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Supplementation of P with rhizobial inoculants to improve growth of Peanut plants

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Abstract
Biological nitrogen fixation represents the major source of N input in agricultural soils and rhizobia chemically convert the nitrogen from the air to make it available for the plant.The N fixation process is influenced by many factors and P is one of them. Due to the important role played by P in the physiological processes of plants, application of P to soil deficient in this nutrient leads to increase plant growth and yield. This research was carried out to investigate the effects of biological and chemical fertilizers on some physiological characters of peanut plants. Five different rhizobial inoculants (approximately 1.9 x 10^8 cfu) alone and in combination with DAP (as phosphate fertilizer @ 60kg/ha) were used as treatments. After 30 days of germination all the treatments showed significant effects on physical parameters of peanut plants, more significant results observed when plants treated with rhizobial inoculations with combination of DAP. Due to the imperative role played by P in the physiological processes of plants, application of P with rhizobial inoculants are beneficial for leguminous plants growth.

Keywords: Integrated nutrient management, Microbial inoculants, Phosphate fertilizers, Leguminous plants,

Introduction
Stumpy crop yield is a general problem facing most agricultural systems. These small yields are prominent in grain legumes and are often connected with declining soil fertility and reduced N2-fixation due to biological and environmental factors. Biological nitrogen fixation (BNF), a key resource of N for farmers using little fertilizer, represents one of the possible solutions and participate a key role in sustainable grain legumes production (Mfilinge *et al.* 2014) [1]. Soil fertility can be re-established efficiently through adopting the conception of integrated soil fertility management (ISFM) encompassing a policy for nutrient management-based on natural reserve protection, biological nitrogen fixation (BNF) and better efficiency of the input (Mohammadi and Sohrabi, 2012) [2]. Biofertilizers are vital components of integrated nutrients management. They are cost effective, ecofriendly and renewable supply of plant nutrients to supplement chemical fertilizers in sustainable farming system. Beneficial microorganisms in biofertilizers hasten and get better plant development and defend plants from pests and diseases (Mohammadi and Sohrabi, 2012) [2]. Because of avoidance of environmental problems, human health, and additional crop production to meet the rising requirement of world population, integrated nutrient management by the mixture of chemical and biofertilizers may be a helpful method as mentioned by Shiri Janagard (Shiri Janagard et al., 2013).
Generally, *Rhizobium* inoculation in legumes is recognized for stimulating growth and is an substitute to the costly inorganic nitrogen fertilizers (Tairo and Ndakidemi, 2013) [4]. PGPB can contribute to plant growth in different manners: by rising nitrogen uptake, production of phytohormones (auxin, cytokinin), minerals solubilization and iron chelation (Robin et al., 2008; Adesemoyea *et al.* 2010). The biocontrol effect of rhizobia is due to the oozing of secondary metabolites such as antibiotics and HCN (Deshwal *et al.* 2013) [7]. Rhizobial actions and N fixation is down without appropriate application of P. It promotes early root development and the formation of lateral, fibrous and healthy roots, which is extremely

significant for nodule formation and to fix atmospheric N. It was reported that application of P along with *Rhizobium* inoculant influenced nodulation and N fixation of legume crops (Bhuiyan *et al.* 2008) [8].

Phosphorus is considered as a limiting issue in plant nutrition due to the deficit of available soluble phosphate in the soil (Uma and Sathiyavani, 2012) [9]. P as well increased the efficiency of plants to photosynthesis, improves the actions of rhizobia and raise the number of branches and pod /plants, therefore produces a better total yield of pea**,** lupine, faba bean and peanut (Nadia, 2012) [10].It has been reported that legumes inoculated with rhizobium species and supplemented with P, react differently in the growth, yield and nitrogen fixation (Mmbaga *et al.* 2014) [11]. Groundnut (*Arachis hypogea* L.) is one of the principal economic crops of the world that ranks 13th among the food crops (Kabir *et al.* 2013) [12]. Groundnut (*Arachis hypogaea*) belongs to the family Leguminosae. Groundnut seeds have 40 to 50 % oil and 20 to 30 % protein, and they are an outstanding resource of vitamins B (Sajid, 2010) [13]. The bio and organic sources, like biofertilizers and organic manures as supplement to plant nutrients are gaining worldwide value in groundnut farming (Mathivanan, 2014) [14]. The present study was aimed to evaluating the effect of rhizobial inoculations alone and in combination with phosphorus fertilizer on growth of groundnut plants.

2. Material and methods:

2.1 Collection of root samples:

The samples for root nodules were collected from localities of Jinnah University For Women, Nuclear Institute of Agriculture Tando Jam, University of Karachi and Memon Ghoth Malir. Plants were dug out carefully (table 1).

Table: 1 Code of rhizobial treatments, their host plants and location of collection.

S.no	Code	Host plant	Locality
1	JUWR1	Chick pea	Nuclear Institute of Agriculture, Tando Jam
2	JUWR3	Mung	Jinnah University For Women
3	JUWR4	White Lobia	MamongothMalir, Karachi
4	JUWR5	Masoor	Nuclear Institute of Agriculture, Tando Jam
5	JUWR6	Methi	University of karachi,Karachi

2.2 Isolation of Rhizobia from nodule:

Rhizobium species were isolated by crushed nodule methods (Aneja, 1993) [15]. The rhizobial isolates were subculture, purified and maintained on YEMA slants and stored at 4 to8°C for further use in the present study.

2.3 Experimental plant:

<u>*Arachis hypogea* L</u>. (peanut plants)

2.4 Phosphorus fertilizer:

DAP @ 60kg/ha

2.5 Treatments used in Experimental setup:

Table: 2 List of treatments

1	Control	7	Control + P
2	JUWR1	8	JUWR1 + P
3	JUWR2	9	JUWR2 + P
4	JUWR3	10	JUWR3 + P
5	JUWR4	11	JUWR4 + P
6	JUWR5	12	JUWR5 + P

2.6 Experimental setup:

The complete randomized block designed pot trial was conducted in net house of Department of Botany, Jinnah University for Women, Nazimabad Karachi, Pakistan to check the effects of rhizobial *inoculants* alone and in combination with phosphorus fertilizer on the growth of Arachis hypogea L. Seeds of Arachis hypogea L. were sown in pots filled with 2 kg soil each. After 5 days of germination, developing seedlings in each pot were inoculated with different treatments (table 2). Twenty five milliliters of suspension of each treatment (approximately 1.9 x 10^8 cfu of *Rhizobium spp.*) were used. Three replicates were used for each treatment. Three plants of each treatment (1plant/replicate/treatment) were uprooted at 30^{th} day of growth to determine the selected physical (number of nodules, root & shoot length, fresh& dry plants weight) parameters.

2.7 Statistical analysis:

The analysis of variance procedure treatments was followed to determine difference in means among treatments by SPSS 16 (version 4). All treatment means were compared using the Least Significant Difference (LSD) at 5 % level of significance.

3. Results:

The application of rhizobial inoculants with and without phosphorus had no significant effect on number of nodules after 30 days of germination.JUWR1 and JUWR3alone increased maximum number of nodules (Fig.1). JUWR3 with phosphorus application increase maximum number of nodules (Fig.1).

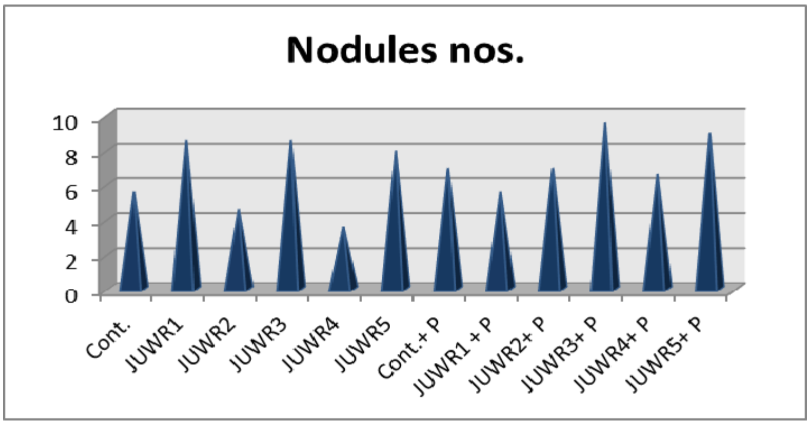

Fig: 1 Effect of treatments on number of nodules of experimental Plants after 30 days of germination. Columns bearing superscript are statistically significant (p<0.05 LSD) with respective control.

The application of rhizobial inoculants alone increase length of root but JUWR2, JUWR3 and JUWR5 significantly promote the root length (Table 3). However, phosphorus application with rhizobial inoculants significantly ($p < 0.05$) affected the length of root (Table 3) upto 25-115% over the control. The results presented in Table 3 show that inoculation with JUWR3 and JUWR5 had significant effect on shoot length in absence of phosphorus. On the other hand, there was noteworthy result on shoot length of experimental plants when phosphorus was applied with rhizobial inoculants with compare to control.

Table: 3 Effect of rhizobial treatments alone and in combination with phosphorus fertilizer on shoot and root length after 30 days of germination.

S.No.	Treatments	Shoot length (cm)	Root length (cm)
1	Control	28.60 ± 1.93	16.93 ± .23
2	JUWR1	34.58 ± 3.97	20.50 ± .80
3	JUWR2	33.72 ± 1.14	21.12 ± 1.42[d]
4	JUWR3	35.79 ± 3.22[d]	21.36 ± 1.01[d]
5	JUWR4	22.48 ± 1.21	18.95 ± 1.81
6	JUWR5	17.67 ± 4.32	29.92 ± 2.39
7	Control + P	30.56 ± 1.74	18.61 ± .83
8	JUWR1 + P	35.78 ± 1.48[d]	21.52 ± 3.49[d]
9	JUWR2 + P	44.45 ± 8.16[a]	21.88 ± 1.26[d]
10	JUWR3 + P	36.32 ± 2.45[d]	21.57 ± 1.21[d]
11	JUWR4 + P	28.92 ± 6.39	36.55 ± 4.90[a]
12	JUWR5 + P	36.05 ± .72[d]	35.94 ± 4.48[a]

Each value is a mean ± S.D (standard deviation) of 3 replicates. Means bearing superscripts in each column are significantly different with respective control at $P < 0.05$.

Roots fresh and dry weight not significantly affected by rhizobial inoculation with out phosphorus application, only JUWR3 significantly promote dry mass of roots. JUWR2, JUWR3 and JUWR5 with phosphorus application significantly improve fresh and dry weight of roots (Fig 2).

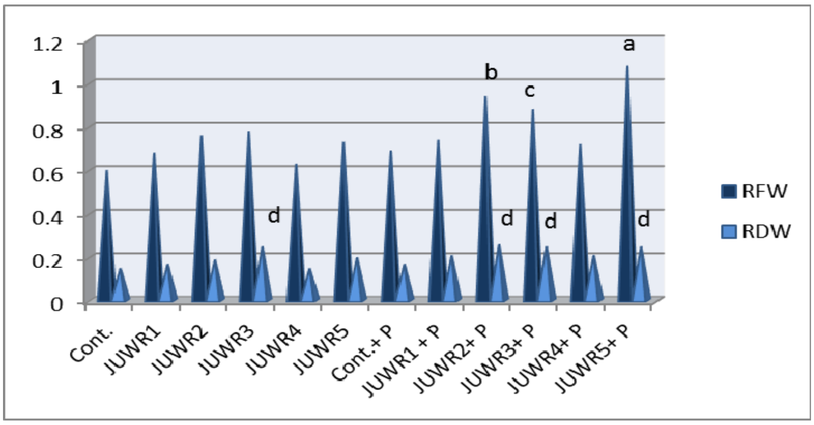

Fig: 2 Effect of treatments on root fresh and dry weights of experimental plants after 30 days of germination. Columns bearing superscript are
statistically significant (p<0.05 LSD) with respective control. [RFW=Root fresh weight (gm), RDW=Root dry weight (gm)].

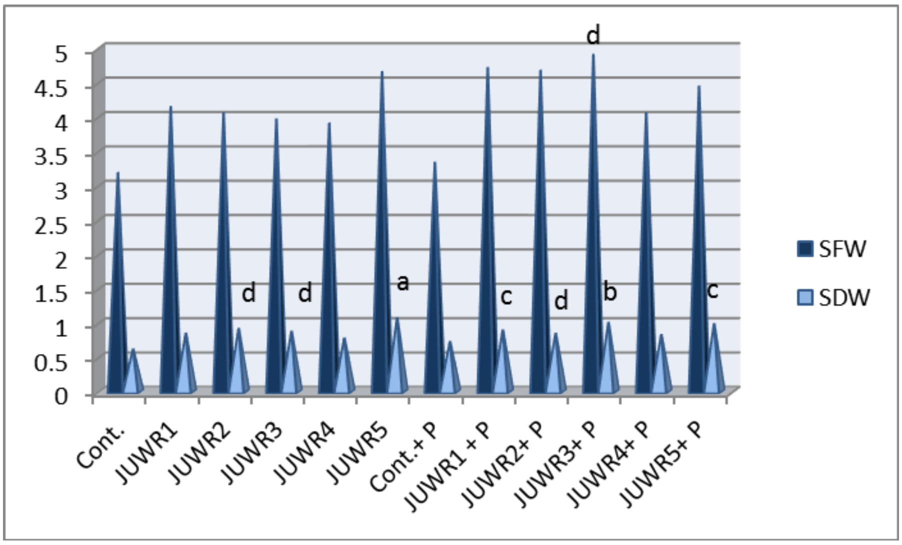

Fig: 3 Effect of treatments on root fresh and dry weights of experimental plants after 30 days of germination. Columns bearing superscript are statistically significant ($p<0.05$ LSD) with respective control. [SFW=Shoot fresh weight (gm), SDW=Shoot dry weight (gm)].

Shoot fresh weight of plants was not significantly increased by rhizobial inoculants alone and with combination of phosphorus, only JUWR3 with phosphorus significantly improve the shoot fresh weight. All treatments of rhizobial inoculants alone and in combination with phosphorus significantly increased shoot dry weight except JUWR4 in both forms (Fig 3). P application significantly improved plant height and total dry weight.

4. Discussions

Rhizobial activities and N2 fixation without suitable fertilization by phosphorus (P) is depressed as it promotes early root development and the formation of lateral, fibrous and healthy roots. Leguminous crops gather their N requirement through BNF. The requirements of host plants for best growth and symbiotic dinitrogen fixation processes for P have been evaluated by determination of nodule growth and working (Chaudhary, 2008) [16]. The probable uses of biofertilizers in farming participate vital role to increase yield (Alsamowal, 2013) [17].

Application of bacterial inoculum leads to boost the number of root nodules that raise the capability of nitrogen fixation. Nodulation, N2 fixation, and specific nodule activity are directly associated to the P contribution (Abdulameer, 2011) [18]. Phosphorus is basically required for healthy growth with well-organized root system and abundant nodulation which in turn can influence the N2-fixation potential (Mohamed and Abdalla, 2013) [19].

Phosphorus application and rhizobium inoculation in combination tended to enhance root development i.e. root length. (Ahmad *et al.* 2008) [20]. reported an enhance in root length and nodules number per plant with the use of P-fertilizer and rhizobium inoculation. Comparable outcomes were also reported by Bhuiyan et al. (2008) [23]. Rhizobia inoculation in cowpea notably enhanced the plant height measured at four, six and eight weeks after planting (WAP) in both screen house and field experiments comparative to the control treatment (Nyoki and Ndakidemi, 2014) [21]. The results of present study are in line with Mohamed & Abdalla, (2013) [19]. and Singh *et al.* (2011) [22]. Who reported that seed inoculation with Rhizobium and phosphorus solubilizing microorganisms enhanced growth and yield of groundnut. Comparable results have been reported by Rahman *et al.* (2008) and Kabir, *et al.* (2013) [12].

Microbial inoculants have confirmed their importance as biological alternatives to compensate agro-chemicals and to maintain environment friendly crop production (Roychowdhury *et al.* 2013) [24]. A variety of mechanisms involved in plant growth promotion due to these inoculants are N2-fixation, hormone regulation, improvement in nutrient uptake and enzymatic reactions in plants, phosphate Solubilization and stress resistance. The necessity of P in nodulating legumes is elevated compared to non-nodulating crops as it plays a important role in nodule formation and fixation of atmospheric nitrogen (Weisany, 2013). Due to the significant role played by P in the physiological processes of plants, application of P to soil poor in this nutrient leads to enhance groundnut yield (Kabir *et al.* 2013) [12]. Current studies confirmed that, a number of bacterial species generally connected with the plant rhizosphere, are found to be useful for plant growth, yield and crop value (El-Sayed et al., 2014) [26].

5. Conclusion:

The use of plant growth-promoting bacteria with phosphorus fertilizers boost the soil fertility and get better growth of agronomical important crops. P nourishment improved host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation and function.

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