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Application of Biofertilizers for improving the growth of fenugreek plants (*Trigonella Foenum-Graecum* L.)

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Abstract
The present study is design to investigate the effects of rhizobium species as Biofertilizers for improving the growth parameters of fenugreek plants in Department of Botany, Jinnah University for Women. Two rhizobial species I and II, isolated from fenugreek and Melilotus alba respectively were used as Biofertilizers. Results showed that both treatments promote all the growth parameters as root and shoot lengths, seedling fresh weights, number of nodules and number of leaves after 21 days of growth of fenugreek plants. Application of Biofertilizers improved the growth of plants.

Keywords: Biofertilizers, fenugreek plants, physical parameters, growth.

Introduction
Fenugreek (*Trigonella foenum-graecum* L.), the annual herbaceous legume crop belonging to the family Fabacecae is usually known as Fenugreek and is deliberated as one of the oldest multipurpose medicinal herbs [1]. It was named, Trigonella, from Latin language that means ‘‘little triangle” due to its yellowish-white triangular flowers [2]. Fenugreek, like other legumes, is a worthy source of dietary protein for feeding by man and animals. From earliest times, Greeks (and the Romans) used it as medicine, spice and cattle fodder and so it was and still known as Greek hay. Seeds of fenugreek are used as a yellow dye, in cosmetics and for medicinal commitments. Fenugreek is a good soil renovator and commonly used as a green manure [3].

Seeds are used as a condiment for flavoring of foods and leaves as vegetable. It has also got medicinal importance, therefore used for cure of flatulence, dysentery, diarrhoea, enlargement of liver and spleen, rickets, diabetes, and many others [4]. It is assumed that the class of flavonoids which fenugreek contains may play a substantial role in the prevention of cancer [5]. Shekhawat *et al.*, in 2012 [6] highlighted the requirement for incorporation of biofertilizers in the fertilizer programme to meet about one third of plant nutrient needs. Biofertilizers are environment friendly, less costly, and therefore lead to sustainable crop production [7]. In addition, they produce hormones, vitamins and other growth factors essential for plant growth. Fenugreek, being a legume crop, responds to inoculation with *Rhizobium* to meet the partial requirement of nitrogen. *Rhizobium* inoculation of fenugreek has been reported to intensification the biomass of plant and seed production [8]. Many studies have shown that simultaneous infection with rhizobia and rhizosphere bacteria increases nodulation and growth in a extensive variety of legumes [9]. This experiment was, therefore, designed with the objective of studying the effect of biofertilizer on physical growth of fenugreek.

Materials and method
The present research work conducted in green house of Department of Botany, Jinnah University for Women in complete randomized block design. Farm yard manure used as organic material. Two rhizobial species used as Biofertilizers, isolated from fenugreek (rhizobial sp. I) and Melilotus alba (rhizobial sp. II) by crushed nodule method [10]. An optical density of 0.5nm recorded at a wavelength of 535 nm, was achieved by dilution to maintain uniform cell density (10^8-10^9 cfu mL-1) [11].

Three treatments were used as experimental setup. Treatments are as follows, T1= control, T2= rhizobial sp.I, T3= rhizobial sp. II. Each treatment has three replicates. Fenugreek was used as experimental plants. 4 seeds of Fenugreek were planted in each pot. Plants were

irrigated with tap water to maintain soil moisture. After 21 days plants were harvested for physical analysis. Root & shoot lengths (cm), seedling fresh weights (g), number of nodules and number of leaves were measured as physical parameters.
The data was analyzed by using *One way* ANOVA followed by LSD (least significant difference) test through SPSS 16 (version 4). The differences were considered significant at $p<0.05$ when treatments' mean compared with control.

Results

The inoculation of two different rhizobial species promoted the growth of fenugreek plants when observed after 21 days of germination. The results of table 2 showed that rhizobial sp.I significantly promoted the root length of experimental plants up to 105 % as compared to control. While rhizobial sp.II also promoted the root lengths non significantly (table, 1). Both treatments with rhizobial species (I & II) significantly increased the shoot length of experimental plants (table, 1). Fresh weights of experimental plants were also promoted with both treatments as compared with control but significant promotion observed with the treatment of rhizobial sp.I after 21 days of growth in fenugreek plants (table, 1).

Table 1: Effect of Biofertilizers on root lengths, shoot lengths and fresh weights of fenugreek plants.

S.no.	Treatments	Root length (cm)	Shoot length (cm)	Seedling Fresh weight (g)
1	Control	6.13 ± 0.29	5.67 ± 0.45	0.16 ± 0.03
2	Rhizobial sp.I	12.6^{b}± 3.8 (105.5 %)	8.15^{b}± 1.14 (43.73 %)	0.22^{d}± 0.02 (37.5 %)
3	Rhizobial sp.II	7.25 ± 0.95 (15.44 %)	6.22^{b} ± 0.29 (9.7 %)	0.17 ± 0.04 (6.25 %)

Values in parenthesis are showing the percentage increased over untreated control. Columns bearing superscript are statistically significant ($p< 0.05$ LSD) with respective control. a = $p< 0.0001$, b = $p< 0.001$ and c= $p< 0.01$, d = p< .05.

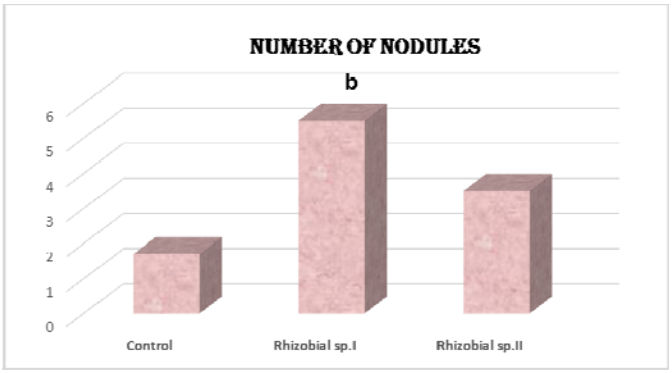

Fig 1: Effect of Biofertilizers on number of nodules of fenugreek plants. Column bearing superscript are statistically significant ($p< 0.05$ LSD) with respective control. a = $p< 0.0001$, b = $p< 0.001$, c = $p< 0.01$ and d = .05

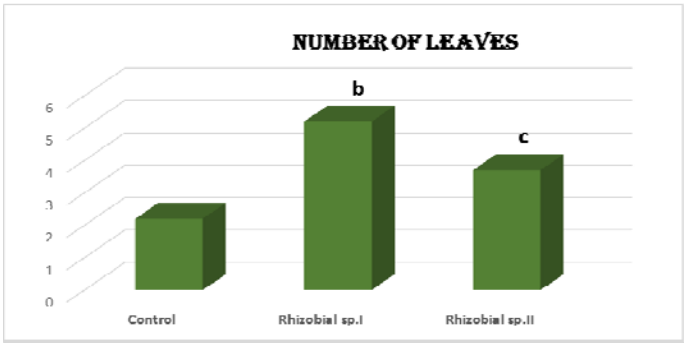

Fig 2: Effect of Biofertilizers on number of leaves of fenugreek plants. Column bearing superscript are statistically significant ($p< 0.05$ LSD) with respective control. a = $p< 0.0001$, b = $p< 0.001$, c = $p< 0.01$ and d = .05

Both treatments increased number of nodules in experimental plants as compared to control. Inoculation with rhizobial sp.I showed significant promotion in nodulation upto 223% in fenugreek plants (fig.1). Both treatments significantly enhanced the number of leaves in experimental plants ranges 68-136% as compared to control (fig.2).ss

Discussions

Nutrient augmentation of soils by nitrogen fixing symbiotic bacteria present in legumes has been famous for centuries [12]. Biofertilizers have revealed great potential as additional, renewable and environmental friendly sources of plant nutrients. These encourage growth by increasing the availability of primary nutrients and/or growth incentive to the target crop when applied to seed, plant surfaces, or soil [13]. *Rhizobium* spp. are well known group of microbes that acts as the major symbiotic fixer of nitrogen. These bacteria infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place. This symbiosis decreases the requirement for N fertilizers in the course of growth of leguminous crops [14]. Rhizobial species stimulate plant growth either directly (nitrogen fixation, phosphate solubilization, iron chelation and phytohormone production) or indirectly (destruction of plant pathogenic organisms, initiation of resistance in host plants against plant pathogens and abiotic stresses), are stated as plant growth promoting rhizobacteria [15].
Result showed the effect of rhizobial species (sp.I & sp.II) were more beneficial and promotory in the growth of nodules as compared to control (fig.1). The improved nodulation, N fixation and yield of legume crops following inoculation with biofertilizers have been reported by many workers [16, 17]. Managements with pure cultures of *Rhizobium* significantly amplified the number of nodules [18]. Use of bacterial inoculum leads to increase the number of root nodules that rise the capability of nitrogen fixation [19].
In the present study, inoculation of rhizobial isolates enhanced the growth parameters particularly root and shoot lengths of test plants. The obtained positive effects of rhizobial isolates on growth parameters of fenugreek plants in present study support the earlier reports that described the abilities of *rhizobium* strains in producing phytohormones in reaction to their inoculation *via* seed dressing or root drench which facilitated to speed up the growth of plants as described by the Aher and Khapke in 2009 [20]. Another study also verified that Biofertilizers considerably stimulate growth parameters of fenugreek plants as obtained in present study in terms of root & shoot lengths, fresh weights of seedlings and number of leaves [21]. Sunanda *et al*., in 2014 [22] detected significant results of growth parameters when put on Biofertilizers for the management of kasuri methi. Soundari *et al*., in 2015 [23] also demonstrated the significant effects of biofertilizer inoculation on fenugreek growth as compared with control plants.
The outcomes of present study corroborate with the outcomes of Meena *et al*., (2014) [24] in fenugreek, that these 289

biofertilizers are also accomplished to secret some biologically active compounds such as auxin, gibberellins, vitamins etc. which are deliberated to be imperative for suitable growth and development of plants. Rhizosphere is an exclusive niche that offers habitation and nutrition to PGP microorganisms. In turn, these microorganisms produce multiple benefits of induced plant growth, resistance against diseases and survival under stress with many other unknown benefits [15].

Conclusion

Application of Biofertilizers is improve the growth of fenugreek plants. It is therefore concluded that effective use of 290iofertilizers play a vital role in increasing the plants growth.

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