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Research Article

Evaluation of *Rhizobium* Inoculation in Combination with Phosphorus and Nitrogen Fertilization on Groundnut Growth and Yield

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Abstract

Background and Objective: Low soil fertility status, nutritional imbalance and inappropriate agronomic practices are the major problems limiting the current production and yield of groundnut, therefore, a study was conducted during 2015/2016 at the greenhouse of the Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia, to evaluate the effect of *Rhizobium* inoculation, in combination with Phosphorus (P) and Nitrogen (N) fertilization on the growth and yield of groundnut. **Methodology:** The experiment included plots with and without *Rhizobium* inoculated treatments, two rates of Phosphorus (P) fertilizer (0.82 kg ha^{-1}) and two rates of Nitrogen (N) fertilizer (0.27 kg ha^{-1}). The treatments were laid out in a split-split plot design with two main plots (plots with and without *Rhizobium*), two subplots (for two nitrogen levels), two sub-sub-plots (for two phosphorus levels) and the experiment replicated thrice. **Results:** The highest growth and yield parameters such as maximum plant height (75 cm), number of branches (21.3 per plant), number of nodules (45 per plant), number of pods (18.3 per plant), pod yield ($2661.7 \text{ kg ha}^{-1}$), 100 seed weight (39.2 g), shelling percentage (43.1%), oil content (54.3%), protein content (25.6%), seed moisture (7.9%) and seed germination (86%) were observed in artificially inoculated *Rhizobium* treatment plots where the recommended dosage of inorganic P and N fertilizers were applied. **Conclusion:** Based on the results of the study it can be tentatively concluded that groundnut seed inoculated with *Rhizobium* and subsequently fertilized with P_2O_5 and N at rates of 82 and 27 kg ha^{-1} , respectively during the growth stage showed improved growth and yield parameters.

Key words: Inorganic fertilizers, growth, yield, synthetic *Rhizobium*, *Arachis hypogaea*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Groundnut (*Arachis hypogaea*) belongs to the family Leguminosae. It has its origin in South America, possibly Brazil and has been grown for ages by Native Americans. Groundnut seeds have 40-50% oil and 20-30% protein. Furthermore, they are a great source of vitamin B¹. In leguminous plants such as soybean and groundnut, nitrogen-fixing bacteria in the root zones/nodules supply a reasonable quantity of nitrogen to the soil, which is used by the current crop as well by the subsequent crops.

Phosphorus is basically necessary for healthy growth with the effectual root system and profuse nodulation which can then impact the N₂-fixation potential². Nodulated legumes need greater levels of P for optimal symbiotic performance. Furthermore, in legumes, there exists a close relationship between the P levels and the symbiotic mechanism³. In other words, N fixation, nodulation and specific nodule activity are directly associated with the P level in the soil^{4,5}.

Often, nitrogen is the most important element in the growth and development of plants⁶. The usage of nitrogenous fertilizers is linked with extensive pollution issues. Therefore, the interaction between legumes and *Rhizobium* sp., for Biological Nitrogen Fixation (BNF) is the most preferred alternative as it utilizes photosynthetic energy and atmospheric N plus it is ecologically cleaner⁷.

Farmers have taken to inoculating their crops with the bacteria (*Rhizobium*) infused soil. The use of this soil can turn out to be a successful strategy to improve symbiotic N fixation by legumes. This, in turn, improves crop yield². The practice of inoculation with *Rhizobium* in conjunction with the application of recommended fertilizers is said to increase crop yield by 40% on the average⁸. Many farmers currently inoculate groundnut seeds with *Rhizobium* bacteria as it has been proven to produce remarkably higher yields⁹.

When groundnut yields are low, the problem is often associated with low nodulation and to competition from indigenous, undesirable strains¹⁰. Inoculation of groundnut with *Rhizobium* is deemed a useful practice; as the native *Rhizobium* strains are unable to produce sufficient N efficiently to meet the overall nitrogen requirement of the groundnut plant¹¹.

It has been shown that plants inoculated with *Rhizobium* had increased levels of phosphorus, dry matter and nitrogen content^{12,13}. In the study, it was observed that inoculated legumes exhibited improved nodulation and nitrogen fixation.

One of the most serious threats particularly to legumes is acid-rich soils. The most detrimental impact of acidic soils is

low pH, owing to low calcium content in the soil. Soil acidity severely impacts the continuance of *Rhizobium* strains and hence nodulation and nitrogen fixation in legumes in temperate and tropical soils.

Stumpy crop yield is a generic issue facing the majority of agricultural systems. Stumpy crops produce a low yield of the agricultural matter. Such low yields are noticeable in grain legumes. The yields are frequently associated with reduced soil fertility and decreased N₂-fixation which can be attributed primarily to ecological and biological aspects. This reduction in fertility has created a need for technical alternatives geared towards improving soil fertility and enhancing agricultural yield. This can be accomplished through the usage of inexpensive and locally available agricultural resources such as *Rhizobium* and other soil enhancers. The current study was carried out to assess the impact of *Rhizobium* inoculation in combination with phosphorus and nitrogen fertilization. The combination of these inputs has demonstrated improvement in growth and productivity of groundnut plants.

MATERIALS AND METHODS

Study site: The present research was conducted during 2015/2016 at the greenhouse of UKM, Bangi, Malaysia (2°55'13.1" N 101°47'01.4" E). The experiment was set up to study the effects of *Rhizobium* inoculation, in combination with P and N fertilization on the growth and yield of groundnut. The experimental soil (0-30 cm depth) was analyzed according to the method described by Jackson¹⁴. The physicochemical properties of the soil are shown in Table 1.

Experimental design, treatments and measurements: This study was conducted under greenhouse, conditions which included planting in an area of 2.5×2.5 m for each plot. Polyethylene pots were used, each pot containing 5 kg of soil. Four groundnut seeds were sown in each pot and thinned to two plants per pot, 1 week after seeding. The pots were

Table 1: Initial physicochemical properties of the experimental soil

Parameters	Concentration
pH	4.28
CEC meq/100 g soil	7.237
Soil moisture (%)	11.581
Organic matter (%)	6.638
Ca ²⁺ (µg g ⁻¹)	465.17
Mg ²⁺ (µg g ⁻¹)	381.165
K ⁺ (µg g ⁻¹)	838.22
Phosphorus (µg g ⁻¹)	43.919
Silt (%)	6.47
Clay (%)	31.42
Sand (%)	62.11
Nitrate (µg g ⁻¹)	17.5
Texture	Sandy clay

frequently irrigated to maintain moisture content at field capacity, during the crop growth period.

The experiment was conducted using a split-split plot design with two main plots (namely with and without *Rhizobium*), two subplots (for two nitrogen levels) and another two sub-sub-plots (for two phosphorus levels). The experiment was replicated three times.

The details of the treatments:

Treatments

- **Main plots:** *Rhizobium*:
 - R⁺: *Rhizobium*
 - R⁻: Without *Rhizobium*
- **Sub-plots:** Nitrogen levels:
 - N₀: No nitrogen application
 - N₁: 100% of nitrogen requirement (45 kg ha⁻¹)
- **Sub sub-plots:** Phosphorus levels:
 - P₀: No phosphorus application
 - P₁: 100% recommended dose of phosphorus (P₂O₅ 82 kg ha⁻¹)

After preparation of the pots, half of the groundnut seeds was inoculated with *Rhizobium* inoculum prior to sowing and the other half was left uninoculated as control, phosphorus and nitrogen fertilizers were applied at sowing stage.

The plant samples were collected at harvest stage (115 days after sowing) to record data on crop growth and yield parameters. Measurement of growth parameters included plant height, number of branches per plant, number of nodules per plant and total dry matter production. The yield parameters included the number of unfilled pods per plant, number of pods per plant, pod yield (kg ha⁻¹), seed weight and shelling percentage, whereas seed quality parameters included oil protein content, seed moisture and percentage germination.

Data analysis: The statistical analysis of data on growth and yield were done using the analysis of variance (ANOVA). Significant differences among treatment effects were tested using the F-test. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of treatments on the growth of groundnut: The statistical analysis pointed out the significant impact of the distinct treatments on the height of the plant, the number of branches, number of nodules and dry weight (Table 2). The plants that were inoculated with *Rhizobium* in combination

Table 2: Effect of different treatments on groundnut growth

Main plots	Plant height (cm)	No. of branches	No. of nodules	Dry weight (g per plant)
R⁺				
N ₁ P ₁	75	21.3	45	36.6
N ₁ P ₀	70	17.6	38	30.1
N ₀ P ₁	66	13.1	33	28.3
N ₀ P ₀	62	12.0	29	24.2
R⁻				
N ₁ P ₁	68	13.0	37	29.5
N ₁ P ₀	62	7.0	21	23.1
N ₀ P ₁	63	11.0	31	24.3
N ₀ P ₀	60	8.0	20	20.7
LSD value	6.52*	4.36*	9.21*	5.44*

*Significant by the F-test at 5%

with P and N had the maximum height (75 cm), while those that were not inoculated with *Rhizobium* and not fertilized with P and N (control) had lower plant height (60 cm). Similarly, the plants that were inoculated with *Rhizobium* and fertilized with P and N had more branches (21.3) in comparison to the plants that were not inoculated fertilized. The maximum number of nodules per plant (45) was produced in the plants that were treated with R⁺P₁N₁. The groundnut plants that were artificially inoculated *Rhizobium* and fertilized with P and N at the recommend dosage produced the maximum dry weight of 36.6 g per plant, while the control plants had the minimum dry weight of 20.7 g per plant.

Thus, it is apparent that inoculation with *Rhizobium* increases the number of root nodules that boost the efficiency of nitrogen fixation. The ability to nodulate, fix N₂ and carry out any other specific activity is directly related to the P contribution¹⁵. Similarly, root development or root length was reported to be strengthened by the application of phosphorus and the introduction of *Rhizobium*¹⁶. Milev⁴ studied the elaborate nodulation pattern in pea with raised P levels, while Amba *et al.*² documented the elaborate nodulation pattern that occurred in selected legume grain species with the application of P.

Effect of different treatments on groundnut yield: The treatment with *Rhizobium*, P and N had a major impact on the quantity, yield and shelling percentage of the pods as well as the hundred seed weight (Table 3). The plants that were inoculated yielded the highest number of pods per plant (79.8) while the ones that were not inoculated yielded the minimum number of pods (56.40) per plant. Similarly, the inoculated plants were the ones that produced higher 100 seed weight (39.2 g), compared to the plants that were not inoculated which had a much lower yield (15.8 g). The highest pod yield (2661.7 kg ha⁻¹) was produced by plants that were inoculated, while the lowest pod yield

Table 3: Effect of different treatments on groundnut yield

Main plots	No. of filled	No. of unfilled	No. of pods per plant	Pod yield (kg ha ⁻¹)	100 seed weight (g)	Shelling (%)
R+						
N ₁ P ₁	16	2	18.3	2661.7	39.2	43.1
N ₁ P ₀	11	3	14	2490.3	35.3	59.4
N ₀ P ₁	10	1	11	2401.8	31.5	55.1
N ₀ P ₀	6	3	10	2368.1	29.1	63.7
R-						
N ₁ P ₁	11	3	14	2480.2	24.7	68.0
N ₁ P ₀	7	1	7	2109.2	19.3	82.0
N ₀ P ₁	8	1	10	2379.6	20.9	76.0
N ₀ P ₀	5	2	8	2005.3	15.8	87.0
LSD value	3.89*	1.75*	4.19*	139.6*	7.31*	11.92*

*Significant by the F-test at 5%

Table 4: Effect of different treatments on seed quality of groundnut

Main plots	Oil content (%)	Protein content (%)	Seed moisture (%)	Germination (%)
R+				
N ₁ P ₁	54.3	25.6	7.9	86
N ₁ P ₀	49.1	23.4	6.8	77
N ₀ P ₁	47.2	22.5	6.7	72
N ₀ P ₀	45.3	19.1	5.2	62
R-				
N ₁ P ₁	49.4	21.9	6.8	71
N ₁ P ₀	45.2	16.6	6.1	68
N ₀ P ₁	47.7	18.5	5.8	66
N ₀ P ₀	43.1	15.8	5.7	59
LSD value	6.74*	6.08*	1.29*	7.53*

*Significant by the F-test at 5%

(2005.3 kg ha⁻¹) was produced by the plants that were not inoculated. The combined usage of commercial *Rhizobium* inoculants with inorganic P and N also gave the highest percentage shelling (43.1%).

The above results are in consonance with those of Singh *et al.*⁵, where seed immunization with phosphorous solubilizing microorganisms combined with *Rhizobium* tended to boost the yield and growth of groundnut. Furthermore, higher yields can be obtained by inoculation of the legume seed with the right *Rhizobium* strain, in combination with the correct application rate of phosphorus during the early growth period¹⁷. In addition, the anticipated usage of bio-fertilisers in agriculture may also play a pivotal role in elevating the output¹⁸.

Effects on the quality of the groundnut seed: The variance data analysis suggested that the oil and protein content, viability (germination %) and seed moisture were highly significant at (p<0.05) (Table 4). The highest oil content (54.3%) was obtained from treatments with inoculation of *Rhizobium* combined with fertilizing with P and N. The control plants with no inoculation and no fertilizer application had the lowest oil content (43.1%). The protein content of 25.6 and

15.8% was recorded for plants inoculated with *Rhizobium* and fertilized (P and N) and for un-inoculated and unfertilized plants, respectively.

In terms of moisture content, the highest value of 7.6 was again obtained from the *Rhizobium* inoculated/fertilized plants in (P and N) plants contrast to the meager value of (5.7) from the un-inoculated, non-fertilised control plants. The highest germination rate (86%) was also obtained from the *Rhizobium* inoculated/fertilized (P and N) plants whereas the lowest rate (59%) came from the control (untreated and unfertilized).

The above findings are in consonance with those of Mohamed and Abdalla¹⁹, where improved growth rate and output were recorded for treatments with *Rhizobium* inoculation in combination with phosphorus fertilization. Furthermore, results of the present study fall in line with the findings of Basu *et al.*¹⁰ with regard to the number of beneficial bacterial species, in the plant rhizosphere that is responsible for increased yield and growth rates²⁰.

CONCLUSION

Rhizobium inoculation, supplemented with phosphorus and nitrogen fertilization improved plant growth and yield parameters of groundnut. *Rhizobium* inoculation combined with fertilizers at the recommended dosage had a significant effect on all growth and yield parameters in the present study. Higher yields were recorded in plots sown with *Rhizobium* inoculated seeds in combination with fertilization with phosphorus and nitrogen at 82 and 27 kg ha⁻¹, respectively at the growth stage. The significant interactive effects from *Rhizobium* inoculation, combined with phosphorus and nitrogen fertilization suggest that this technology could be recommended for adoption in areas with similar soil characteristics.

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