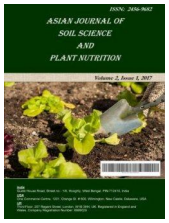

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Microbial Isolates for Enhancement of Seed Germination

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bacteria that colonise plant roots and promote plant growth are referred to as Plant Growth-Promoting Rhizobacteria (PGPR). Rhizobium bacterial isolated from soil samples (PEC 1 and PEC 2) collected from prathyusa college garden soil. The emergence of seedlings, from the seed at a height of 2 mm was treated as germination. PEC1 and PEC2 inoculated seed germination, coefficient and vigor index value remarkably changed compared to control groups. PEC2 showed better responses compared to PEC 1 and control groups.

Keywords: Plant Growth-Promoting Rhizobacteria (PGPR); germination; bhendi and vigor index.

1. INTRODUCTION

Green revolution has changed the poverty globally. The conventional agricultural practices are modernised and crop yield has been increasing. The synthetic fertiliser input in the form of macronutrients like nitrogen, phosphorus and potassium and many micronutrient supplements has been enhancing agricultural output but at the cost of human health. Nitrogen

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enrichment in the soil and its leaching into portable water source causes several pollution related problems. Though nitrogen is needed it should not be applied in discriminately. For the regulated provision of nitrogen to the plants, the natural nitrogen production sources in the plants can be achieved. The root nodules of plants harbor many microbes that are capable of fixing atmospheric nitrogen and convert it into nitrate for plant utilisation. This nitrogen fixing bacteria called rhizobium and studied by many people [1-5].

In order to enhance agricultural productivity plants need a good nitrogen supply. But due to many manmade activities soil nitrogen level got reduced an 24 billion tons of fertile soil from the world's crops land was lost [6]. In 2030 the demand from agriculture land may increase greatly due to population growth so the nitrogen source must compensate the nearly demand [7]. So, microbial source for the supply of nitrogen has to be revitalised. Rhizobacteria near the roots [Rhizospere], root surface (Rhizoplane), root tissue (Endophytic) and root nodules (Root attached) are to be enhanced to produce more nitrogen [8]. In this work, Rhizobacterial action on specific plants productivity was analysed. In the experimental trial the common vegetable, bhendi (*Abelmoschus esculentus*) was chosen. The rhizobium stains were isolated from local soil (Prtahyusha college garden soil). The objective of this study is to trace plant growth promoting action Rhizobacteria isolated from local soil.

2. MATERIALS AND METHODS

For the present study, the vegetable crop *Abelmoschus esculentus* was chosen.

Soil sampling: For the isolation of plant growth promoting rhizobacteria (PGPR) soils were collected from a garden in prathyusha engineering college where the plant was cultivated. The soil attached to the roots [Rhizosphere soil] was collected from 10 plants separately and transferred to ice box for transport to the laboratory. The moisture content in the sample was estimated after the removal of the root material or other plants remain. The storage of the sample was done at 4ºC.

For the isolation to PGPR 10g of moist soil was placed in 100 ml of sterile water shaken for 10 minutes. Then 10 ml of this suspension was transferred to 9 ml blank and serially diluted to 1% concentration. Different types of medium (TSA and NA) and basal medium amended with glucose, manitol, sorbital, inosital and sucrose. The plates were incubated at 37ºC for 2-3 days. The individual colonies were selected for estimating the population of Rhizobia. This was expressed as number of CFU (Colony Forming Units)/ gram soil.

The individual bacterial colonies were isolated and subcultured on nutrient agar. A total of 100 isolates thus obtained were cryopreserved. The isolates were analysed for morphological characters, gram staining, motility. Biochemical test were done the test indole, methyl red, voges-proskaver, citrate, oxidase presence and other tests.

Plant study: The study was carried out October 2017 to November 2017. The seeds of these plants were purchased from the certified seed suppliers from Tiruvallur.

Seed germination study: The PGPR strains isolated were grown in yeast manitol broth and nutrient broth. Healthy seeds of bhendi were recruited and surface sterilised using 100% ethanol in an Erlenmayer flask and the seeds were treated with 1% N hypochlorite for I minute followed by 5 times wash in sterile water. The seeds were then soaked in the two rhizobial broth seeds in normal broth were control. The 25 seeds treated with PGPR inoculums and 25 control seeds placed in petridishes. In the petri dishes were laid over What man filter paper and incubated at 30ºC for 120 hrs. Triplicates were maintained for each experiment. The percentage of seed germination in the control and inoculums treated were calculated.

Percentage of germination = (No. of germinated seeds/ 25) x 100

The speed of germination was counted using a coefficient of germination and vigour index values. The coefficient of germination was estimated by counting the emergence of seed germination depends on days. The Vigor index value of seed germination was calculated as per Copeland formula [9].

Coefficient of emergence % = [100 (A1+ A2+ A3+ A4+ A5) / A1T1+ A2T2+ A3T3+ A4T4+ A5T5].

A= no. of. seeds germinated on different days.
T= times corresponding to days.

Vigor index = (A1/T1)+(A2/T2)+(A3/T3)….. (An/Tn)

Where, A = Number of seeds germinated T = Time (days) corresponding to A and n = No. of days to final count.

Plant growth promotion study: Like the testing for seed germination efficiencies, the meristamatic growth of the plant was followed after administrating the PGPR – PEC1 and PGPR-PEC-2. Observations were noted for the growth in root length (cm), shoot length (cm) and total seedling length for 7 days. From the observed results the PEC1 and PEC2 treated plants and control plant in the petri dish was given the inoculums treatment every day at 6 am and contributed till 7 days.

3. RESULTS

The emergence of seedlings, from the seed at a height of 2 mm was treated as germination. This was carefully recorded for 120 hours (Table 1). In the present study 2 types of plant growth promoter rhizobium (PGPR) strains were isolated and identified by various biochemical test (Table 2). The isolated and plant growth property confirmed strains were used as inoculums PEC1 and PEC2. The coefficient of bhendi seed emergence and vigour index treated with PGPR inoculums recorded in Figs. 1 and 2 respectively. The speed of germination was counted using coefficient of germination in treated seed were remarkably changed compared to control. Among the treatment groups PEC2 treated groups was better compared to PEC1 and control groups (Fig. 1). The high vigour index value showed PGPR treated seeds compared to control groups. PEC2 was better than control and PEC1 treated groups in vigour index value also. From this PGPR enhance the seed germination coefficient and vigour index.

4. DISCUSSION

In current scenario shifting of microbial land influence, the population of microbes was severely affected [10-12]. These kinds of problems were solved by green manure and biological methods [13]. The present study also indicates that the germination efficiency of the bhendi seeds is influenced by the rhizobium isolated from the Prathyusha college garden soil.

Table 1. Percentage germination of bhendi seedlings treated with PEC1 & PEC2 of PGPR inoculums

Microbial isolates	Germination (%)	Germination (%) at different time intervals				
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Control	82 ± 1	20 ± 1	42 ± 2	56 ± 3	68 ± 4	76 ± 2
PEC1	89 ± 3	20 ± 2	44 ± 3	68 ± 2	81 ± 3	92 ± 4
PEC2	85 ± 1	20 ± 1	43 ± 2	64 ± 1	74 ± 2	88 ± 2

Table 2. Biochemical screening of microbial isolated for PGPR inoculums

PGPR inoculums	Biochemical screening test				
	Phosphate solubilization	Indole production	Siderophore production	Ammonia production	Catalase activity
PEC1	+	+	+	+	+
PEC2	+	-	+	-	+

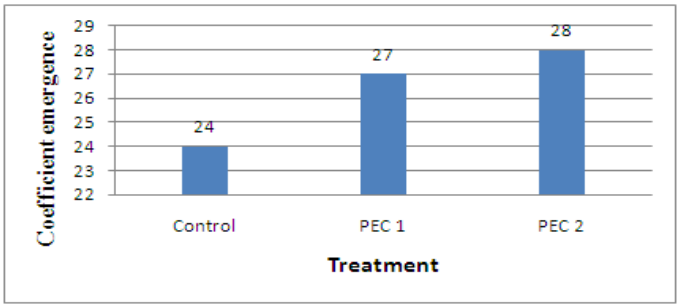

Fig. 1. Coefficient of emergence of bhendi seeds on treatment with PGPR

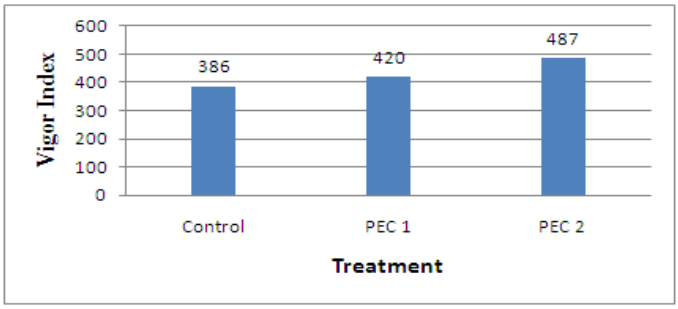

Fig. 2. Vigor index of bhendi plants on treatment with PGPR

In total effective two isolated sample PEC 1 and PEC 2 showed higher influence in germination efficiency, germination co efficiency and vigour index. Rotainal cultivation and proper usage of biological based fertilisers only helpful to maintain the fertility of soil [14]. The differential germination may be due to the activation of gibberellins secretion as it is reported earlier by various people [15-16]. From this study confirm the isolates of rhizobium from soil to be used as plant enhancer.

5. CONCLUSION

Beneficial microbial growths in the agricultural soil retain fertility of the soil and save sustainability of living organisms. The present study indicates that the germination efficiency of the bhendi seeds was influenced by the rhizobium isolated from the Prathyusha college garden soil. In total effective two isolated sample PEC 1 and PEC 2 showed higher influence in germination efficiency, germination co efficiency and vigour index. From the result microbes and plant interaction helps the plant adaptability of tolerance of extreme conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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