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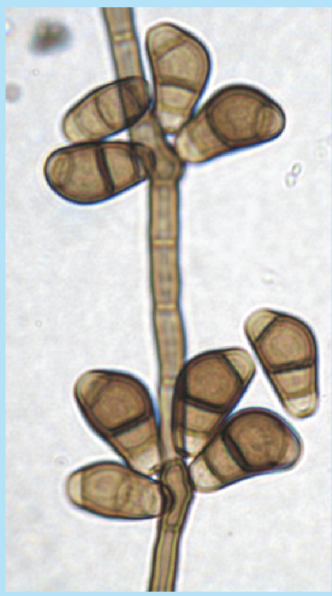
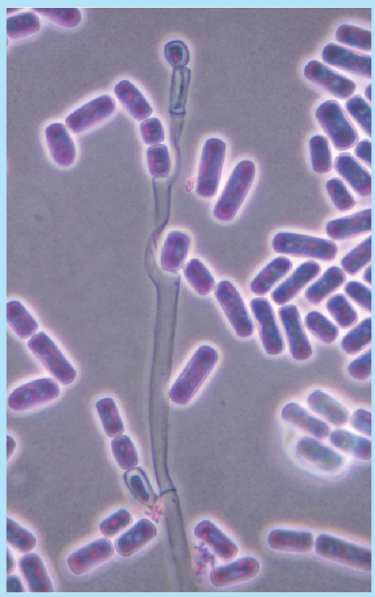

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Promoting effects of *Piriformospora indica* on growth and some physiological aspects of fenugreek (*Trigonella foenum-graecum* L.) under salt stress

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Abstract: Effects of the root endophytic fungus *(Piriformospora indica*) on growth and some physiological aspects such as pigments of photosynthesis, proline content, peroxidase enzyme and mineral acquisition of fenugreek (*Trigonella foenum-graecum*) plants under different levels of salinity (0, 50, 100, 150 and 200 mM NaCl) were studied. Based on the results, *Piriformospora indica* plays an important role in promoting and alleviating the fenugreek plant against the stress of salinity on both roots and stems (lengths and weights). The fungus stimulated the root lengths (0% - 35.58%), root weights (2.08% - 33%), stem lengths (1.2% - 34.28%) and stem weights (34.09% - 106.31%). Concerning the pigments of photosynthesis (chlorophyll a & b and carotenoids) of fenugreek (non-inoculated and inoculated plants), it is clear thatthe three pigments tested were faintly decreased in both control and the lowest concentrations of NaCl (-4.01%, -5.51% & -2.88% and -8.24%, -8.50% & 6.50%, respectively). Whereas, *P. indica* stimulated the biosynthesis of pigments at highest concentrations (14.58% - 38.83%, 12.64% - 84.32% and 0.49% - 43.33% of the three pigments, respectively). The fungus increased the content of proline in control (5.73%) and treated plants with NaCl (4 doses) with inoculation compared with un-inoculated plants (5.87- 9.45%). Regarding the antioxidant enzyme (peroxidase), the fungus stimulated the enzyme activity in both control by 8.55% and in three concentrations of NaCl (50, 150&200 mM NaCl) by 106.51%, 186.72% and 166.22%, respectively. *P. indica* stimulated the uptake of the mono-equivalent (Na^+& K^+, 106.6% & 1.33%, respectively) with a sharp decrease in the bi-equivalent (Mg^{++},-70.28%). The fungus had high uptake of the three metals, followed by negative uptake at 100 mM NaCl. Of the highest doses (150 & 200 mM NaCl). Also, the fungus stimulated the absorption of K^+ ions (32.61% & 31.53%) and Mg^{++} ions (24.04% & 24.44%) with decrease Na^+ ions (-58.32% & -21.42).

Keywords: *Piriformospora indica*, fungi, salinity, fenugreek, growth, photosynthesis, proline, peroxidase, minerals.

Introduction

Since the domestication of plants started, man has been attempting to improve the yield of agricultural plants by different means to secure his growing needs for food, fiber, biofuel, medicinal and other products to sustain and enhance his life. In these attempts, the man tried to improve agricultural practices, choose the best adapted plants and use any growth-enhancing factor and micro-organism in order to increase productivity.

Micro-organism are rich in diversity, complexity of interaction with numerous metabolic pathways. They are an amazing resource for biological activity (Haas and defago, 2005). More recently, the biological methods are most widely used to alleviate the soil stresses where they have received a great attention (Arora *et al*. 2020). Over the past 30 years, micro-organisms have been described characterized and tested for their use as biocontrol agents against diseases (El-Maraghy *et al*. 2020; Wachowska and Perkowskii 2020 and Abdel-Kareem *et al*.

2021), Heavy metals (Suksabye *et al*. 2016 and Shi *et al*. 2017). Salinity is widespread abiotic stress that restricts yield on almost one-third of the irrigated land on the earth (Hussein and Joo 2018). Also, approximately 50% of the arable land will be affected by salinity stress by year 2050. Being a crucial environmental factor, salinity is known to effect plant metabolism and result in rising of organic solutes that come to aid of turgor maintains. (Shrivastava and Kumar 2015).

Of the aforementioned reasons, Egypt and the whole world need to begin to greatly increase of agricultural productivity, and doing so in a sustainable and environmentally friendly manner must be taken into consideration. It is necessary to re-examine many of existing approaches to agriculture e.g. biological fertilizers, pest control, soil and water conservation and the use of improved plant varieties instead of chemical fertilizers, herbicides, fungicides and insecticides. This includes the use of transgenic plants and plant growth promoting micro-organisms as part of mainstream agricultural practices. It is envisioned thatin the not-too-distant future, plant growth promoting micro-organisms will begin to replace the use of chemicals in agriculture **(Lucy *et al.* 2004).** Plant growth promoting rhizomicro-organisms include diverse microbes that influence plant health by colonizing roots, enhancing plant growth, reducing plant pathogen population and activating plant defenses against biotic stresses. Also, micro-organisms regulate nutritional and hormonal balance. In addition to their interactions with plants, with both synergistic and antagonistic interactions with other microbes in the soil environment. These interactions may be vital for sustainable agriculture because they mainly depend on biological processes rather than agrochemicals to maintain plant growth and development as well as proper soil health under stress conditions **(Nadeem *et al.* 2014).** In developing countries (e.g. Egypt) in the arid and semi-arid regions of the world, the problem is more acute due to dramatic and continuous increase in population, the scarcity of fresh water, and the current food storage dilemma. Also in Egypt, salinity, drought, and high temperature are major abiotic stresses.

Fenugreek (*Trigonella foenum-graecum* L.) is one of the most promising medicinal herbs known for a long times that has nutritional value. A wide range of uses was found for it in ancient times. The leaves and seeds of the plant are widely consumed as a spice in food preparations and as an ingredient in traditional medicine. The aqueous extract of fenugreek contains bioactive constituents that may be beneficial as a spice in food and the management of diseases. This supports the traditional use of the plant as a food supplement and in the management of diseases (Buba *et al*. 2015). Therefore and based on the anticipated researches, the aim was to exploring the potentiality of using the fungus (*Piriformospora indica*) to enhance the tolerance of economicallyvaluable Egyptian medicinal plant to salinity.

Materials and Methods

1. Fungus and plant seed source:

Piriformospora indica is a Basidomycetous fungus, Order: Sebacinales, fungus kindly provided by **(Verma *et al.* 1998)**. Fenugreek (***Trigonella foenum-graecum* L.**) is a medicinally and economically-valuable Egyptian plant. The seeds of plant kindly provided by Agriculture Research Center "ARC", Egypt. The seeds were cultivated in plastic pots containing a mixture of soil (sand & clay**,** w: w, 1:1).

2. Sub-culture and inoculation of *Piriformospora indica*:

The fungus was maintained on Kafer's agar medium **(Kafer 1977)** in Petri dishes to obtain a mass of conidia and mycelia after 7-9 days of incubation at 28 ºC. A disc (1 cm) of growing fungus including agar medium was transferred to Kafer's broth medium and incubated in the dark as shaking growth (100 rpm) at 28 ºC for 14 days. At the end of incubation period, the fermented broth medium and mycelium (pellets) were homogenized using a blender (2000 rpm) for 30 sec. and used for inoculation the soil of fenugreek plant.

3. Experiment design and growth conditions:

Plastic pots containing soil (each, 2 kg) were divided into two groups and each group was re-divided into 5 sub-groups. The first group was treated with NaCl (50, 100, 150 and 200 mM NaCl) in addition to the control (only tap H_2O).Whereas, the second group was also divided to the same sub-groups, which were treated with NaCl (the same doses) and inoculated by the fungus (*P. indica*). The experiment was carried out in the open field during growing season (natural conditions). The plants were carefully watered every three days (tap water) for 14 days. After 14 days of cultivation, the second group was treated by homogenate broth medium (200 ml) for another 14 days.

4. Determination of growth parameters:

At the end of the experimental period, growth parameters such as root and shoot lengths were directly estimated as cm. Whereas, fresh and dry weights of the two plant organs were washed several times by tap followed by distilled water for removing air and soil particles. The detached fresh roots and shoots (each, 3 organs) were dried at 35 ^{0}C and 80 ^{0}C for 24 h. during which successive weights were determined as gram.

4. Biochemical analysis of the plant:

4.1. Estimation of photosynthetic pigments:

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method at the wave lengths of 663, 647 and 470 nm, respectively **(Lichtenthaler 1987)**.

4.2. Estimation of proline:

Dry tissue (DW= 100 mg) of shoots was homogenized in 5 ml of 3% sulfosalicylic acid for 3 h, centrifuged of the extract and the supernatant was decanted. Proline was estimated using the spectrophotometric method at 520 nm using a standard curve and calculated on a dry weight basis as mg/g DW **(Bates *et al.* 1973)**.

4.3. Determination of some minerals (Na^+, K^+, and Mg^{++}):

Dry samples of shoots were ground into a fine powder using a micro mill and assayed for mineral ion (Na^+, $K^+$and Mg^{++}) concentrations. The wet digestion method **(Humphrjes 1956)** was used,and the data were expressed as mg/g dry weight.

5. Stress markers determinations (antioxidant enzymes activity):

5.1. Enzyme extraction:

A fresh leaf sample (FW= 500 mg) was added to 5 ml of 50 m M phosphate buffer (Na_2HPO_4/K_2HPO_4) at pH7.0 containing 0.1mM Na_2EDTA and 1% of polyvinylpyrrolidone (PVP), ground by pestle and motor on ice. The homogenate was centrifuged at 13500 rpm for 20 min at 4^{o}C. The supernatant was re-centrifuged at 13500 rpm for 15min at 4^{o}C; the resulted supernatant was collected and stored at-20°C for analysis.

5.2. Determination of peroxidaseactivity:

Total peroxidase activity (POX) was determined as described by **MacAdams *et al.*1992**. Thereaction mixture was measured at 470 nm. POX activity was calculated in terms of µM of guaiacol oxidized $min^{-1}g^{-1}$ fresh weight at 25±2^{o}C.

Statistical analysis:

The experiments were designed as completely randomized with three replications. The experimental data were statistically analyzed by Mstat-C program and ANOVA. Data were compared using the least significant difference (LSD) test at 0.05 levels **(Snedecor and Cochran 1980)**.

Results

Based on the results obtained, concerning the effects of *Piriformospora indica* treatment against salinity stress of fenugreek plant (each, 3 replicates of 4 doses of NaCl). The plants were classified into two groups (group I& group II). Group I was grownunder thestress of salinity in addition to control I (without treatment), whereas group II treated with salinity (same doses) and the fungus in addition to control II (treated only with fungus dose). Six parameters were taken into consideration and these were:

1- Plant growth :

Of plant growth, the length and weight of plant organs (roots & shoots) were estimated. The shoot of group I at 50 mMNaCl was more or less parallel to control I (31.33& 31.00 cm, respectively), followed by regular decrease (-8.61%, -11.84%&-21.52% at 100, 150& 200 mMNaCl, respectively). Of group II that treated with salinity and the fungus, the lowest NaCl dose enhanced the length of shoot compared with control II (18.37%) followed by a faintly decrease in the length (-1.14% at 100 mMNaCl), parallel in length (100% at 150 mM), finally increase in length of the shoot at 200 mM NaCl (12.65%).In the roots of group I, the length was regularly decreased at first 3 doses (-16.98%,-22.98%&-40.98 %, respectively) and retarded faintly(-30.01%) at 200 mM. Whereas, of group II (NaCl treatment and fungus inoculation), the length of roots was, in general, less than control by -2.24% to-16.67%.

Also and based on the results obtained, the weight of shoots of fenugreek group I had a moderate decrease at 50 and 100 mM NaCl(-21,57% & 9.74 %, respectively), followed by a more decrease at 150 mM (-32.21%) and reduced sharply at 200 mM (-54.09%). In group II, the fungus treatment stimulated the weight at 50 and 100 mM (23.04 % &52.02 %, respectively) followed by a moderate decrease at 150 mM (-7.48%) and a sharp decrease at 200 mM (-49.73%).

Of fenugreek roots, NaCl doses (group I) had clearly a decrease in length (-36.92 %, -44.79 %, -64.06 %&-52.16 % at 50, 100, 150& 200 mM, respectively). Whereas, with fungus inoculation, increases in root lengths were occurred (26.69 %, 24.69 %, 19.02%& 55.76 % of the doses, respectively).

The collective effects (means) of salinity with/ without fungal (*P. indica*) inoculation on the fenugreek plant growth including stems and roots (length and weight) revealed tha tthe fungus stimulated the lengths of stems and roots by 7.98% and 5.4%, and the weights were sharply increased by 65.93% and 40.26%, respectively (Table, 1&2; Fig. 1&2).

Table (1): Effect of salinity (50, 100, 150& 200 mMNaCl) and salinity with fungal (*P. indica*) inoculation on the growth (length of root and stem) of fenugreek plants.

Shoot length (cm) / NaCl (mM)	Salinity	Salinity & *P. indica*	Mean	LSD
0	31.00 C	29.00 D	30.00 B	0.4392
50	31.33 C	34.33 A	32.83 A	
100	28.33 D	28.67 D	28.50 C	
150	27.33 E	29.00 D	28.17 C	
200	24.33 F	32.67 B	28.50 C	
Mean	28.467A	30.733A	0.8267	
LSD	2.57			
Root length (cm) / NaCl (mM)	**Salinity**	**Salinity&*P. indica***	**Mean**	**LSD**
0	33.33 A	30.00 AB	31.67 A	6.702
50	27.67 AB	29.33 AB	28.50 AB	
100	25.67 AB	25.67 AB	25.67 AB	
150	19.67 B	26.67 AB	23.17 B	
200	23.33 AB	25.00 AB	24.17 B	
Mean	25.933 A	27.333 A	12.62	
LSD	4.042			

Table (2): Effect of salinity (50, 100, 150& 200 mMNaCl) and salinity with fungal (*P. indica*) inoculation on the growth (weight of root and stem) of fenugreek plants.

Shoot wt.(g) / NaCl(mM)	Salinity	Salinity&*P. indica*	Mean	LSD
0	10.71 BCD	13.11 ABC	11.91 AB	4.117
50	8.400 BCD	16.13 AB	12.27 AB	
100	9.667 BCD	19.93 A	14.80 A	
150	7.260 CD	12.13 BCD	9.697 BC	
200	4.917 D	6.593 CD	5.755 C	
Mean	8.19 B	13.58 A	7.749	
LSD	2.659			
Root wt.(g) / NaCl (mM)	**Salinity**	**Salinity& *P. indica***	**Mean**	**LSD**
0	8.033 AB	8.867 A	8.450 A	3.048
50	5.067 AB	6.500 AB	5.783 AB	
100	4.433 AB	6.677 AB	5.555 AB	
150	2.887 B	8.067 AB	5.477 AB	
200	3.843 AB	3.923 AB	3.883 B	
Mean	4.853 B	6.807 A	5.738	
LSD	1.979			

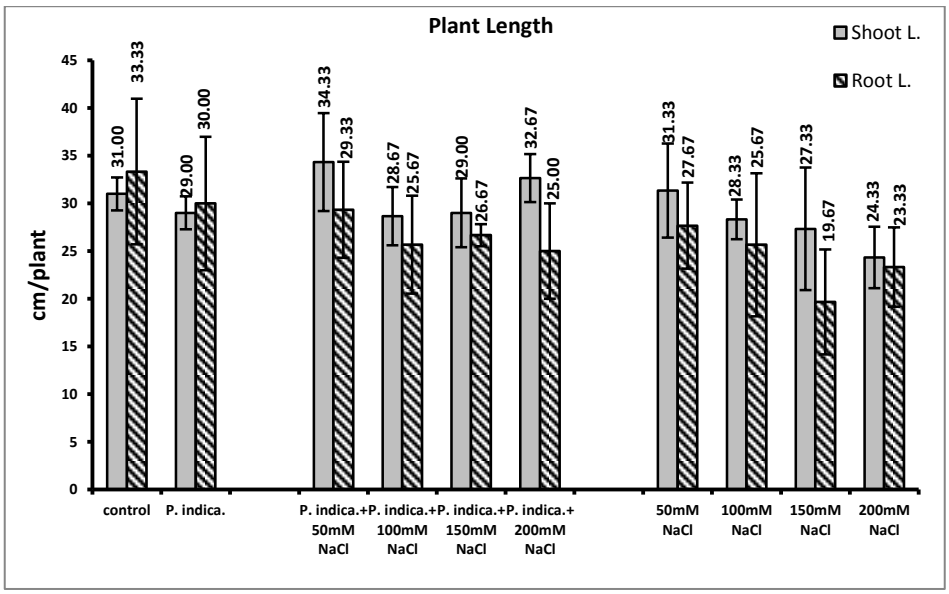

Fig. (1): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on the shoot and root lengths of fenugreek plants.

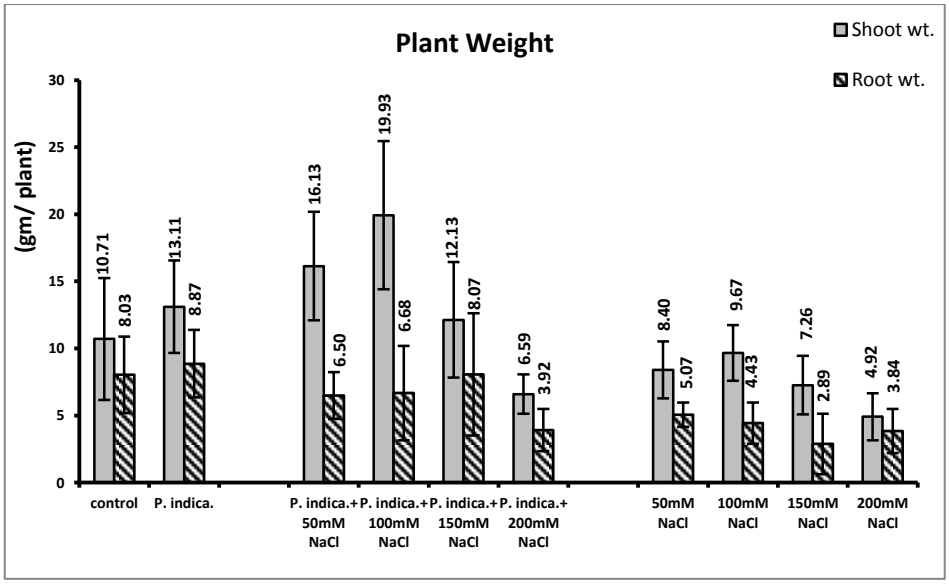

Fig. (2): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on the shoot and root weights of fenugreek plants.

2- Pigments of Photosynthesis:

Concerning to pigments of photosynthesis, three pigments (chlorophyll a & b and carotenoids) were estimated. Chlorophyll a (group I) was irregularly decreased or increased at the concentrations of NaCl (-22.68%, -43.81%, -19.41%&-29.21 % at 50, 100, 150&200 mMNaCl, respectively). Whereas, in the case of fungus inoculation (group II), an increase in chlorophylla content was observed (-26.01 %, 18.74 %, 3.80%& 0.37 % of the doses, respectively) in all doses except for 50 mM compared with salinity alone. Chlorophyll b contents were parallel to that of chlorophyll a. Of group I, stress of salinity, the contents (-23.11 %, -44.44 %, -19.54%&-28.14 % at 50, 100, 150 &200 mM NaCl, respectively) were irregularly decreased compared with control I. Based on chlorophyll b contents in group II, the fungus sharply increased the chlorophyll b contents at 200 mM (40.17 %) with its contents was decreased at the remaining doses (-25.54 %, -19.87 %&-4.08 % at 50, 100&150 mM NaCl, respectively). Finally, the carotenoid contents were in general un-constant in group I toward a quantity decrease at all (-16.83 %, -30.63 %, -8.90 %&-15.59 % at 50, 100, 150& 200 mM NaCl, respectively). But with fungus inoculation with stress of salinity, the contents of carotenoid were less in the first 3 treatments (-19.93 %, -19.65 %&-6.68 %, respectively) with sharply increases at 200 mM (15.78%).

In this respect, treatment of the fenugreek plant (under stress conditions) by *P.indica* had enhanced effects (the means) of 3 pigments (12.5 %, 20.37 % & 8.01 % of chlorophyll a, b & carotenoids, respectively). Also, the contents of the three pigments tested after inoculation (control II) by the fungus were less (1.078, 0.458& 1.077 mg/g) compared with untreated plants (1.123, 0.4847& 1.109 mg/g dry weight, respectively) as shown in table 3 & Fig. (3).

3- Proline content:

Based on the results obtained, proline was sharply decreased at 50 mM NaCl (-53.69 % of 0% NaCl), followed by a regular increase (-2.86 %, 1.56%& 5.81 % at 100, 150& 200 mM NaCl) as clear in group I (only NaCl treatment). Of group II (NaCl treatment and fungus inoculation), the proline content was increased at 50 mM (10.37 %), followed by a slight decrease (-0.31 %) and retarded the increase (3.27 %& 5.75 % at 150 &200 mM NaCl, respectively).

It is clear that *P. indica* stimulated the proline content of fenugreek plants compared with un-inoculated plants (mean, 21.79%). Also, control II had proline content more than un-treated plants by 5.72% (Table, 4; Fig. 4).

4- Peroxidase activity:

Concerning the peroxidase activity of fenugreek plants under salinity stress (group I, 4 doses) and salinity with inoculation by *P. indica*, unclear effects were detected, which was increased at low concentrations of NaCl (10.29% & 136.47% at 50& 100 mM) and slightly was decreased at high doses (-2.52 & -0.02% at 150 & 200 mM, respectively). Whereas, the peroxidase activity was clearly increased with the fungus treatment compared with control I(109.84 %, 104.19 %, 157.48 %& 145.01 % at 50, 100, 150& 200 mM NaCl, respectively).Finally, the mean content of the peroxidase activity in inoculated plants with salinity stress was higher than in plants under stress only by 71.35%. Also, the peroxidase activity in control II (fungus only) was higher than control I (without treatment) by 8.55 % (Table, 5; Fig. 5).

Table (3): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungal (*P. indica*) inoculation on pigments (chlor. a, chlor. b and carotenoids) of photosynthesis of fenugreek plants.

Chlor.a(mg/g. DW) NaCl (mM)	Salinity	Salinity&*P. indica*	Mean	LSD
0	1.123 A	1.078 A	1.101 A	0.3023
50	0.8683 A	0.7967 A	0.8325 AB	
100	0.6310 A	0.8760 A	0.7535 B	
150	0.9050 A	1.037 A	0.9712 AB	
200	0.7950 A	1.074 A	0.9343 AB	
Mean	0.864 A	0.972 A	0.569	
LSD	0.2003			
Chlor.b (mg/g. DW) NaCl (mM)	**Salinity**	**Salinity&*P. indica***	**Mean**	**LSD**
0	0.4847 A	0.4580 A	0.4713 A	0.2188
50	0.3727 A	0.3410 A	0.3568 A	
100	0.2693 A	0.3670 A	0.3182 A	
150	0.3900 A	0.4393 A	0.4147 A	
200	0.3483 A	0.6420 A	0.4952 A	
Mean	0.373 A	0.449 A	0.4118	
LSD	0.1436			
Cartenoids (mg/g. DW) NaCl (mM)	**Salinity**	**Salinity&*P. indica***	**Mean**	**LSD**
0	1.109 A	1.077 A	1.093 A	0.2925
50	0.9223 A	0.8623 A	0.8923 A	
100	0.7693 A	0.8653 A	0.8173 A	
150	1.010 A	1.005 A	1.008 A	
200	0.870 A	1.247 A	1.058 A	
Mean	0.936 A	1.011 A	0.5506	
LSD	0.1917			

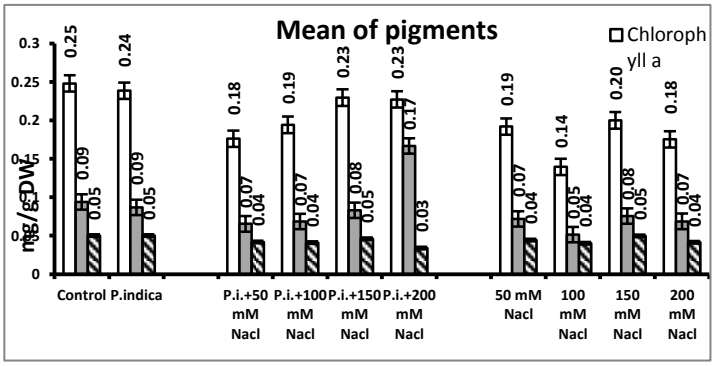

Fig. (3): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on pigments (chlor. a, chlor. B & carot.) of fenugreek plants.

Table (4):Effect of salinity (50, 100, 150& 200 mMNaCl) and salinity with fungal (*P. indica*) inoculation on theproline content of fenugreek plants.

Proline cont. ((mg/gDW) NaCl(mM)	Salinity	Salinity&*P. indica*	mean	LSD
0	3.317 AB	**3.507 AB**	**3.412 A**	
50	**1.536 C**	**3.871 A**	**2.704 B**	
100	**3.222 B**	**3.496 AB**	**3.359 A**	**0.2974**
150	**3.369 AB**	**3.622 AB**	**3.496 A**	
200	**3.510 AB**	**3.716 AB**	**3.613 A**	
Mean	**2.991 B**	**3.643 A**	**0.5599**	
LSD	**0.1772**			

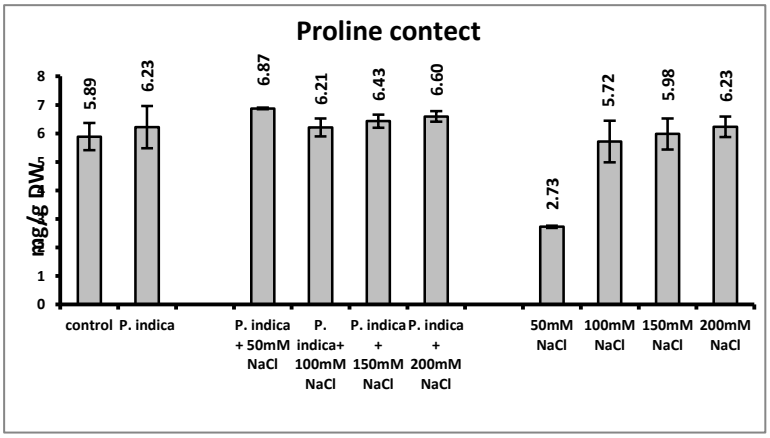

Fig. (4): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on the proline content of fenugreek plants.

Table (5): Effect of salinity (50, 100, 150& 200 mMNaCl) and salinity with fungal (*P. indica*) inoculation onperoxidaseactivityof fenugreek plants.

Peroxidase activity (U/g.sec) NaCl(mM)	Salinity	Salinity & *P. indica*	mean	LSD
0	**0.7020 C**	**0.7620 BC**	**0.7320 B**	
50	**0.7743 BC**	**1.599 A**	**1.187 A**	
100	**1.660 A**	**1.556 AB**	**1.608 A**	**0.4359**
150	**0.6843 C**	**1.962 A**	**1.323 A**	
200	**0.7013 C**	**1.867 A**	**1.284 A**	
Mean	**0.904 B**	**1.549 A**	**0.8205**	
LSD	**0.2885**			

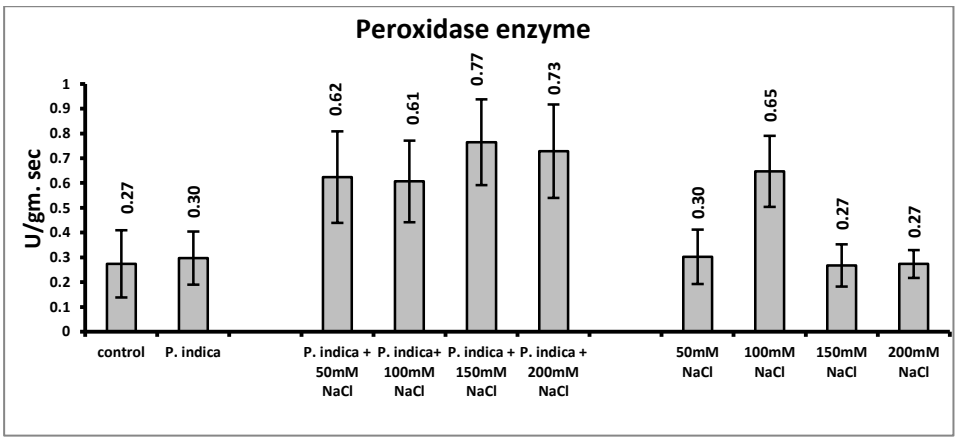

Fig. (5): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on peroxidase activity of fenugreek plants.

5- Mineral contents:

The changes in mineral contents (Na^+, K^+& Mg^{++}) related to salt stress with/ without fungus inoculation were examined. the concentrations of sodium (group I) had the highest values at 100, 150& 200 mM (98.75%, 67.27% 55.95%, respectively) with a decrease at 50 mM (-18.45%) in fenugreek plants under salinity stress. *P. indica* stimulated sodium uptake at 50 mM (96.8%) with irregularly detection at the remaining doses (-46.99%, 28.96% &-40.65, respectively). Also, the fungal inoculation increased sodium uptake by 56.96% as a mean of group II compared to group I, and also increased sodium concentration (106.61%) in control II compared with control I (native soil).

Concerning the level of potassium ions (K^+), NaCl treatment (group I) increased the element at 100& 150 mM (25.21%& 17.58%) with decreased at 50 and 200 mM (32.99%& 17.58%). Whereas inoculation by *P. indica* increasing the uptake of potassium ions at all doses of NaCl (21.47 %, 2.7 %, 37.94 %& 6.84 % at 50, 100, 150& 200 mM NaCl, respectively) was observed. Also, based on the results obtained, the fungus inoculation increased the potassium uptake as means of treatments by 20.08% and in controls of two treatments (group I & II).

Magnesium (Mg^{++}) uptake was sharply depressed at 50 mM NaCl (-63.81%) compared with control I (group I), followed by a moderate increase at 100 mM (-42.91%) and a return to a slight decrease (-47.45%& -52.13% at 150& 200 mM NaCl, respectively).Of group II, there was a sharp increase in magnesium uptake compared with control II (+297.91%) and at 50 m MNaCl of group I (+201.31%). At 100 mM NaCl, there was a sharp decrease in magnesium (Mg^{++}) uptake compared with the same dose of group I (-55.74%) and a slight decrease (-14.69%) compared with control II of group II. Magnesium content (Mg^{++}) was increased at the highest levels of NaCl compared with control II (120.06% & 101.11% at 150 & 200 mM NaCl, respectively). Regarding the results of the two groups, the fungus *P. indica* reduced magnesium uptake by -2.38% and sharply depressed in control II compared with control I by -337.59% (Table, 6; Fig. 6).

Table (6): Effect of salinity (50, 100, 150& 200 mMNaCl) and salinity with fungal (*P. indica*) inoculation onthe mineral (Na^+, K^+& Mg^{++}) contentof fenugreek plants.

Na^+cont. NaCl(mM)	Salinity	Salinity&*P. indica*	Mean	LSD
0	0.05600 BC	0.1157 BC	0.08583 AB	0.0538
50	0.04567 C	0.2277 A	0.1367 A	
100	0.1113 BC	0.06133 BC	0.08633 AB	
150	0.09367 BC	0.1483 AB	0.1210 AB	
200	0.08733 BC	0.06867 BC	0.07800 B	
Mean	0.079 B	0.124 A	0.1014	
LSD	0.0301			
K^+ cont. NaCl(mM)	Salinity	Salinity&*P. indica*	Mean	LSD
0	0.2697 ABC	0.2733 ABC	0.2715 A	0.0762
50	0.1807 C	0.3320 AB	0.2563 A	
100	0.3377 AB	0.2807 ABC	0.3092 A	
150	0.2843 ABC	0.3770 A	0.3307 A	
200	0.2223 BC	0.2920 ABC	0.2572 A	
Mean	0.259 B	0.311 A	0.1434	
LSD	0.0472			
Mg^{++} cont. NaCl(mM)	Salinity	Salinity&*P. indica*	mean	LSD
0	0.2137 AB	0.06333 C	0.1385 AB	0.06596
50	0.07733 C	0.2330 A	0.1552 A	
100	0.1220 ABC	0.0540 C	0.0880 B	
150	0.1123 ABC	0.1393 ABC	0.1258 AB	
200	0.1023 BC	0.1273 ABC	0.1148 AB	
Mean	0.126 A	0.123 A	0.1242	
LSD	0.0404			

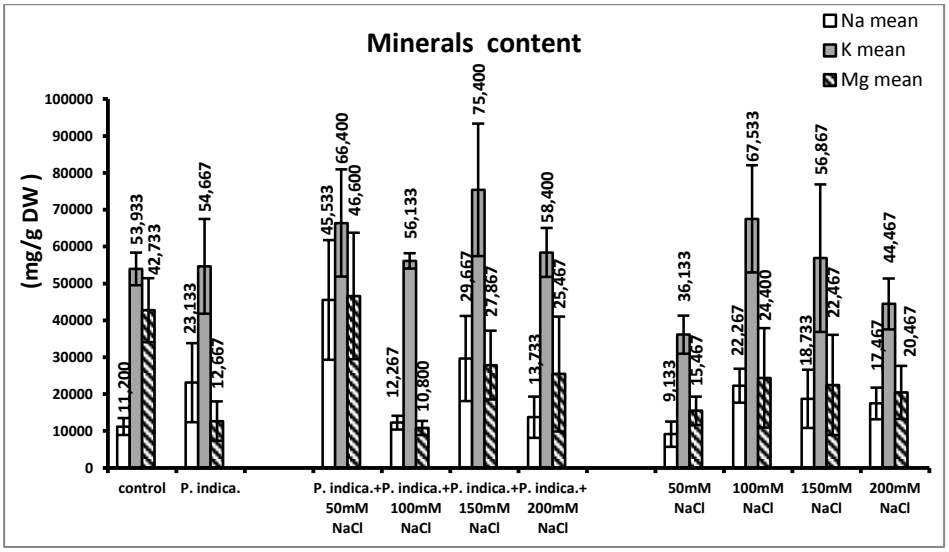

Fig. (6): Effect of salinity (50, 100, 150& 200 mM NaCl) and salinity with fungus (*P. indica)* treatment on the mineral (Na^+, K^+& Mg^{++}) content of fenugreek plants.

Discussion

Concerning the correlation between plants and micro-organisms, rhizobacteria and mycorrhiza alone and/ or in combination are superior in using for enhancing plant growth under stress conditions **(Nadeem *et al.* 2014)**. The most common mutualistic association between fungi and plant roots is mycorrhizal symbiosis. Where the most common type of association is arbuscular mycorrhizae (AM), which are obligate biotrophs, provide the plant with enhanced access to water and nutrients, able to produce enzymes involved in the hydrolysis of nitrogen and phosphorus compoundsfrom organic matter in the soil as well as, alleviate abiotic and biotic stresses by increasing the plant fitness **(Smith and Read 2010)**. Recently, AM-like organisms were discovered that colonizes plant roots of woody shrubs *Prosopis juilifora* and *Zizuphus hummularia* in sandy desert of Rajasthan, India with a strong promotive effect on host growth and its physiological processes is the Basidiomycetous fungus (*Piriformospora indica*). The fungus is cultivable and can be cultured with the plant host as well as can be easily grown on a few various complex and substrates **(Verma *et al.* 1998).**

Piriformospora indica plays an important role in promoting and alleviating fenugreek plants against against salinity stress on both roots and stems (lengths and weights). The fungus stimulated root lengths (0% - 35.58%), root weights (2.08% - 33%), stem lengths (1.2% - 34.28%) and stem weights (34.09% - 106.31%).The root and shoot lengths are the most important factors for salt stress where the roots are in a direct contact (adhered) to soil and absorb water from soil and nutritional supply source to the rest of the plant. Therefore, root and shoot lengths provides an important clue to the response of plants to salt stress. Salt stress declined the seedling growth (root and shoot length), fresh root and shoot weight but, root length was more affected than shoot length. The reduction in root and shoot development related to NaCl used as well as unbalanced nutrient uptake by seedling whereas, inhibition of plant growth by salinity

may related to effect of ions (**Farulla** *et al.* 2017).

In this respect and based on the previously literature, *P. indica* interact with a wide range of hosts, including bryophytes, pteridophytes, Gymnosperms and a large number of monocot and dicot plants **(Varma *et al.* 2012, Fakhro *et al.* 2010, Oelmüller *et al.* 2009).**Itis a wide host root colonizing endophytic fungus, which allows the plant to grow under extreme physical and nutrient stresses, enhanced the growth of the host plant and provides protection against biotic and abiotic stresses **(Varma *et al.* 1999, Peškan-Berghöfer *et al.* 2004)**, reprograms barley to salt stress tolerance, resistance to diseases and higher yield. The fungus provided disease resistance not only to roots but also to shoots **(Achatz *et al*. 2010)**.

Concerning the pigments of photosynthesis (chlorophyll a & b and carotenoids) of fenugreek (non-inoculated and inoculated plants), it is clear that the three tested pigments were slightly decreased in both control and lowest at lowest concentrations of NaCl (-4.01%, -5.51%&-2.88% and -8.24%, -8.50% & 6.50%, respectively). Whereas, *P. indica* stimulated the biosynthesis of pigments at highest concentrations (14.58%-38.83%, 12.64%-84.32% and 0.49%- 43.33% of the three pigments, respectively).

In this respect, a non-significant increasing in intercellular CO_2 concentrations of *Plantago lanceolate* was observed under the influence of arbascular mycorrhiza (AM) **(Parádi *et al.* 2003).** In otherwise, *P. indicant* enhanced the photosynthetic rates of colonized barley plants **(Oelmüller *et al.* 2009)** and enhanced the rate of photosynthesis at low light related to higher chlorophyll content with increasesd grain yield of barley plants **(Achatz *et al*. 2010)**.

Of proline contents in fenugreek (inoculated by *P. indica* and non-inoculated) plants under salinity stress (NaCl), the fungus increased the content of proline in control (5.73%) and treated plants with NaCl (4 doses) with inoculation compared with un-inoculated plants (5.87- 9.45%). Proline is a non-essential amino acid and along with hydroxyl proline, is the only amino acid without primary amino groups and therefore has a unique metabolic pathway in the mitochondria **(Kohlmeier 2003)**. Proline isomerization is ubiquitous in protein and is important for regulating important processes such as folding and enzymatic activity **(Burkholder *et al.* 2018)**. Free proline accumulates in Plants in response to stresses **(Delauney and Verma 1993).** Therefore, the endophytic fungus *P. indica* reprograms barley to salt-stress tolerance, disease resistance and higher yield **(Waller *et al.* 2005)**.

Regarding the antioxidant enzyme (peroxidase), the fungus stimulated the enzyme activity in both controls by 8.55% and in three concentrations of NaCl (50, 150 & 200 mM NaCl) by 106.51%, 186.72% and 166.22%, respectively. Peroxidases represent a family of isoenzymes actively involved in oxidizing reactive oxygen species, innate immunity, hormone biosynthesis and pathogenesis of several diseases **(Khan *et al.* 2014)**. Peroxidase has a prominent place in biotechnology and associated areas such as microbiology, biochemistry, medicine, genetics and clinical chemistry due to its versatility, viability and economic nature **(Kumar *et al.* 2017).** Concerning to the result obtained in this manuscript plants, the antioxidant enzyme system was activated by *P. indica* **(Baltruschat *et al.* 2008).** Also, the increase in peroxidase activity in inoculated plants might be responsible for enhanced redox balance, resulting in reduction and oxidation damage **(Balestrini *et al.* 2012).**

P. indica stimulated mono-equivalent uptake (Na^+& K^+, 106.6% & 1.33%, respectively) with a sharp decrease in bi-equivalent (Mg^{++},-70.28%). The fungus had high uptake effects on the three minerals the three metals, followed by negative uptake at 100 mM NaCl. Of the highest doses (150 & 200 mM NaCl), the fungus stimulated the absorption of K^+ ion (32.61% & 31.53%) and Mg^{++} ion (24.04% & 24.44%) with decreasing Na^+ ion (-58.32% & -21.42). Regarding the previous, results concerning the uptake of the three minerals (Na^+, K^+& Mg^{++}) with other literature in this respect, sodium (Na^+) concentrations were slightly higher in tissues of non-inoculated plants (fenugreek) than inoculated with mycorrhizal fungus (*Glomus intraradices*) and the symbiosis of fungus can control uptake of sodium ion when it becomes toxic of plants **(Evelin *et al.* 2012).** Also, mycorrhizal plants accumulated less sodium ion **(Bohra *et al.* 1995)**, which may be due to improved potassium ion uptake **(Giri *et al.* 2004)**, and mycorrhiza prevent the disruption of K^+ ion homeostasis **(Shokri and Maadi 2009)**.

In conclusion and based on the results obtained, concerning the enhancement and mitigation of fenugreek plants under salinity stress using a saprophytic fungus (*Priformospora indica*) in several parameters including plant growth and some physiological aspects. The authors suggest using the fungus until the final yield of the plants. Also, another isolated fungus must be taken in consideration for the mass production of cultivated plants.

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