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Piriformospora indica Culture Filtrate and Biofertilizer (Nitrokara) Promote Chicory (*Cichorium intybus* L.) Growth and Morpho-physiological Traits in an Aeroponic System and Soil Culture

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Abstract
In two independent experiments, acclimatized chicory plants were transferred into an aeroponic system and to the soil. Then, the effects of biofertilizer (Nitrokara) and culture filtrate of *Piriformospora indica* were investigated on some plant characteristics in both aeroponic and soil cultivation system under greenhouse conditions. The plants were foliar sprayed with three different biofertilizer concentrations (1, 2, and 3 g/L) and *P. indica* culture filtrate (2.5, 5, and 7.5 mL in 100 mL water) following 20, 40 and 60 days after transplanting to the aeroponic and soil culture media. Results showed that the highest vegetative growth (e.g. plant height, root length, number of leaves per plant, root and shoot dry weights) and physiological traits (e.g. relative water content, proline, anthocyanin content, chlorophyll a, and chlorophyll b) were observed with 7.5 mL of *P. indica* culture filtrate in both aeroponic and soil culture media. Besides, the best results for all studied traits were obtained from 3 g/L of biofertilizer application in the both culture media. In both experiments, better results were obtained from the aeroponic system than the soil culture for *P. indica* production.

Keywords: Biofertilizer, medicinal plant, soilless culture, chicory.

Introduction

Chicory (*Cichorium intybus* L.) is a member of the family *Asteraceae*. According to Vavilov (1951), the whole plant (including root, leaf, and seed) can be used for medicinal purposes. The plant is used to treat AIDS, cancer, diabetes, dysmenorrhoea, impotence, insomnia, spleen, and tachycardia (Duke, 1983; Velayutham et al., 2006).

The cultivation of this plant often requires 3-6 years before roots reach maturity. Harvesting requires a lot of labor work and the extraction of the roots from the soil is energy-intensive. Even with modern farming techniques such as trenching or building raised beds of loose soil, most fragile secondary roots are lost during the harvest process, representing a significant loss of the potential yield. Additionally, commercial products derived from roots cultivated in the soil are at the risk of contamination with soil and soil-

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borne organisms, as well as the roots of weed species. Good quality control and standardization of botanical products have proven to be a difficult and expensive challenge. Many growers are looking for alternative methods of cultivation that reduce contamination risks and improve the quality and consistency of the raw material (Pagilarulo et al., 2005). Aeroponics is a form of hydroponic plant cultivation in which plant roots are suspended in a closed chamber and misted with a complete nutrient solution. Aeroponics does not require solid or aggregate growing medium and allows for easy access to roots. The chamber and a misting system allow complete control of the root zone environment, including temperature, nutrient level, pH, humidity, misting frequency, and duration and oxygen availability. Plants often exhibit accelerated growth and maturation in aeroponic systems (Mirza et al., 1998). These qualities have made aeroponics as a popular research tool for studying root growth and plant nutrient uptake (Barak et al., 1996).

Bio-fertilizers are more environmentally friendly and have given the same or even better crop yields compared to mineral fertilizers in many cases (Saghir Khan et al., 2007; Vessey, 2003). Bio-fertilizers mainly include nitrogen-fixing, phosphate solubilizing and plant growth-promoting microorganisms (Goel et al., 1999) providing more balanced nutrition for plants (Belimov et al., 1995). Presently, Azotobacter, Azospirillium, Cyanobacteria, Rhizobium, Endophytic diazotrophs, blue-green algae, Azolla, Mycorrhizae, and Sinorhizobiumare are used as bio-fertilizers to improve crop production (Hegde et al., 1999).

Piriformospora indica, which discovered in the Indian Thar desert in 1997, is a symbiotic mycorrhizal-like fungus that plays a beneficial role in plant growth and yield under normal and stressed conditions (Varma et al., 2012). This axenically cultivable root endophytic organism has been used as a model to study the mechanisms and evolution of mutualistic symbiosis. Like the fungi *Abuscular mycorrhiza*, *P. indica* promotes growth and yield in its host plants, but it possesses a broader host range among mono- and dicotyledonous plants (Pham et al., 2004; Sherameti et al., 2008; Ghaffari et al., 2016). This fungus can be considered as a successful symbiotic partner to provide a growth-promoting activity to a diverse range of plant species, including agricultural and medicinal crops (Tsimilli-Michael and Strasser, 2013). Because of these features, *P. indica* has a good potential to be used as a plant biofertilizer, probiotic and bio-hardening tools (Sun et al., 2010; Stein et al., 2008).

There are reports concerning the effects of the above-mentioned biofertilizer (Nitrokara) and *Piriformospora indica* on the medicinal plant production in the *in vitro*, greenhouse and field conditions; however, based on our knowledge, the effects of biofertilizer (Nitrokara) and *Piriformospora indica* have not been investigated on the chicory production in an aeroponic system yet. Therefore, the present study aimed to evaluate the effects of different concentrations of the biofertilizer (Nitrokara) and *Piriformospora indica* culture filtrate on chicory growth and morpho-physiological traits following 20, 40 and 60 days of transplanting *in vitro* plants to the aeroponic and soil culture media.

Materials and Methods

Plant material and cultivation systems

In the first experiment, chicory seeds were planted in small pots in a greenhouse and after 30 days, the plants with similar traits (≈20 cm height) were transferred into an aeroponic system and pots filled with the soil in a greenhouse with controlled conditions. In this study, the effects of the biofertilizer (Nitrokara) concentrations (1, 2 and 3 g/L) and *Piriformospora indica* culture filtrate (2.5, 5 and 7.5 ml in 100 ml water) were investigated in two cultivation systems

including conventional planting in soil (pots in 25×30 cm sizes) and an aeroponic system as independent experiments. The pots were filled with perlite: peat moss (1:2 v/v) medium. These pots were irrigated with a nutrient solution used in the aeroponic system. The plants were foliar sprayed with biofertilizer (Nitrokara) containing the fungi *Azorhizobium*and *Arbuscular mycorrhizal* (*Glomus versiforme*and *Glomus intraradices*) and *Piriformospora indica* 20, 40 and 60 days after transplanting in the aeroponic and soil culture media. The experiment was carried out based on a completely randomized design (CRD) in four replications.

Aeroponic system

In this study, we conducted a research on an aeroponic system (phytorhizotron) that was designed for the production of medicinal plants. The phytorhizotron consisted of two compartments: the upper compartment was supplied with a photoperiod control and the lower compartment was kept in darkness. The plants were planted on the board of the upper compartment spaced by 13 × 13 cm and about one-third of the length of the stems was placed inside the lower compartment. The shoots were grown in the upper compartment and the roots were developed in the lower compartment under dark conditions. The lower compartment was a closed container (180 × 120 × 120 cm; depth, width, and length, respectively), which had a removable front panel for root monitoring and harvesting. The plant roots were periodically sprayed (every 20 min for 20 s) with a nutrient solution using 15 fog nozzles. The nutrient solution was renewed weekly. The residual nutrient solution was allowed to flow back into a collecting tank and was recirculated.

Nutrient solution and growing conditions

The ingredients of the nutrient solution used for plant nutrition are shown in Table 1. The electrical conductivity (EC) of the nutrient solution was adjusted to 1.6 ± 0.2 dS/m. The initial pH was adjusted to 5.8 ± 0.2, whereas pH beyond that was not controlled. The plants were grown in a greenhouse under a 16/8 h light/dark photoperiod.

Table1. Concentrations of nutrients used in the aeroponic system (mg/L)

Elements	Concentration(mg/L)	Elements	Concentration (mg/L)
K	200	Fe	1
N	190	Mn	0.5
Ca	150	B	0.5
S	70	Zn	0.15
Mg	45	Cu	0.1
P	35	Mo	0.05

Growth of P. indica and preparation of culture filtrate

P. indica was grown on slants containing complex medium (CM) supplemented with 15 g/L agar (Ghabooli et al., 2013). After 8 days of growth, 5 mm agar discs (fully grown fungus) were inoculated in 250 mL Erlenmeyer flasks containing 100 mL CM without agar on an incubator shaker at 200 rpm at 28 ± 1°C. The fungal biomass was harvested at the mid-log phase and was separated by passing through a Miracloth filter. The spent medium was centrifuged at 5000 g for 15 min to remove suspended particles and filtered through a Whatman No. 1 filter paper. The obtained clear solution was designated as culture filtrate and was used at different concentrations (Kumar et al., 2012).

Data acquisition

The plants were harvested six months after transplanting. The total length of the plant, root length, number of leaves, root

fresh and dry weight, shoot dry weight, and also the amount of photosynthetic pigments, relative water content (RWC), and carbohydrate concentration were measured. The photosynthetic pigments of the chicory leaves were determined according to Zhongfu et al. (2009). The leaf relative water content (RWC) was determined based on the following equation:

$$RWC = (FW - DW)/(TW - DW) \times 100.$$

where FW, DW, and TW are the fresh, dry and turgid weight of leaf, respectively. Carbohydrate concentration was determined according to Hedge and Hofreiter (1962).

Statistical analysis

The primary statistical analyses such as normality test (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test) were conducted. After the analysis of variance (ANOVA), the means of the treatment combinations were compared using Duncan's Multiple Range Test (DMRT). All the above statistical analyses were carried out using the SPSS version 21 software package.

Results

Effect of biofertilizer (Nitrokara) on growth and physiological characteristics of chicory (Cichorium intybus L.) in aeroponic system

The results of analysis of variance indicated significant ($p<1\%$) differences in morpho-physiological characteristics of the chicory plants between different applied concentrations of biofertilizer (Nitrokara) for the studied traits. The means comparison showed that the plants treated with 3 g/L of biofertilizer had the highest vegetative growth traits (Table 2) including plant height (198.7 cm), root length (123.7 cm), number of leaves per plant (76.2), root fresh weight (250.7 g/plant), root dry weight (38.6 g/plant), shoot fresh weight (513.4 g/plant), and shoot dry weight (79.7 g/plant) and physiological traits including carbohydrate (0.72 mg/g DW), chlorophyll a (24.9 mg/g), chlorophyll b (13.21 mg/g), carotenoid (14.1 mg/g) concentrations and RWC (88.17%) (Table 3).

Table 2. Effect of different concentrations of biofertilizer (nitrokara) on vegetative growth traits of chicory in the soil culture

Treatment	Leaf number	Dry weight of shoot (mg)	Fresh weight of shoot (mg)	Dry weight of root (mg)	Fresh weight of root (mg)	Root length (cm)	plant height (cm)
Control	36.5 ± 0.91 d	35.2 ± 0.13 c	210.6 ± 2.05 c	11.3 ± 0.28 c	74.5 ± 1.82 c	63.5 ± 1.29 d	91.7 ± 1.23 d
1 g/L	54.4 ± 1.2 c	37.3 ± 0.45 c	218.4 ± 6.8 c	10.2 ± 0.31 c	82.3 ± 2.1 c	98.7 ± 1.31 c	121.6 ± 1.42 c
2 g/L	64.2 ± 1.6 b	49.2 ± 1.01 b	325.4 ± 10.8 b	20.7 ± 0.41 b	186.4 ± 2.31 b	102.3 ± 1.52 b	170.8 ± 2.35 b
3 g/L	76.2 ± 1.7 a	79.7 ± 1.26 a	513.4 ± 13.5 a	38.6 ± 0.74 a	250.7 ± 5.5 a	123.7 ± 1.85 a	198.7 ± 2.47 a

Means followed by the same letter(s) are not significantly different at the 0.01 level of probability

Table 3. Effect of different concentrations of biofertilizer (nitrokara) on physiological traits of chicory in the soil culture

Treatment	Carbohydrate (mg/g dw)	Carotenoid (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a (mg /g)	RWC (%)
Control	0.24 ± 0.03 b	8.4± 0.11 b	9.2 ± 0.37 b	15.9 ± 0.085 c	66.37 ± 0.82 c
1 g/L	0.31 ± 0.026 b	13.2± 0.05 a	11.56±0.17 a	22.5± 0.28 b	83.13 ± 1.01 b
2 g/L	0.7 ± 0.016 a	13.8 ± 0.07 a	12.03±0.15 a	23.7 ± 0.21 b	84.3 ± 1.04 b
3 g/L	0.72 ± 0.013 a	14.1 ± 0.09 a	13.21 ± 0.4 a	24.9 ± 0.15 a	88.17 ± 1.3 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Effect of biofertilizer on growth and physiological characteristics of chicory (Cichorium intybus L.) in soil system

The results obtained from the analysis of variance indicated significant (p<1%) differences in morpho-physiological characteristics of the chicory plants between different applied concentrations of biofertilizer (Nitrokara) for all traits studied. The means comparison (Table 3) showed that the plants applied with 3 g/L of biofertilizer had the highest vegetative growth traits (Table 4) including plant height (45.2 cm), root length (17.8 cm), number of leaves per plant (36), root fresh weight (1.8 g/plant), root dry weight (0.27 g/plant), shoot fresh weight (31 g/plant), and shoot dry weight (6.5 g/plant) and physiological traits including carbohydrate (0.64 mg/g DW), chlorophyll a (22.32 mg/g), chlorophyll b (12.03 mg/g), and carotenoid (12.6 mg/g) concentrations and RWC (81.35%) (Table 5).

Table 4. Effect of different concentrations of biofertilizer (nitrokara) on vegetative growth traits of chicory in the soil culture

Treatment	Leaf number	Dry weight of shoot (mg)	Fresh weight of shoot (mg)	Dry weight of root (mg)	Fresh weight of root (mg)	Root length (cm)	Plant height (cm)
Control	27.7 ± 2.16 d	3.2 ± 0.03 d	17.2 ± 0.2 b	0.2 ± 0.015 b	1.0 ± 0.018 b	13.02 ± 0.09 b	39.2 ± 0.25 d
1 g/L	30.2 ± 2.17 c	4.9 ± 0.047 c	25.8 ± 0.31 b	0.23 ± 0.011 b	1.25 ± 0.055 b	14.2 ± 0.11 b	41.2 ± 0.29 c
2 g/L	34.6 ± 3.09 b	5.89 ± 0.11 b	30.3 ± 0.68 a	0.25 ± 0.018 a	1.7 ± 0.021 a	15.8 ± 0.15 b	43.2 ± 0.36 b
3 g/L	36 ± 3.12 a	6.5 ± 0.16 a	31 ± 1.06 a	0.27 ± 0.013 a	1.8 ± 0.027 a	17.8 ± 0.18 a	45.2 ± 0.37 a

Means followed by the same letter(s) are not significantly different at the 0.01 level of probability

Table 5. Effect of different concentrations of biofertilizer (nitrokara) on physiological traits of chicory in the soil culture

Treatment	Carbohydrate (mg/g)	Carotenoid (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a (mg /g)	RWC (%)
Control	0.22 ± 0.011 b	7.5 ± 0.1 b	8.26 ±0.27 b	14.4 ± 0.07 c	61.7 ± 0.74 c
1 g/L	0.28 ± 0.014 b	11.7 ± 0.05 a	10.54±0.13 a	20.1 ± 0.14 b	76.8 ± 0.93 b
2 g/L	0.63 ± 0.24 a	12.3 ± 0.07 a	10.85±0.15 a	21.36 ±0.19 ab	77.9± 0.91 b
3 g/L	0.64 ± 0.027 a	12.6 ± 0.12 a	12.03±0.34 a	22.32 ± 0.25 a	81.35 ± 1.24 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Effect of Piriformosporaindica on growth and physiological characteristics of chicory (Cichorium intybus L.) in aeroponic system

Based on the results of analysis of variance, there were significant (p<1%) differences in growth and physiological characteristics of the chicory plants as influenced by different levels of *P. indica* for all studied traits, except for carotenoid and carbohydrate concentrations.

Plant height, root length, number of leaves per plant, root fresh and dry weights, and shoot fresh and dry weights were significantly different between the inoculation with *P. indica* and non-inoculation treatment (Table 6). Maximum plant height (176.7 cm), root length (127.5 cm), number of leaves per plant (138.5), root fresh weight (230.8 g/plant), root dry weight (46.2 g/plant), shoot fresh weight (304.1 g/plant), and shoot dry weight (60.8 g/plant) belonged to the plants treated with 7.5 mL of *P. indica* culture filtrate (Table 6).

The highest chlorophyll a (28.5 mg/g) and chlorophyll b (16.04 mg/g) were obtained from the treatment of 7.5 mL of *P. indica* culture filtrate (Table 7).

Effect of Piriformospora indica on morpho-physiological characteristics of chicory (Cichorium intybus L.) in soil system

The results of analysis of variance revealed significant (p<1%) differences in growth and physiological characteristics of the chicory plants treated with different concentrations of *P. indica* for all studied traits, except for carotenoid and carbohydrate concentrations.

Based on the observed data, the highest vegetative growth traits including plant height (42.5 cm), root length (13.5 cm), number of leaves per plant (22), root fresh weight (2.3 g/plant), root dry weight (0.32 g/plant), shoot fresh weight (43.7 g/plant), and shoot dry weight (6.1 g/plant) (Table 8), as well as the highest RWC (81.2), chlorophyll a (24.24 mg/g), and chlorophyll b (13.21 mg/g) concentrations (Table 9) were produced by the plants treated with 7.5 mL of *P. indica* culture filtrate.

Table 6. Effects of different concentrations of *Piriformospora indica* on vegetative growth traits of chicory in the aeroponic system

Treatment	Leaf number	Dry weight of root (mg)	Fresh weight of shoot (mg)	Dry weight of root (mg)	Fresh weight of root (mg)	Root length (cm)	Plant height (cm)
Control	54.7 ± 1.55 d	22.3 ± 0.37 d	111.4 ± 1.8 d	14.5 ± 1.31 b	72.5 ± 3.6 d	83.7 ± 4.6 c	102.3 ± 4.6 c
2.5 mL	62.3 ± 1.27 c	24.1 ± 0.27 c	120.2 ± 1.4 c	16.8 ± 0.97 a	84.3 ± 3.03 c	92.5 ± 2.39 c	117.7 ± 1.49 b
5 mL	88.5 ± 1.71 b	48 ±0.53 b	240.5 ± 2.9 b	29.3 ± 0.43 a	146.7 ± 4.07 b	110 ± 3.5 b	125.2 ± 1.8 b
7.5 mL	138.5 ± 1.73 a	60.8 ± 0.76 a	304.1 ± 3.7 a	46.2 ± 0.62 a	230.8 ± 5.46 a	127.5 ± 2.2 a	176.7 ± 1.65 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Table 7. Effects of different concentrations of *Piriformospora indica* on chlorophyll concentrations of chicory in the aeroponic system

Treatment	Chlorophyll b (mg/g)	Chlorophyll a (mg/g)
Control	10.38 ± 0.14 c	18.15 ± 0.017 c
2.5 mL	56.11 ± 0.26 b	18.6 ± 0.013 c
5 mL	12.98 ± 0.12 b	25.2 ± 0.21 b
7.5 mL	16.04 ± 0.15 a	28.5 ± 0.14 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Table 8. Effects of different concentrations of *Piriformospora indica* on vegetative growth traits of chicory in the soil

Treatment	Leaf number	Dry weight of shoot (mg)	Fresh weight of shoot (mg)	Dry weight of root (mg)	Fresh weight of root (mg)	Root length (cm)	Plant height (cm)
Control	14 ± 0.23 b	3.4 ± 0.13 c	24.2 ± 0.28 b	0.15 ± 0.01 b	1 ± 0.026 b	8.2 ± 0.7 b	35.7 ± 0.69 b
2.5 mL	19.2 ± 0.2 a	5.1 ± 0.45 b	37.4 ± 0.21 a	0.2 ± 0.012 a	1.5± 0.03 b	10.5 ± 0.31 b	39.7 ± 0.22 a
5 mL	21.6 ± 0.26 a	5.3 ± 1.01 b	40.5 ± 0.39 a	0.25 ± 0.012 a	1.7 ± 0.031 b	12.1 ± 0.52 a	41.2 ± 0.27 a
7.5 mL	22 ± 0.25 a	6.1 ± 1.26 a	43.7 ± 0.57 a	0.32 ± 0.013 a	2.3 ± 0.046 a	13.5 ± 0.33 a	42.5 ± 0.25 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Table 9. Effects of different concentrations of *Piriformospora indica* on chlorophyll concentrations and RWC of chicory in the soil

Treatment	Chlorophyll b (mg/g)	Chlorophyll a (mg /g)	RWC (%)
Control	8.75 ±0.37 b	15.42 ± 0. 85 c	60.07 ± 4.44 b
2.5 mL	11.56 ± 0.17 a	15.84 ± 0.11 c	80.56 ± 11.7 a
5 mL	12.03 ± 0.15 a	21.51 ± 0.18 b	68.55 ± 3.05 b
7.5 mL	13.21 ± 0.4 a	24.24 ± 0.12 a	81.2 ± 9.88 a

Means followed by the same letter(s) are not significantly different at 0.01 level of probability

Discussion

The morpho-physiological characteristics of the chicory plants significantly depended on the dose of applied Nitrokara. The effects of some biofertilizers have also been documented on other plants such as wheat (Behl et al., 2006) and corn (Zaied et al., 2007).

The Nitrokara biofertilizer contains *Azorhizobium* bacteria, which were isolated from nature and are very effective in supplying the nitrogen requirement of plants. This bacterium was active in the rhizosphere, on the root surface and in intercellular spaces of leaf tissues, stems and roots of the plants. Besides nitrogen fixation, the bacterium also

provides plants with growth regulators such as auxins, cytokinins, abscisic acid, and also provides riboflavin and vitamins, which are effective in improving plant growth and functioning. The PhosphoNitrokara biofertilizer contains the bacteria *Bacillus coagulans*, *Azotobacter chroococcum*, and *Azospirillum lipoferum*. The latter two bacteria are nitrogen-fixing and, in addition to nitrogen provision, promote plant growth and health through the production of plant growth regulators such as indole acetic acid (IAA), gibberellins, B-group vitamins, and antifungal substances. *Bacillus coagulans* is a bacterium effective in solubilizing mineral and organic phosphate in soils. *Bacillus coagulans* increases the solubility of insoluble phosphates occurring in the soil and the leaf surface through the production of organic acids such as lactic acid and phosphatase and phytase enzymes and thereby provides higher amounts of phosphate for the plant. *Azospirillum lipoferum* is also among the most important bacteria living in root and leaf environments, having symbiotic relationships with the plant and naturally fixing the nitrogen from the air, which could be absorbed by the plant. This bacterium, in addition to nitrogen fixing, produces growth stimulants and thus helps plant growth. *Azotobacter chroococcum* is also one of the well-known symbiotic nitrogen-fixing bacteria living naturally in small populations in root and leaf environments. This bacterium can fix nitrogen from the air and thereby provide plants with the required nitrogen. The improvement in plant growth by using biofertilizers may be due to the effects of microorganisms on the physiological and metabolic activities of plants and also nitrogen fixation. The positive effects of biofertilizers on plant growth can also be attributed to the effect of secretions of hormones such as cytokinin and auxin, which stimulate water and nutrient uptake (EL-Zeiny, 2007).

The results of both experiments (biofertilizer Nitrokara) and culture filtrate of *Piriformospora indica*) showed that the aeroponic system significantly increased morpho-physiological characteristics compared to soil culture and these results are in agreement with the previous reports for other plants. Weathers and Zobel (1992) revealed that the aeroponic system remarkably increased plant growth compared to the classic hydroponic system and correlated with an increase in fresh and dry weights of shoot and roots and also an increase in shoot length, the number of leaves and tubers in tuberous plants. Cultivation of vegetable such as lettuce (He and Lee, 1998) and tomato (Cho et al., 1996), ornamental plants such as chrysanthemum (Molitor et al., 1999) and anthurium (Fascella and Zizzo, 2007) and and medicinal plants such as asparagus (Christie and Nichols, 2004) in aeroponic system resulted in remarkable increases in vegetative growth compared to classic hydroponic systems. Leaf area is a major factor for plant growth and its increase enhances photosynthesis and assimilation production, which in turn increases the amounts of plant biomass (Taiz and Zeiger, 2006). In a study on *Urtica dioica*, the greenhouse soilless medium treatments yielded 72% more shoot biomass and 140% more root biomass per plant than the field plants are grown for a similar duration (Pagilarulo et al., 2005). In another study on valerian, the growth of this plant was significantly higher in soilless systems compared to its production in soil (Tabatabaei, 2008). Besides, it have been shown that aeroponic production of cucumber, tomato, spinach, red cabbage, and pepper resulted in a higher yield and comparable phenolics, flavonoids, and antioxidant properties as compared to those grown in the soil (Chandra et al., 2014).

The production of medicinal plants in aeroponic systems has various advantages, considerable efficiency in water and nutrient solution usage, high root growth due to better availability of oxygen and higher levels of nutrient solution uptake, increases

in the number of crop cycles due to faster growth and maturity, possibility of controlling the temperature of the root environment, the lack of weeds, and consequently optimum plant growth, which as a result, decreases herbicide usage and enhances growth of the plants. Further advantages are better availability of carbon dioxide for photosynthesis and production of clean roots devoid of soil pathogens. Taking into account the water scarcity problem and the necessity of more efficient water usage, it is necessary to use this system for plants of economic value such as the production of virus-free potato micro-tubers, medicinal plants, saffron and etc.

In nature, *P. indica* mimics the typical arbuscular mycorrhiza (Verma et al., 1998), but it can easily be cultivated on several synthetic media without a host (Pham et al., 2004). This fungus is associated with the roots of diverse groups of plant species (Varma et al., 2012) and it has been proposed that *P. indica* promotes plant growth by producing or manipulating phytohormones, especially auxin (Lee et al., 2011), cytokinins (Vadassery et al., 2008), gibberellins, abscisic acid, and brassinosteroids (Schafer et al., 2009).

The present study is the first research that studied the effect of biofertilizer and different concentrations of *P. indica* culture filtrate on chicory growth in aeroponic and soil culture media. The main goal of our study was to investigate the application of biofertilizer and *P. indica* culture filtrate in aeroponic culture. The results showed that *P. indica* filtrate enhanced growth and photosynthetic capacity in chicory plants so that there were significant differences in plant growth parameters between the *P. indica* culture filtrate-treated plants and the control plants.

In this research, plant growth promotion was evidenced by the increases in the production of photosynthetic pigments, proline, and anthocyanin, higher number of leaves per plant, and higher root and shoot dry weight in the *P. indica* culture-filtrate-treated plants compared to the control plants. The observations for fresh and dry weights were in correlation with shoot and root length, where the plants with a higher root and shoot length revealed maximum values of biomass. The increase in chlorophyll and carotenoid contents may have resulted in an increase in biomass accumulation in the culture filtrate-treated plants. Overall, the culture-filtrate-treated plants promoted plant growth than the control plants at the concentration of 7.5 mL culture filtrate (as the concentration of culture filtrate increased, the improving effect was increased).

The improvement of plant growth by *P. indica* culture-filtrate has been reported in some medicinal plants before. *P. indica* has been reported to improve the growth, endogenous levels of bacosides, antioxidant activity, and hypertrophy of nuclei in *B. monnieri* (Prasad et al., 2013). Baishya et al. (2015) reported that *P. indica* filtrate had a positive effect on the overall growth of biomass in *Artemisia annua*. There was an increase in growth parameters such as root and shoot length, dry and fresh weight, and, most importantly, pigment contents in *P. indica* inoculated plants compared to non-inoculated plants. Ahlawat et al. (2017) reported that an endophytic fungus promoted the production of withaferin A in the cultures of *Withania somnifera*. In most studies, the effects of *P. indica* on plant growth have been reported on pot-/field-grown or tissue culture-raised plants; while there is lack of reports for the effects of *P. indica* on plant growth in soilless cultures like hydroponic and aeroponic systems.

Therefore, based on our finding and the results of other studies, it can be hypothesized that the plant growth-promoting effect of *P. indica* culture filtrate might be due to the release of some metabolites in the medium during fungus growth. We have found that the *P. indica* filtrate can promote growth and some

physiological parameters in chicory plants. Another critical aspect of the study is the possibility of the application of biofertilizer and *P. indica* filtrate in aeroponic systems.

Conclusion

The present research focused on chicory plant growth-promoting effects of the Nitrokara biofertilizer and *P. indica* culture filtrate, which may be due to the production or induction of growth-promoting metabolites by the fungus in the culture medium. To the best of our knowledge, this study is the first research studied the effect of *P. indica* culture filtrate on the growth and some metabolite production of chicory in an aeroponic system. The results indicated the promotion in the growth of the plants treated with biofertilizer and *P. indica* culture filtrate when compared to the control plants. These treatments enhanced the growth and metabolite production of *Cichorium intybus*. The results represent a successful application of biofertilizer and fungus culture filtrate in the aeroponic system.

Conflict of interest

The authors indicate no conflict of interest for this work.

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