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Research Article

IMPACT OF *BACILLUS SUBTILIS* ON FENUGREEK (*TRIGONELLA FOENUMGRAECUM* L.): NATURAL MEDICINE FOR RURAL INDIA.

Shashi Kant Shukla*

Centre of Rural Technology and Development, IIDS, University of Allahabad, Allahabad-211002

Abstract:

The present study was design to observe the impact of *Bacillus subtilis* as Biofertilizers for improvement in the growth parameters of fenugreek a leguminous plant to support the requirement of food, protein along with their medicinal value in the rural areas of India. A experiment was arranged based on completely randomized design with four replications at Biological Product Laboratory, Botany Department, University of Allahabad. Treatments were given at the seed level with one of control. Results indicated that application of *Bacillus subtilis* significantly improved vegetative growth and had a best positive effect on fenugreek as compared to the control.

Keywords: *Bacillus, Fenugreek, Leguminous, Medicinal, Rural etc.***Corresponding author:**

Shashi Kant Shukla*

Centre of Rural Technology and Development,
IIDS, University of Allahabad,
Allahabad-211002

Email: shashibplau@gmail.com

QR code



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INTRODUCTION:

India is a country of second largest population in the world where agriculture is the principal source of livelihood for more than 58% peoples where food supply to everyone in ample amount is the major challenge. In India 68.8% people are living in the rural areas. A rural area can be defined as a remote area for from urban one are deprived with basic amenities. The people who are living, are not able to get all medical facilities there resulting their dependency in the traditional medicines obtained from plants. There are several medicinal crops which are cultivated in the rural areas and villagers are using them as a food and medicines both. Several plants have been reported to be medicinal Fenugreek (*Trigonella foenum graecum* L.) is one of them. Fenugreek is a flowering annual plant, with bisexual flowers. This crop is native of Iran to northern India and is extensively cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. [1]. It is one of most cultivated spice crop in India. Through the globe it is greatly cultivated in Tunisia [2] and normally used as the condiments as well as folk medicines for severe diseases such as diabetes [3]. The seed is produced as a spice, as a vegetable for humans, as rummage for cattle, and for medicinal purposes. Fenugreek is incorporated into short-term rotation for hay and silage (livestock feed), and for soil fertility (fixation of nitrogen) [4]. According to the ancient literature fenugreek was said to be good for everything. But the production of fenugreek is hassled, due to the escalating global population the arable land quickly getting reduced and inadequate energy resources provoked the problem; synthetic fertilizers are one of the responsible factors for the present situation [5]. This situation can be avoided by using Biofertilizers such as rhizobacteria, whose habitat is rhizosphere. The rhizosphere is the volume of soil, surrounds plant roots, and Rhizoplane is the area of strongly adhered soil particles on plant root surfaces and most important as biological control[6]. Many rhizobacteria are reported which are responsible for soil fertility; *Bacillus subtilis* is one of them. *B. subtilis* is a Gram-positive bacterium.

Classification/taxonomical position:

Kingdom: Plantae
 Division: Phenarogames
 Class: Dicotyledons
 S. class: Polypetalae
 Order: Rosales
 Family: Fabaceae
 Genus: *Trigonella*
 Species: *foenum-graecum*



Fig1: Trigonella foenum graecum L.

MATERIAL AND METHODS:**Procurement of bacterium culture, plant seeds and inoculum preparation:**

The selected culture, *Bacillus subtilis* was procured from Microbial Type Culture Collection (MTCC-2274), Chandigarh, India and the seeds of *Trigonella foenum-graecum* L. were procured from Alopi Agro Company, Allahabad and bed was prepared using muslin cloth. Seeds were surface sterilized with 0.02% (w/v) HgCl₂ and washed three to five times with sterile distilled water to remove traces of HgCl₂. Inoculum was prepared according to CLSI recommendations [7].

Potassium solubilising activity:

This activity was observed in modified Aleksandrov medium plates by the spot test method [8]. The composition of aforesaid medium (in gms/ltr) with 5.0 glucose, 0.5 magnesium sulphate, 0.1 calcium carbonate, 0.006 ferric chloride, 2.0 calcium phosphate, 3.0 mica powder (potassium aluminium silicate) and 20.0 agar mixed in 1 ltr deionised water, were autoclaved at 121°C and 15 lbs pressure for 15 minutes.

Catalase activity:

The 24 hrs old cultures of *B. subtilis* grown on Luria Bertani medium, put as a drop on a glass slide which was followed by the addition of 3-5 drop of 0.3 % of Hydrogen Peroxide. The appearance of air bubbles was considered as a Catalase positive result.

Citrate reduction activity:

The microorganisms have the ability to utilize the citrate as a carbon and energy source for the growth and ammonium salts as the source of nitrogen. The Simmons citrate agar medium was prepared and the pH was set at 6.8. The plates were streaked with different bacterial cultures and incubated at 30±2°C for 48 hours. Blue colour and growth indicates positive result while original green colour and no growth indicated the negative result.

Experimental details:

All the experiments were performed in Biological Product Lab., Botany department, University of Allahabad. A stock solution of *B. subtilis* was prepared according to McFarland i.e. 10^7 CFU, and matched visually in Muller Hinton Broth (MHB) 60 ml. 10 ml of inocula were added in each pods. The present study was done in triplicates i.e., three pods of control for 10 days; three pods of treated for 10 days; three pods of control for 25 days; and three pods of treated for 25 days. The treatment was given for two periods i.e., for 25 days observation treatments of *B. subtilis* was given in three pods and after 15 days. Treatment for 10 days observation was given on the germination of seeds in three pods observation. Controls were also planted accordingly in the six pods. Each pod contains 5 seeds. The effect of *B. subtilis* was measured in the form root growth, shoot growth, total growth, number of leaves and leaf size.

RESULTS AND DISCUSSION:

Table 1: Morphological and biochemical characterisation of *B. subtilis*

S.No	Characteristics/Tests	Results
1.	Grams stain	Gram-positive
2.	Shape	Ellipsoidal and rod
3.	Potassium Solubilization test	+ve
4.	Catalase test	+ve
5.	Citrate activity	+ve

Citrate reduction activity:

The growth and change of colour was considered for citrate positive result. As a result based on the growth and colour change in Simon citrate agar medium (SCA), *B. subtilis* was found positive in citrate reduction (Table-1). Citrate utilization shows the ability of the microbes to convert citrate into oxaloacetate. Colour change of the medium is due to the utilization of available citrate in the medium and increase of the pH and also because of the secretion of some organic acids [9].

Catalase test:

B. subtilis was found to be Catalase positive (Table-1). It is usually observed that the bacterial strains in which the catalase activity is prevalent are highly resistant to environmental, mechanical and chemical stress. Catalase is one of the most important enzymes which break hydrogen peroxide into water and Oxygen. Peroxide is widely used as an antiseptic, and the bubbling seen is due to evolution of oxygen gas. The current findings also supports the observation obtained by Rashmin et al (2013) [9].

Potassium solubilizing activity:

Potassium (K) is the third abundantly available macronutrient in the environment, absorbed in the form of cation, playing a crucial role in the growth and development of the plants. More than 90% of potassium exists in silicate minerals and insoluble rocks[10] *B. subtilis* was found as a good source of phosphate solubilizer. The present study also supports the finding observed by Shukla et al (2017) [5].

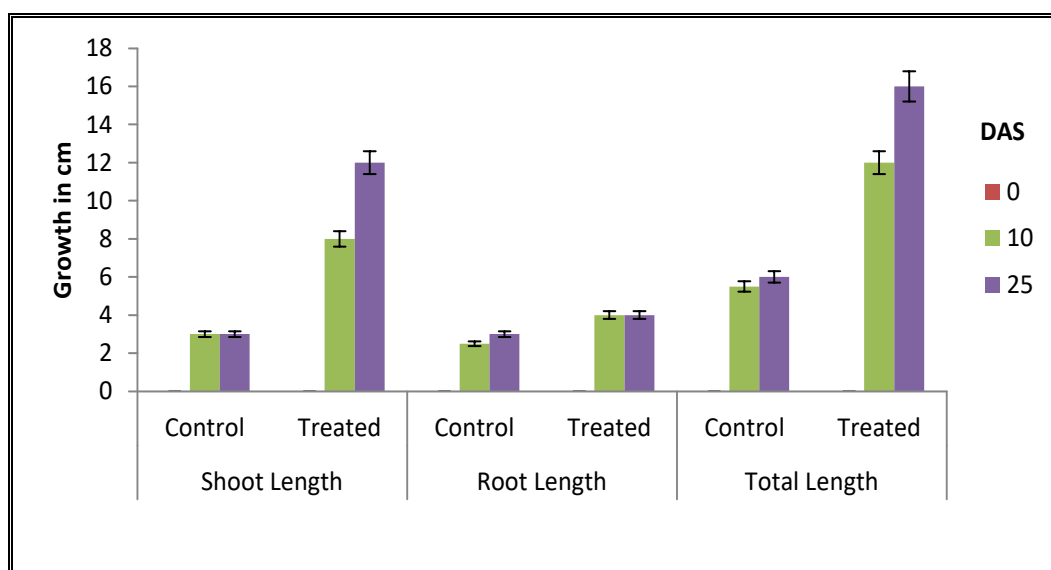
Effect of *Bacillus subtilis* on growth of *T. foenum-graecum* L. –

Fig.2. Graph representing the increased growth of treated plants when compared to control ones.

Graph (Fig. 2) drawn on the basis of observations of experiments set which clearly shows an increase in the growth of plants. After 10 days there was 166.67 % growth in shoot length; 60 % growth increase in root length; 118.19 % growth in the total length of plants. After 25 days 300 %, 33.33 %, and 166.67 % increase in the growth observed in shoot, root and total length respectively when compared with control. Along with the increase in the growth there is an increase in the number of leaves and leaf size. The results clearly demonstrates that *B. subtilis* is a plant growth promoting rhizobacteria (PGPR) and helps in the growth and differentiation of plants by providing phosphates to the plants. Growth promotion can be observed by morphological parameters like length of the root, shoot, pod size and weight [11,12] in response to higher plants, whereas population density of PGPR in broth was measured using turbidity by qualitative method, indicating to positive or negative responses. The present study also support the findings obtained by Shukla et al. in 2017 [5] which clearly says that these microbes enhances the growth and productivity of the crops via diverse mechanisms such by producing hormones, fixation of atmospheric nitrogen, potassium solubilization etc.

CONCLUSION:

Application of rhizospheric bacterium such as *B. subtilis* drastically improved vegetative and generative growth as well as nutrient uptake in fenugreek. Fenugreek is being widely used in spices with their highly medicinal values since thousands of years ago. *B. subtilis* improves root system and created deeper and more abundant roots and caused better nutrient absorption. The effect of *B. subtilis* was measured in pod tests which was filled with the autoclaved soil and performed in its triplicates. The observed result clearly indicates the positive growth on the taken parameters of fenugreek.

Conflict of interest-

The authors declare no conflict of interest.

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