (Bajaj et al., 2015). Tomato seeds (*Solanum lycopersicum* CV. Early Urbana), obtained from Pakanbazr Co., were surface-sterilized by immersion in 70% v/v ethanol for 30 seconds and then in 10% sodium hypochlorite (NaOCl) for 5 min. Then, the seeds were rinsed in water and were germinated for seven days. Four-day tomato seedlings were inoculated by

immersing in the spore suspension solution with gentle shaking for 1-2 h. For mycelium treatment, 1% (w/v) of mycelium suspension was directly added to each plant. The mock-treated seedlings whether were dipped in sterile water or treated with autoclaved mycelium. The seedlings were later transferred into boxes (5 kg plastic boxes), filled with normal soil, and then placed at 25 ± 2 °C, relative humidity of 55% ± 5%, under a 16 h light and 8 h dark cycle condition for 105 days in the Research Greenhouse of Agricultural Faculty, Malayer University. The seeding growth till fruiting period and the samples for morpho-physiological analysis were harvested in three stages (four weeks after inoculation, eight weeks after inoculation, and finally, at flowering stage and yield).

Shoot and root dry weight, and chlorophyll content

For dry weight measurement, the shoot and root samples were incubated in an oven at 70 °C for 48h. Photosynthetic pigments (chlorophyll a, chlorophyll b, and total chlorophyll) were extracted from leaf samples in 80% acetone (v/v) as described by Arnon (1949). After centrifugation, the optical density of the supernatant was recorded at 645, 663, and 470 nm using a UV-Vis spectrophotometer.

Carbohydrate and protein content

Carbohydrate content in tomato leaves was quantified in 95% ethanol extracts (Irigoyen et al., 1992). First, 0.5 g freshly harvested leaves were ground in 5 ml of 95% (v/v) ethanol. The insoluble fraction of the extract was washed twice with 5 ml of 70% ethanol. All soluble fractions were centrifuged at 3500 g for 10 min. The supernatants were collected and stored at 4 °C for carbohydrate determination. Carbohydrates were analyzed by reacting 0.1ml of the alcoholic extract with 3 ml freshly prepared anthrone (150 mg anthrone + 100 ml H_2SO_4 72%) and placed in a boiling water bath for 10 min. After cooling, the absorbance at 625 nm was measured in a spectrophotometer (Analytik Jena Spekol, 2000).

Bradford method (1976) was used for the determination of total soluble protein content. Five ml of the protein reagent was added to 0.1 ml of the extract, and the contents were mixed in a vortex mixer. The absorbance was measured at 595 nm after 1 h. Bovine serum albumin (BSA) was used as a standard.

Enzyme activity

To measure the enzyme, 0.5 g of leaf powder was transferred to 2 ml microtubes and centrifuged by adding 1 ml of the extraction buffer (50 mM K-phosphate buffer [pH 7.0]) for 15 minutes at 14000 rpm at 4 °C. After centrifugation, the supernatant was transferred to 1.5 ml microtubes and centrifuged again for 10 minutes at 10000 rpm at 4 °C. After centrifugation, the supernatant was placed in a new microtube of the same volume and was stored in an ice container. Other samples were centrifuged and stored at – 80 °C if not used (Beauchamp and Fridovich, 1971). This extract was used to assay superoxide dismutase, ascorbate peroxidase, and catalase. The total activity of the superoxide dismutase enzyme was determined by measuring its ability to prevent the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977). Ascorbate peroxidase activity was done as described by Nakano and Asada (1981) and by measuring the ascorbate oxidation at 290 nm for 1 minute. Catalase activity was measured by monitoring the decomposition of H_2O_2 by a spectrophotometer at 240 nm for 1 minute (Chance and Maehly, 1955).

Leaf elements (K and P)

Potassium and phosphorous contents were extracted from 0.2 g of dry leaves. Acid digestion was carried out by mixing samples with 4 ml HNO_3 and 1 ml $HCLO_4$ and heated to 220 °C for 20 min. The resulting mixture was extracted with 5 ml HNO_3 and adjusted to the final volume of 250 ml of distilled water. The K and P contents were analyzed by inductively coupled plasma spectrometry. The element contents were expressed as mg/g F.W. (Abdel-Shafy et al., 1994).

Results

Four weeks after inoculation

We first studied the interaction of *P. indica* with tomato roots. Tomato roots inoculated with *P.*

Table 1
Effects of different fungus inoculants on the measured morphological parameters of tomato (4W AI)

Fungus treatments	Root length (cm)	Shoot length (cm)	Leaf No.	Root D.W. (g)	Shoot D.W. (g)
P_0	8.40^b	13^b	4.333^c	0.094^b	0.588^b
P_1	13.00^a	18.4^a	8.667^a	0.149^a	0.727^a
P_2	10.567^{ab}	12.967^b	6.33^b	0.141^a	0.721^a

Mean values marked with different letters are significantly different (P≤0.05) by Duncan's multiple range test (P_0: non-inoculated; P_1: *P. indica* mycelium; P_2: *P. indica* spore).

Table 2
Effects of different fungus inoculants on the measured morphological parameters of tomato (8 WAI)

Fungus treatments	No. of branch	Plant height (cm)	Leaf No.	Root D.W. (gr)	Shoot D.W. (gr)
P_0	4.00^b	40.00^c	26.67^b	1.69^b	20.88^b
P_1	7.33^a	69.33^a	50.67^a	2.28^{ab}	34.41^a
P_2	6.67^a	55.67^b	47.33^a	2.40^a	34.17^a

Mean values marked with different letters are significantly different (P≤0.05) by Duncan's multiple range test (P_0: non-inoculated; P_1: *P. indica* mycelium; P_2: *P. indica* spore).

indica showed a high degree of root colonization. *P. indica* had a growth-promoting effect on inoculated plants as they grew faster and more vigorously than non-inoculated plants. Microscopic analyses showed efficient colonized tomato roots as measured by the production of the number of chlamydospores, two weeks after spore inoculation. No colonization was observed in non-inoculated plant roots (Data not shown).

Morpho-physiological traits

Shoot length, root length, number of leaves, and dry weight of shoots and roots were evaluated four weeks after inoculation (Table 1). *P. indica* increased leaf number of colonized tomato plants by two times higher than non-inoculated plants (8.66, 6.33, and 4.33 in mycelium, spore-treated, and non-inoculated plants, respectively). As expected, fungus inoculation had a significant positive effect on root dry weight. The root dry weight of *P. indica*-colonized tomato plants was up to 58% higher than non-inoculated plants. The roots of the *P. indica*-inoculated plants were 54% longer than those of the non-inoculated plants. Similarly, results showed that shoot length of the inoculated plants increased by 41% compared to the control plants. Furthermore, the results revealed an increase in shoot dry weight of the inoculated as compared with non-inoculated tomato plants (Table 1).

Morpho-physiological traits (eight weeks after inoculation)

To evaluate the durability of the positive effects of the fungus inoculation, the same morpho-physiological traits were measured eight weeks after inoculation. The mean comparison results showed that eight weeks after inoculation, nearly all measured traits were improved in *P. indica*-inoculated plants compared to non-inoculated plants (Table 2). Like previous results, *P. indica* increased leaf number (89%) and branch number (83%) in colonized tomato plants compared to non-inoculated plants (Table 2). Also, results showed that inoculation with both inoculums (mycelium and spore) increased the plant height by an average of 73%. The effect of fungus inoculation on root dry weight was significant. Root dry weight of *P. indica* spore-colonized plants increased up to 42%, compared to control plants. In the same way, the results revealed an increase in shoot dry weight of colonized plants compared to non-inoculated tomato plants, as inoculation with fungus spore and mycelium increased shoot dry weight by 64%. Overall, the results of analysis of morphological traits showed that inoculation with *P. indica* had a positive effect both in 4 WAI and 8 WAI. Also, spore suspension and mycelium had almost the same effect as inoculated plants.

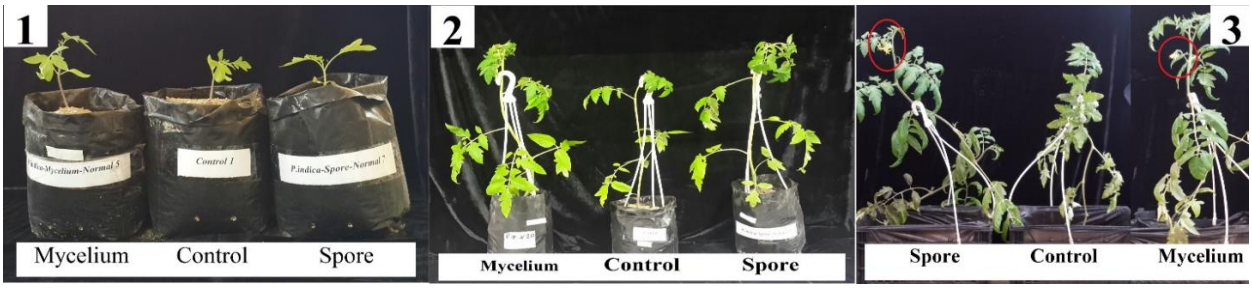

Fig. I. Effect of *Piriformospora indica* inoculants on tomato growth rates (1): 4 weeks after inoculation; (2): 8 weeks after inoculation; (3): 12 weeks after inoculation

Flowering and fruiting period (12 weeks after inoculation)

At the final step, the effect of *P. indica* inoculation on some morphological and physiological traits was studied during the flowering and fruiting period.

Morpho-physiological traits

The endophytic fungus, *P. indica* maintained its positive effect on morphological traits from the beginning of seedling growth until the flowering and fruiting stage (Fig. I). The same positive effects were seen in this step as well (Table 3). *P. indica* increased shoot dry (47%) weight in colonized tomato plants compared to non-inoculated plants (Table 3). The results also showed that root dry weight increased by 66% in inoculated tomato plants. These results indicate that shoot and root weight were higher in inoculated plants compared to control plants in all three stages. Tomato plant colonization with *P. indica* also increased stem diameter by 40%.

Another factor studied in this experiment was the number of flowers. Tomato flowers were measured in two steps: 10 and 12 weeks after inoculation. Flower numbers were increased by 1.5 times and 40% in 10 and 12 WAI, respectively. *P. indica* promoted flower-fruit conversion ratio and increased this trait by 44% in the colonized plants. Also, the fungal root colonization had a significant and positive effect on the number of tomato fruits. The number of fruits of *P. indica*-colonized plants increased by 96% compared to the control plants (Table 4).

P. indica also had a clear effect on the time of fruit coloring. *P. indica* spore and mycelium caused the

Table 3
Effects of different fungus inoculants on measured morphological parameters of tomato at final step (12 WAI)

Fungus treatment	Shoot D.W. (g)	Root D.W. (g)	Stem Diameter (cm)
P_0	61.15^b	9.19^b	1.33^c
P_1	90.08^a	14.47^a	1.87^a
P_2	87.54^a	15.34^a	1.63^b

Mean values marked with different letters are significantly different (P≤0.05) by Duncan's multiple range test (P_0: non-inoculated; P_1: *P. indica* mycelium; P_2: *P. indica* spore).

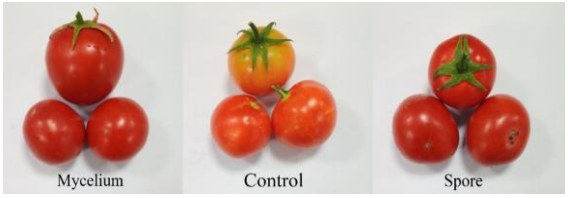

Fig. II. Effect of different fungus inoculants (mycelium and spore) on tomato fruit coloring

early coloring of tomato by 12 and 10 times higher, respectively compared to the color of tomato fruit of non-inoculated plants (Fig. II). The results revealed an increase in fruit diameter and fruit fresh weight of colonized plants compared to non-inoculated tomato plants as inoculation with fungus increased fruit fresh and dry weights by 45% and 51%, respectively. An increase of 34% in fruit diameter was seen in *P. indica*-colonized tomato plants. Eventually, inoculation with *P. indica* improved tomato yield by 73% in inoculated plants (Table 4).

Photosynthetic pigments

There was a significant difference concerning chlorophyll a, chlorophyll b, and total chlorophyll

Table 4
Effects of different fungus inoculants on the measured morphological fruit parameters of tomato at final step (12 WAI)

Fungus treatment	Flower No. in 10 WAI	Total Flower No.	flower-fruit conversion ratio (%)	Fruit No.	Fruit F.W. (g)	Fruit D.W. (g)	Fruit Diameter (cm)	Red Fruit No. in 14 WAI	Yield (g)
P_0	11.00^b	20.67^b	46.81^c	9.67^c	39.38^b	2.76^c	3.86^b	1.00^b	801.67^b
P_1	27.67^a	23^b	62.34^b	14.33^b	57.48^a	3.87^b	5.05^a	10.67^a	1192.67^a
P_2	26^a	28.00^a	67.83^a	19.00^a	53.78^a	4.17^a	5.21^a	12.00^a	1393.33^a

Mean values marked with different letters are significantly different (P≤0.05) by Duncan's multiple range test (P_0: non-inoculated; P_1: *P. indica* mycelium; P_2: *P. indica* spore).

Table 5
Effects of different fungus inoculants on the measured physiological parameters of tomato at final step (12 WAI)

Fungus treatment	Chl. a (mg/g)	Chl. b (mg/g)	Total Chl. (mg/g)	Carbohydrate (% in 100 mg)	Protein (mg/g)	CAT (ΔOD/min.FW)	SOD (Unit/mg fw.min)	APX (ΔOD/min.FW)	K (mg/g DW)	P (mg/g DW)
P_0	1.62^b	0.38^b	2.005^b	16.30^b	0.1007^b	0.593^b	1.950^a	11.997^b	11.803^c	2.997^b
P_1	1.73^a	0.79^a	2.523^a	16.91^b	0.2450^a	0.860^a	1.890^b	54.670^a	32.200^a	4.240^a
P_2	1.67^b	0.45^{ab}	2.122^{ab}	23.50^a	0.1557^{ab}	0.863^a	1.870^b	51.633^a	26.157^b	3.973^a

Mean values marked with different letters are significantly different (P≤0.05) by Duncan's multiple range test (P_0: non-inoculated; P_1: *P. indica* mycelium; P_2: *P. indica* spore).

content between the inoculated and non-inoculated tomato plants when analyzed after eight weeks of inoculation. Both mycelium and spore increased photosynthetic pigments. The content of Chl. a, Chl. B, and total Chl. of *P. indica* spore-colonized plants increased up to 6%, 107%, and 25% compared to control plants, respectively (Table 5).

Protein and carbohydrate content

Total protein contents of *P. indica*-colonized tomato leaves were higher than those of the non-colonized controls. The results showed that the protein content of inoculated plants increased by 1.43 times compared to control plants. The carbohydrate content in the inoculated treatment recorded an increase compared to that of the control plants. Our results revealed that carbohydrate content increased root growth by 44% (Table 5).

Antioxidant enzyme

The antioxidant enzyme activities of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) were assayed, and the results are depicted in Table 5. The level of CAT and APX increased in *P. indica* co-inoculated plants. An increase by 45% and 35% on CAT and APX activity was seen in *P. indica*-colonized tomato plants, respectively. In contrast to CAT and APX, inoculation with *P. indica* slightly decreased the SOD activity by 3.17%.

K and P content

To determine whether the fungal effect on growth and yield is reflected by an increase in nutrient uptake, we measured the K and P contents in the leaves of inoculated and non-inoculated plants. K and P contents significantly increased in plants inoculated with both *P. indica* inoculums. Accordingly, *P. indica* increased K and P by 172% and 41%, respectively in inoculated tomato plants compared to non-inoculated plants. Thus, it appears that *P. indica* specifically promotes K and P uptake.

Discussion

In the present study, we established a symbiosis of tomato with different *P. indica* inoculants. The interesting finding of this research is the significant effects of using *P. indica* spore and mycelium in enhancing tomato growth and yield. It was demonstrated that the growth and biomass of leaf, root, and fruit promoted in tomato plants following *P. indica* spore and mycelium colonization. Overall, results clearly showed a

positive effect of *P. indica* on all measured traits in three measurement steps. To determine whether *P. indica* increases the growth of tomato, the biomass of the above and underground parts of plants were monitored after 4, 8, and 12 weeks of inoculation with *P. indica*. Our results showed a strong promotion of shoot and root formation and development in *P. indica*-colonized plants compared to that in non-colonized plants. *P. indica* directly enhances plant root biomass by producing indole-3-acetic acid (IAA) (Sirrenberg et al., 2007). Ghabooli et al. (2015) showed that inoculation of rice plants with *P. indica* increases morphological and physiological traits and also enhances drought tolerance (Ghabooli et al., 2015). Fakhro et al. (2010) also reported that the number of leaves, root length, plant height, shoot, and root fresh and dry weights increased in *P. indica*-inoculated plants (Fakhro et al., 2010). Similarly, *P. indica*-colonized barley plants showed an increase in dry weight and root length and number compared with non-colonized plants (Ghabooli, 2014). The present study revealed that both *P. indica* inoculants stimulate tomato plant growth at the vegetative and also reproductive phases which is in line with the above studies. Growth-promoting microorganisms are involved in increasing plant branching (Vessey, 2003) and height (Burd et al., 2000) by producing plant growth hormones such as auxin and cytokinin.

P. indica also increased tomato leaf number which can increase light absorption surface. Consistent with these findings, an increased number of leaves has been reported in former studies *(Anith et al., 2015; Wang et al.,* 2015). Increasing stem diameter could improve the stability of the plant. It also increases the vessel diameter, which facilitates the exchange of water, minerals, amino acids, carbohydrates, and signal molecules (Dickison, 2000; Olson and Rosell, 2013). Accumulation of cytokinin hormone could be one of the possible mechanisms included in increasing stem diameter, which plays a crucial role in cell division and expansion (Kieber and Schaller, 2018). There have also been reports that the level of gibberellin increased in endophytic fungus-inoculated plants (Khan et al., 2013; Waqas et al., 2012). Tomato inoculated with *P. indica* inoculants had greater plant height at 8 WAI. Our data at 12 WAI showed that the root and shoot dry weights of inoculated plants were higher than non-inoculated plants. These results showed that both fungus inoculants had a long-term effect on plant biomass and growth.

P. indica inoculation promotes earlier flowering of tomato plants. Tomato plants inoculated with *P. indica* had a higher number of flowers. Also, the flower-fruit conversion ratio was promoted in inoculated plants. In addition, the rate of fruit color change was higher in inoculated plant compared to non-inoculated plants. Recently, early flowering is more considered as an effective way to shorten the growth period and thus enhance yield (Pan et al., 2017). The fungus *P. indica* also enhanced fruit number and this was accompanied by increases in flower number and flower-fruit conversion ratio. *P. indica*-inoculated plants also showed an increase in fruit fresh and dry weights that in turn had a positive effect on total yield. Positive effects of *P. indica* fungus on yield have been attributed to improved photosynthetic pigments and water and mineral nutrients uptake, with greater absorption through the enhanced surface area provided by extensive fungal hyphae and increased root length and surface. An increase in the number of flowers and fresh weight of the fruits has been reported (Gill et al., 2016).

The results of the present study demonstrated the beneficial effects of *P. indica* inoculation on some physiological traits of tomato. Photosynthetic efficiency is widely accepted as vital for enhancing plant biomass and yield. The content of photosynthetic pigments are the most important factors in the photosynthetic capacity of plants, and it is also considered an indicator of plant growth and fitness because they directly affect the speed and amount of photosynthesis and biomass production (Guerfel et al*.,* 2009). The mycorrhizal fungus helps to increase chlorophyll content and subsequent photosynthesis by establishing symbiotic relationships with plants in the uptake and availability of some elements such as phosphorus, which is a key element in the energy transfer chain during photosynthesis (Sharifi et al., 2011). In the present work, the co-cultivation of tomato plants with *P. indica* had significantly

higher contents of Chl a, Chl b, and total Chl, than non-colonized plants. An increase in total chlorophyll due to inoculation with mycorrhiza has been previously reported by other researchers (Pal and Pandey, 2017), which is consistent with our results.

Like chlorophyll, carbohydrate content was also found to increase in *P. indica*-inoculated plants compared to control plants. Changes in carbohydrate content are of special importance because of their direct dependence on physiological processes like photosynthesis, translocation, and respiration (Mane et al., 2011). Wang et al. (2015) reported increased carbohydrate content due to inoculation with *P. indica* in tomato, which is in line with the results obtained in this research. The results of the present study showed a positive relationship between carbohydrate accumulation and performance of tomato in terms of photosynthesis as the inoculated plants had higher biomass.

In this study, higher protein concentrations were seen in *P. indica*-inoculated plants compared with non-inoculated plants. Improved N uptake promotes the activity of key N-assimilating enzymes and increases protein concentration. Similarly, nitrate reductase shows increased expression in roots colonized by *P. indica*, which could emphasize that the fungus also supports N nutrition of plants (Sherameti et al., 2005).

The results showed that the level of CAT and APX significantly increased in *P. indica*-inoculated plants whereas inoculation with *P. indica* slightly decreased the SOD activity. There are abundant evidences that *P. indica* can regulate the major antioxidant enzymes and other constituents of ROS- scavenging system (Hamilton et al., 2012; Sun et al., 2010). *P. indica* enhanced the antioxidant enzyme activities which help in defense and tolerance mediation against abiotic stresses. Baltruschat et al. (2008) have suggested that antioxidant enzyme system was activated in inoculated plant leaves (Baltruschat et al., 2008). This clearly shows that *P. indica* might have used the same mechanism to activate antioxidant enzymes to prevent ROS-induced oxidative damage. Catalase, ascorbate peroxidase, and superoxide dismutase are among the metalloenzymes, and micronutrients can play an effective role in reducing or increasing their production in plants. Deficiency or increased availability of micronutrients such as iron, zinc, copper, manganese, and magnesium affect the expression of metalloenzymes (Del Río et al., 1991). Increased enzymatic activity due to AM colonization may be related to the effective role of AM in the availability of various elements (Ruiz-Lozano et al., 2012). It seems that *P. indica* can help produce antioxidant enzymes indirectly by facilitating the availability of various macro and micronutrients.

Faster growth of inoculated plants may be analyzed by an enhanced efficiency of nutrient uptake (Debbarma and Das, 2017). In our study, plant inoculation with *P. indica* led to a remarkable increase in K and P contents. This indicates that the inoculated plants were capable of transporting much more K and P to the shoots. The higher K availability may be thus instrumental for the fully functional stomatal operation and conserve a pool of available ATP that is used for the process of de novo synthesis of organic osmolytes (Yun et al., 2018). The effect of *P. indica* on K content was reported in several studies (Ghabooli, 2014; Ghaffari et al., 2016; Kord et al., 2019).

Phosphorous is the second most important macronutrient after nitrogen for crop production. However, it is also the least accessible macronutrient and plants cannot use it directly. The accessibility of P for the hosts in the soil depends on the available P form, the exudation of organic acids and/or protons, and phosphatase enzyme activities in the rhizosphere (Richardson and Simpson, 2011). *P. indica* fungus helps the host plant by producing complex and insoluble forms of phosphate in the soil through releasing large amounts of phosphatase acid (Yadav et al., 2010). Ngwene et al. (2016) showed that *P. indica* solubilizes P to inorganic form. In our data, the P content in tomato leaves was strongly stimulated by *P. indica*. Shahollari et al. (2005) reported that *P. indica* promotes the phosphate uptake 2-3 times higher in Arabidopsis seedlings and suggested that *P. indica* enhances Arabidopsis growth in a manner parallel to mycorrhizal fungi.

This study showed that *P. indica* inoculants retained their stimulatory effect on the measured traits at different stages. The results obtained from the measurements of the first stage (4 weeks after inoculation) showed that the symbiosis of *P. indica* fungus with the plant has an increasing effect on all measured traits. After eight weeks of inoculation, the effect of fungus inoculation in most traits increased significantly compared to the former period. In the final step, the vegetative growth rate of the plant was lower than in the previous steps, but the inoculated plants still had better growth than the non-inoculated plants. It seems that at this step, the plant, by activating the flowering and fruit set mechanism, reduces vegetative growth and provides the materials needed for this process. In summary, the stimulatory effect of *P. indica* is detectable during the critical early phase to the late phase of plant growth.

Conclusion

Inoculation of *P. indica* fungus inoculants significantly increased growth and yield compared with non-inoculated plants. Our data showed that both *P. indica* spore and mycelium had a long-term effect on tomato growth as nearly all measured traits were increased in three stages. So, the application of fungus inoculants can help tomato seedlings to grow vigorously during their life. In field application, *P. indica* would probably remain active in the soil for several months. Overall, the employment of *P. indica* as a plant-stimulating biofertilizer offers novel opportunities for its application in sustainable crop production. Further studies should be performed to optimize the productivity of inoculants and to prove the efficacy of the fungus mycelium and with other plants.

References

Abdel-Shafy, H. I., W. Hegemann and A. Teiner, 1994. Accumulation of metals by vascular plants. *Environmental Management and Health*. 5, (2) 1-4.

Abdelaziz, M. E., M. Abdelsattar, E. A. Abdeldaym, M. A. Atia, A. W. M. Mahmoud, M. M. Saad and H. Hirt, 2019. *Piriformospora indica* alters Na+/K+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Scientia Horticulturae*. 256, 108532.

Anith, K., A. Sreekumar and J. Sreekumar, 2015. The growth of tomato seedlings inoculated with co-cultivated *Piriformospora indica* and Bacillus pumilus. *Symbiosis*. 65, (1) 9-16.

Arnon, D. I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant physiology*. 24, (1) 1.

Bajaj, R., W. Hu, Y. Huang, S. Chen, R. Prasad, A. Varma and K. E. Bushley, 2015. The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode. *Biological control*. 90, 193-199.

Baltruschat, H., J. Fodor, B. D. Harrach, E. Niemczyk, B. Barna, G. Gullner, A. Janeczko, K. H. Kogel, P. Schäfer and I. Schwarczinger, 2008. Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytologist*. 180, (2) 501-510.

Barrs, H. and P. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian journal of biological sciences*. 15, (3) 413-428.

Beauchamp, C. and I. Fridovich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*. 44, (1) 276-287.

Burd, G. I., D. G. Dixon and B. R. Glick, 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Canadian journal of microbiology*. 46, (3) 237-245.

Chance, B. and A. Maehly, 1955. [136] Assay of catalases and peroxidases.

Costa, J. M. and J. E. Loper, 1994. Characterization of siderophore production by the biological control agent Enterobacter cloacae. *MPMI-Molecular Plant Microbe Interactions*. 7, (4) 440-448.

Debbarma, M. and S. P. Das, 2017. Priming of seed: enhancing growth and development. *Int J Curr Microbiol App Sci*. 6, (12) 2390-2396.

Del Río, L. A., F. Sevilla, L. M. Sandalio and J. M. Palma, 1991. Nutritional effect and expression of SODs: induction and gene expression; diagnostics; prospective protection against oxygen toxicity. *Free Radical Research Communications*. 13, (1) 819-827.

Dickison, W. 2000. Integrative plant anatomy.,(Harcourt Academic Press: San Diego, CA). Calif

Fakhro, A., D. R. Andrade-Linares, S. Von Bargen, M. Bandte, C. Büttner, R. Grosch, D. Schwarz and P. Franken, 2010. Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza*. 20, (3) 191-200.

Ghabooli, M., 2014. Effect of *Piriformospora indica* inoculation on some physiological traits of barley (Hordeum vulgare) under salt stress. *Chemistry of natural compounds*. 50, (6) 1082-1087.

Ghabooli, M., G. Hosseini Salekdeh and M. Sepehri, 2015. The effect of mycorrhiza-like fungus *Piriformospora indica* on some morphophysiological traits of rice under normal and drought stress conditions.

Ghabooli, M., E. Rezaei, Z. Movahedi and E. Mohsenifard, 2020. Effect of *Piriformospora indica* inoculation on some morphophysiological parameters in licorice (Glycyrrhiza glabra L.) under drought stress. *Iranian Journal of Plant Physiology*. 10, (4) 3379-3389.

Ghaffari, M. R., M. Ghabooli, B. Khatabi, M. R. Hajirezaei, P. Schweizer and G. H. Salekdeh, 2016. Metabolic and transcriptional response of central metabolism affected by root endophytic fungus *Piriformospora indica* under salinity in barley. *Plant molecular biology*. 90, (6) 699-717.

Giannopolitis, C. N. and S. K. Ries, 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology*. 59, (2) 309-314.

Gill, S. S., R. Gill, D. K. Trivedi, N. A. Anjum, K. K. Sharma, M. W. Ansari, A. A. Ansari, A. K. Johri, R. Prasad and E. Pereira, 2016. *Piriformospora indica*: potential and significance in plant stress tolerance. *Frontiers in Microbiology*. 7, 332.

Guerfel, M., O. Baccouri, D. Boujnah, W. Chaïbi and M. Zarrouk, 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (Olea europaea L.) cultivars. *Scientia Horticulturae*. 119, (3) 257-263.

Haas, D. and G. Défago, 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature reviews microbiology*. 3, (4) 307-319.

Hamilton, C. E., P. E. Gundel, M. Helander and K. Saikkonen, 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. *Fungal Diversity*. 54, (1) 1-10.

Irigoyen, J., D. Einerich and M. Sánchez-Díaz, 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativd) plants. *Physiologia plantarum*. 84, (1) 55-60.

Justice, A. H., J. E. Faust and J. L. Kerrigan, 2018. Evaluating a novel method to introduce a mycorrhizal-like fungus, *Piriformospora indica*, via an inoculated rooting substrate to improve adventitious root formation. *Horttechnology*. 28, (2) 149-153.

Khan, A. L., M. Waqas, M. Hamayun, A. Al-Harrasi, A. Al-Rawahi and I.-J. Lee, 2013. Co-synergism of endophyte Penicillium resedanum LK6 with salicylic acid helped Capsicum annuum in biomass recovery and osmotic stress mitigation. *BMC microbiology*. 13, (1) 1-13.

Kieber, J. J. and G. E. Schaller, 2018. Cytokinin signaling in plant development. *Development*. 145, (4)

Kloepper, J. W., J. Leong, M. Teintze and M. N. Schroth, 1980. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*. 286, (5776) 885-886.

Kokalis–Burelle, N., C. Vavrina, E. Rosskopf and R. Shelby, 2002. Field evaluation of plant growth-promoting rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant and Soil*. 238, (2) 257-266.

Kord, H., B. Fakheri, M. Ghabooli, M. Solouki, A. Emamjomeh, B. Khatabi, M. Sepehri, G. H. Salekdeh and M. R. Ghaffari, 2019. Salinity-associated microRNAs and their potential roles in mediating salt tolerance in rice colonized by the endophytic root fungus *Piriformospora indica*. *Functional & integrative genomics*. 19, (4) 659-672.

Krishnaveni, N., B. Dipika, D. Ramani and C. Chakravarthy, 2014. Cultivable endophytic mycobiont *Piriformospora indica* in millet growth promotion. *Int J Sci Eng Technol Res*. 3, (7) 2033-2047.

Lahrmann, U. and A. Zuccaro, 2012. Opprimo ergo sum—evasion and suppression in the root endophytic fungus *Piriformospora indica*. *Molecular plant-microbe interactions*. 25, (6) 727-737.

Malla, R., R. Prasad, R. Kumari, P. Giang, U. Pokharel, R. Oelmüller and A. Varma, 2004. Phosphorus solubilizing symbiotic fungus: Piriformospora in-dica. *Endocytobiosis Cell Res*. 15, (2) 579-600.

Mane, A., T. Deshpande, V. Wagh, B. Karadge and J. Samant, 2011. A critical review on physiological changes associated with reference to salinity. *International Journal of Environmental Sciences*. 1, (6) 1192.

Ngwene, B., S. Boukail, L. Söllner, P. Franken and D. Andrade-Linares, 2016. Phosphate utilization by the fungal root endophyte *Piriformospora indica*. *Plant and Soil*. 405, (1) 231-241.

Nouh, F. a. A., H. H. A. Nahas and A. M. Abdel-Azeem, 2020. *Piriformospora indica*: Endophytic Fungus for Salt Stress Tolerance and Disease Resistance. *Agriculturally Important Fungi for Sustainable Agriculture*. 261-283.

Oelmüller, R., I. Sherameti, S. Tripathi and A. Varma, 2009. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis*. 49, (1) 1-17.

Olson, M. E. and J. A. Rosell, 2013. Vessel diameter–stem diameter scaling across woody angiosperms and the ecological causes of xylem vessel diameter variation. *New phytologist*. 197, (4) 1204-1213.

Pal, A. and S. Pandey, 2017. Symbiosis of arbuscular mycorrhizal fungi and Pennisetum glaucum l. improves plant growth and glomalin-related soil protein in barren soil. *Int J Sci Invent Today*. 6, (6) 783-792.

Pan, R., L. Xu, Q. Wei, C. Wu, W. Tang, R. Oelmüller and W. Zhang, 2017. *Piriformospora indica* promotes early flowering in Arabidopsis through regulation of the photoperiod and gibberellin pathways. *PLoS One*. 12, (12) e0189791.

Ramamoorthy, V., R. Viswanathan, T. Raguchander, V. Prakasam and R. Samiyappan, 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop protection*. 20, (1) 1-11.

Richardson, A. E. and R. J. Simpson, 2011. Soil Microorganisms Mediating Phosphorus Availability: Phosphorus plant physiology. *Plant physiology (Bethesda)*. 156, (3) 989-996.

Ruiz-Lozano, J. M., R. Porcel, C. Azcón and R. Aroca, 2012. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. *Journal of Experimental Botany*. 63, (11) 4033-4044.

Russo, V. M., 2006. Biological amendment, fertilizer rate, and irrigation frequency for organic bell pepper transplant production. *HortScience*. 41, (6) 1402-1407.

Russo, V. M. and P. Perkins-Veazie, 2010. Yield and nutrient content of bell pepper pods from plants developed from seedlings inoculated, or not, with microorganisms. *HortScience*. 45, (3) 352-358.

Sahay, N. and A. Varma, 1999. *Piriformospora indica*: a new biological hardening tool for micropropagated plants. *FEMS Microbiology letters*. 181, (2) 297-302.

Santos-Sánchez, N., R. Valadez-Blanco and R. Salas-Coronado, 2013. Factors affecting the antioxidant content of fresh tomatoes and their processed products. *Tomatoes: Cultivation, Varieties and Nutrition Higashide T, Nova Science Publishers, Hauppauge, NY, USA*.

Shahollari, B., A. Varma and R. Oelmüller, 2005. Expression of a receptor kinase in Arabidopsis roots is stimulated by the basidiomycete

Piriformospora indica and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. *Journal of plant physiology*. 162, (8) 945-958.

Sharifi, M., M. Mohtashamian, H. Riyahi, A. Aghaee and S. Alavi, 2011. The Effects of Vesicular-Arbuscular Mycorrhizal (VAM) Fungus Glomus etunicatum on Growth and some Physiological Parameters of Basil. *Journal of Medicinal Plants*. 10, (38) 85-94.

Sherameti, I., B. Shahollari, Y. Venus, L. Altschmied, A. Varma and R. Oelmüller, 2005. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *Journal of Biological Chemistry*. 280, (28) 26241-26247.

Sirrenberg, A., C. Göbel, S. Grond, N. Czempinski, A. Ratzinger, P. Karlovsky, P. Santos, I. Feussner and K. Pawlowski, 2007. *Piriformospora indica* affects plant growth by auxin production. *Physiologia plantarum*. 131, (4) 581-589.

Sun, C., J. M. Johnson, D. Cai, I. Sherameti, R. Oelmüller and B. Lou, 2010. *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *Journal of plant physiology*. 167, (12) 1009-1017.

Terhonen, E., K. Blumenstein, A. Kovalchuk and F. O. Asiegbu, 2019. Forest tree microbiomes and associated fungal endophytes: Functional roles and impact on forest health. *Forests*. 10, (1) 42.

Vavrina, C. S., 1998. Transplant age in vegetable crops. *HortTechnology*. 8, (4) 550-555.

Verma, S., A. Varma, K.-H. Rexer, A. Hassel, G. Kost, A. Sarbhoy, P. Bisen, B. Bütehorn and P. Franken, 1998. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia*. 90, (5) 896-903.

Vessey, J. K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*. 255, (2) 571-586.

Wakelin, S. A., R. A. Warren, P. R. Harvey and M. H. Ryder, 2004. Phosphate solubilization by Penicillium spp. closely associated with wheat roots. *Biology and Fertility of Soils*. 40, (1) 36-43.

Waller, F., B. Achatz, H. Baltruschat, J. Fodor, K. Becker, M. Fischer, T. Heier, R. Hückelhoven, C. Neumann and D. Von Wettstein, 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*. 102, (38) 13386-13391.

Wang, H., J. Zheng, X. Ren, T. Yu, A. Varma, B. Lou and X. Zheng, 2015. Effects of *Piriformospora indica* on the growth, fruit quality and interaction with Tomato yellow leaf curl virus in tomato cultivars susceptible and resistant to TYCLV. *Plant Growth Regulation*. 76, (3) 303-313.

Waqas, M., A. L. Khan, M. Kamran, M. Hamayun, S.-M. Kang, Y.-H. Kim and I.-J. Lee, 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules*. 17, (9) 10754-10773.

Xu, L., C. Wu, R. Oelmüller and W. Zhang, 2018. Role of phytohormones in *Piriformospora indica*-induced growth promotion and stress tolerance in plants: more questions than answers. *Frontiers in microbiology*. 9, 1646.

Yadav, V., M. Kumar, D. K. Deep, H. Kumar, R. Sharma, T. Tripathi, N. Tuteja, A. K. Saxena and A. K. Johri, 2010. A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *Journal of Biological Chemistry*. 285, (34) 26532-26544.

Yun, P., L. Xu, S.-S. Wang, L. Shabala, S. Shabala and W.-Y. Zhang, 2018. *Piriformospora indica* improves salinity stress tolerance in Zea mays L. plants by regulating Na+ and K+ loading in root and allocating K+ in shoot. *Plant growth regulation*. 86, (2) 323-331.