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Influence on yield and quality of fennel (*Foeniculum vulgare* Mill.) grown under semi-arid saline soil, due to application of native phosphate solubilizing rhizobacterial isolates

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ABSTRACT

Phosphate solubilizing microorganisms play an important role in balancing plant nutrition through enhanced availability of phosphorus (P) to roots. These microbial strains convert the insoluble phosphates into soluble forms. Phosphate solubilizing bacteria (PSB) were isolated from the saline soil of fennel (*Foeniculum vulgare* Mill.) cultivation fields under semi- arid climate of Rajasthan, India. Nine native PSB isolates were applied to study their influence on fennel seed yield and essential oil content. Significant effect of different PSB isolates on seed yield of fennel crop was recorded. The highest seed yield (2148.66 kg ha-1) was recorded with *Bacillus subtilis* NRCSS-II (PSB-36) and the lowest seed yield (1744.35 kg ha^{-1}) was observed with *Bacillus* sp. PS-1 (PSB-20) which was at par with control. The highest essential oil content was recorded with *Bacillus* sp.Fen-17 (PSB-26) (2.80%) as compared to control (2.09%). Though application of all the PSB isolates resulted into increased essential oil yield as compared to control but *Bacillus subtilis* NRCSS-II (PSB-36) resulted into enhanced seed yield and essential oil both. Biochemical assay of the post harvest soil samples revealed that applied PSB isolates caused significant effect on various soil parameters as compared to control. The organic carbon, N, P, K, and EC increased to 0.59%, 78.25 kg ha^{-1}, 28.54 kg ha^{-1}, 178.3 kg ha^{-1} and 0.99 dS m^{-1} as compared to control. On the other hand soil reaction (pH) declined to 7.25 as compared to control.

1. Introduction

Fennel (*Foeniculum vulgare* Mill.), wild or cultivated belongs to the Apiaceae family, and is widely distributed throughout the world. It is known as *Saunf* in Hindi language in India. It is widespread annual/perennial aromatic plant used in folklore medicines. It is native to southern Europe and Mediterranean region. It is being used since long as a condiment, culinary spice as well as for medicinal purposes (Anonymous, 1988). Due to economic importance as a cash crop, fennel is cultivated over large areas in many districts of Gujarat, Rajasthan and other states of India. Total area under fennel cultivation is–55,000 ha producing 70,000 t fennel seed production during 2013–14 (Indian Horticulture Database, 2014). Researchers have identified fennel as a valuable medicinal plant and also as a raw materials for pharmaceutical industry, especially in the steroidal hormones. A significant increase in quantity and quality yields through the suitable management of crop cultivation could make an important contribution to farm income in the naturally resource poor saline soils of semi-arid region area of India.

Phosphorus (P) is one of the essential macronutrients for plant growth and cellular activities. Most of soil P is rendered unavailable and only about 1–2% of it is taken up and assimilated into plant parts. Similarly, only a small proportion (about 10–20%) of applied P fertilizers is used by plants, and the rest is rapidly converted into insoluble organic and inorganic complexes in soil. A major portion of P fertilizers applied to soils are converted to insoluble forms, thus increasing the actual P fertilizer requirement (Podile and Kishore, 2002). The frequent application of P fertilizers is not only costly but also depletes the non-renewable rock phosphate resources used in fertilizer manufacturing processes. In addition, many synthetic P fertilizers are acidic in reaction, which tend to increase the soil acidity. This results in the decrease of the diversity of beneficial organisms in soils as well as microbial population, which

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affects plant growth negatively. Therefore, the attempts to improve P fertilizer efficiency and utilization of the plant-unavailable P in soils have been a very important research topic since long time. Soil salinity resulting from natural processes or from irrigation with saline water is a common phenomenon in arid and semi-arid regions. Saline soils, inapt for cultivation of normal agricultural crops, can be cultivated for the commercial production of seed spices crops such as fennel. Fennel possesses adaptability to a wide range of habitats and can be grown in different environmental conditions on a variety of soils (Malhotra and Vashishtha, 2007). In saline and sodic soils of semi-arid and arid climate the plant available P is drastically reduced due to formation of insoluble complex with calcium, magnesium and other divalent metal ions present in these soils.

The use of microbial inoculants in agriculture has received more attention recently. Many soil microorganisms have the capacity to transform insoluble soil phosphate in available form (Rodríguez and Fraga, 1999). Inoculation of phosphate solubilizing bacteria (PSB) improved solubilization of sparingly soluble phosphate compounds in soils, resulting in a higher crop yield (Sundra et al., 2002). The PSB can solubilize inorganic phosphate through the processes of acidification, chelation, exchange reactions and by production of organic acids (Singh and Reddy, 2011). Many new PSB isolates have attracted the attention of agriculturists as soil inocula to improve plant growth and yield (Fasim et al., 2002; Chen et al., 2006; Sahay and Patra 2014). However, the field efficacies of these biofertilizers are sometime restricted mainly due to rhizospheric competence and survival which varies in different soil conditions. Efficient and native PSB isolates from the prevailing local cropping system may be employed for better and sustainable results of microbial inoculants. Little information is available on the isolation of PSB from fennel rhizosphere and its benefit to fennel plant growth in soils of arid and semi-arid region. The objective of this research work was to study the bioefficacy of native PSB isolates towards various yield attributes of fennel through availability of phosphorus to plants. The significant changes in the soil nutritive profile were also analyzed.

2. Materials and methods

2.1. Field experiment

Field experiments were conducted in the Experimental farm at National Research Centre on Seed Spices, Ajmer, Rajasthan in India for two consecutive years (2013–14 & 2014–15) at same site during winter season. The experimental site located between 26°45′ N latitudes and 74°64′ E longitude with average annual temperature of 24.7 °C and average annual rainfall 557 mm. The experiments was laid out in randomized block design (RBD) having 10 treatments, three replicates each and in all total 30 micro-plots of 12 m^2 area for each treatment and the fennel seeds were sown. The soil under study was sandy loam in texture classifed as Typic Ustorthents (USDA), EC 0.8 dSm^{-1}, pH 8.1 and organic carbon 0.23%. The major available nutrients like N, P_2O_5 and K_2O were quantified as 118.0, 18.50 and 135 kg ha^{-1}, respectively. Fennel seeds (variety Ajmer Fennel-1 developed from National Research Centre on Seed Spices, Ajmer) were separately treated with the PSB isolates from fennel rhizosphere samples of different locations in semi-arid region of Rajasthan state in India (Mishra et al., 2015) using seed coating technique. The bacterial culture suspension in nutrient broth media (Hi-media, India) was prepared in a BOD incubator cum shaker at 28 ± 1 °C with continuous shaking at 100 rpm for 72 h. The bacterial culture broth was mixed with pre-sterilized talc powder along with carboxy methyl cellulose (CMC). To perform the growth-promoting test, PSB isolates along with carrier material were evenly applied on moisten and surface disinfected seeds to get 1×10^8 CFU g^{-1} of PSB treated seed. For control purpose the fennel seeds were coated with nutrient broth mixed talc powder along with CMC without bacterial culture. Seed rate (10 kg/ha) was kept uniform for all treatments and after 15 days of seed germination, thinning was done to maintain spacing of 10 cm x 40 cm between the plants and the rows. The plants were allowed to grow and no chemical fertilizer or pesticides were applied to the soil during the course of the experiment. Weeding was done manually at regular intervals and plots were irrigated as per water requirement of the crop.

2.2. Observations on plant growth

Ten randomly selected plants were harvested after maturity. Shoot dry-weight was determined after drying the shoot tissue in an oven at 80 °C for 48 h. Oven-dried shoot tissue was ground and sieved through a 0.5 mm sieve. 0.2 g ground material was digested in a triple acid mixture (HNO_3, H_2SO_4 and 60% $HClO_4$ in a ratio of 10:1:4) for the analysis of phosphorus. The phosphorus in the digested sample was estimated by the molybdenum blue method (Allen, 1989). Plant and soil samples were also analyzed for residual fertility status using standard protocols for available nitrogen (Subbiah and Asija, 1956), extractable phosphorus (Olsen et al., 1954) and extractable potassium (Jackson, 1973). In addition, plant height, number of branches, number of primary umbels per plant, number of umbellets per umbel and seeds per umbel were also recorded. Soil samples from root zone were collected immediately after harvesting of the fennel and used for assay of dehydrogenase activity and microbial biomass carbon as described by Kaushik et al. (2004).

2.3. Essential oil estimation

The harvested fennel seeds from each treatment were crushed in electric grinder and ground mass was subjected to hydro-distillation using Clevenger's apparatus (Borosil, India). The oil fraction thus collected was used for estimation of total essential oil and expressed in per cent (v/w).

2.4. Identification of PSB isolates by 16S rDNA sequence analysis

Genomic DNA was extracted by the phenol/chloroform extraction method (Sambrook et al., 1989). The 16S rRNA gene was amplified using specific primers (Maatallah et al., 2002). The Polymerase chain reaction (PCR) was carried out in a 100 μl reaction mixture containing 1.5 mM MgCl, 0.2 mM each dNTP, 25 pmoles of forward and reverse primers, 50 ng DNA template and 5U Taq DNA polymerase with its reaction buffer. A 30cycle reaction was performed at 94 °C for 1 min, 62 °C for 30 s and 72 °C for 90 s followed by a final extension of 10 min at 72 °C. The reaction was carried out in a thermocycler. The resulting 1.5 kb DNA fragment was extracted and purified using a gel extraction purification kit. The purified product was sequenced (Sci Genom Labs Pvt. Ltd. Kochin, India). The isolates were identified by aligning the nucleotide sequence with the other nucleotide sequences submitted to NCBI. The gene sequences were also submitted to GenBank and accession numbers were assigned. The 16S rRNA gene-sequence based phylogenetic tree of the PSB isolates was constructed using neighbour-joining method.

2.5. Data analysis

All field experiments were performed in randomized block design with three replications in each treatment and the experiment was repeated twice (2013–14 and 2014–15). The data was analyzed for variance and mean values in each treatment by using SPSS package and the significance of the treatments was calculated