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Comparative Studies of the Applications of Different Fertilizers on the Morphological Properties of *Arachis hypogaea* (L.)

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ABSTRACT

The tremendous increase in population causes an increase in the world's demand for food. Recent agricultural technology involves the applications of pesticides and chemical fertilizers for the rapid and healthy growth of plants. Excessive use of chemical fertilizers for the fast growth of plants causes pollution, environmental hazards as well as affects soil quality poorly. Chemical fertilizers mainly nitrogen, potassium, and phosphorous are though vital micronutrients for plants growth, they cause severe health hazards. Biofertilizers- microbial inoculants are environment friendly, the best alternatives for today's agronomy. The use of bio-inoculants in agriculture improves the quality and fertility of the soil. In the present research, comparative applications of chemical fertilizers with biofertilizers were studied on the growth and yield of Arachis hypogaea (L.). The seeds of Arachis hypogaea (L.) treated with Azotobacter and Rhizobium biofertilizer separately and in combination with both organisms along with chemical fertilizer urea (T_1-T_7) were studied in the present investigation. Data obtained revealed that seeds treated with biofertilizers showed a significant increase in the morphological properties of plants as compared to seeds coated with chemical fertilizer and untreated seeds. Maximum root, shoot length, and the number of leaves were observed in treatment T_4, while maximum plant height was observed in treatment T_5. Analysis of variance also revealed the significant difference between the treatment of different fertilizers.

Keywords: *Arachis hypogaea (L.), Rhizobium, Azotobacter, Urea, Morphological properties, ANOVA*

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INTRODUCTION

To fulfill the increasing demand for food due to the non-stop expansion of the world's population, various crop nutrition techniques are being explored by farmers because demand for agricultural products is increasing constantly. Enhancing crop production, retaining a secured environment is one of the major challenges in front of the farmers in the present century (Dizayee & Maaroof, 2019; Karagodin *et al.,* 2020; Nosheen, *et al.,* 2021)**.** Modern agriculture continuously applies chemical fertilizers and pesticides increase the world's food production, as these serve as a fast-food for plants causing them to grow more efficiently and rapidly (Sneha *et al.*, 2018) Continuous application of chemical fertilizers causes soil quality decay and fertility, degradation of soil structure, pollution of the soil, generally not absorbed and can interfere with both underground and surface water (Ajmal *et al.*, 2018). causing eutrophication, high cost (Asadu *et al.*, 2020), and might lead to heavy metals collection in plant tissues, which affect the fruit edibility and nutritional value (Farnia & Hasanpoor, 2015).

Biofertilizer or microbial inoculants are defined as preparations containing living microorganisms that when applied to the soil or seeds, forms the association with roots or the inner part of the plant and stimulate growth by increasing important nutrients supply to the host plant (Garcha *et al.*, 2019; Htwe *et al.*, 2019; Sudhakar & Ranganathan, 2020). The biofertilizers use in modern agriculture is increasing continuously nowadays as it offers an effective tool to replace the use of pesticides, chemical fertilizers, and other harmful supplements (Ansari *et al.*, 2017; Ansari & Mahmood, 2019a, 2019b; Sumbul *et al.*, 2020). The microorganisms generally applied as biofertilizers are nitrogen-fixing bacteria in soil, algae, cyanobacteria, phosphate-solubilizing bacteria, in combination with some molds and fungi (Kalsoom *et al.*, 2020). Among various essential nutrients required potassium (K), phosphorous (P), and nitrogen (N) are required in very large quantities for the fast growth of plants (Mishra & Dash, 2014). Microorganisms present in Biofertilizers contribute essential micronutrients through their natural processes like phosphorus solubilization, fixation of atmospheric nitrogen, and secretion of plant growth-promoting hormones for stimulation of plant growth. Due to this reason, bio inoculants can replace hazardous chemical fertilizers and pesticides. The microbes in biofertilizers form a symbiotic or non-symbiotic association with the roots of plants. These microbes easily convert complex organic matter into simpler ones to be easily utilizable by plants. By improving soil fertility biofertilizers improves crop yield by 20-30%, replace chemical phosphorus and nitrogen by 25% in addition to stimulating plant growth. Besides this, these microbes' protect plants against some soil-borne diseases. In comparison with chemical fertilizers, biofertilizers have enormous environmental and economic advantages (Saeed *et al.*, 2015). Another most important benefit is the continuous use of biofertilizers for more than 4 to 5 years. There is no need to apply them because initial inoculums are sufficient for multiplication and growth (Bumandalai & Tserennadmid, 2019). The use of biofertilizers is eco-friendly, productive, more efficient (Sandle, 2019), cost-effective (Glick, 2020), non-toxic, and easy to apply; they help to maintain the soil structure and biodiversity of the agricultural land. Thus, they serve as an excellent alternative for chemical fertilizers.

Groundnut (*Arachis hypogaea* L.) is one of the prominent commercial oilseed crops not only in India but in most countries including China, Brazil, Nigeria, and the USA. Among the oilseed crops variety produces in India, groundnut occupies a pre-dominant position (Zalate & Padmani, 2009). It is a necessary source of edible oil with digestible carbohydrates and protein, has been given great attention towards its products, due to its major contribution to human nutrition. Because of the poor soil fertility and nutrition, its productivity and production are low in the country. The macro and micronutrients deficiency in the soil leads to poor quality produce (lower protein and oil content) (Singh *et al.,* 2018). This crop is grown mainly for groundnut seeds which contain 40–50% oil, 20–30% protein, and contains vitamin B as well. All other parts of the plant are used as animal feed (Asante *et al.,* 2019). As it is one of the prominent leguminous plants, inoculation with biofertilizers can promote plant growth and yield. Hence, the present research work was conducted for investigation of the effect of biofertilizers *Azotobacter* and *Rhizobium* separately and in combination along with chemical fertilizer on the growth and yield of groundnut in pot assay. The main aim of this study was to enhance the productivity of groundnut with natural fertilizers as an eco-friendly for the environment.

MATERIALS AND METHODS

The healthy seeds of *Arachis hypogaea* (L.) and chemical fertilizer urea were purchased from the local market of Shahada, Dist- Nandurbar, and used in the present research study. Two organisms *Azotobacter* and *Rhizobium* were isolated from rhizospheric soil samples and used for biofetilillizer production.

Isolation of Azotobacter

Isolation and identification of Azotobacter were carried out as a method described by Dubey and Maheshwari. 1 gm rhizospheric soil sample aseptically collected from the botanical garden was diluted serially up to 10^{-6} in sterile distilled water. 0.1 ml of dilution was inoculated into the sterile nitrogen-free Ashby's mannitol salt broth flask and incubated for 6-7 days at RT. After 7 days surface growth was inoculated into sterile nitrogen-free Ashby's mannitol salt agar plate and incubated plate at RT for more than 3 days. Mucoid, transparent colony of *Azotobacter* on Ashby's mannitol salt agar plate was selected, purified further, and confirmed by morphological and biochemical tests. Pure culture of the isolate was maintained on Sterile nitrogen-free Ashby's mannitol salt agar slant and Nutrient agar slant and stored in the refrigerator.

Isolation of Rhizobium

The culture of *Rhizobium* was isolated from freshly collected healthy nodules of the nodulated plant as the method described by Dubey and Maheshwari. 4-5 nodules were sterilized with 0.1% acidified $HgCl_2$ solution for 5min and then repeatedly washed with distilled water. These nodules were crushed in sterile saline and the loopful was inoculated in a sterile congo red yeast extract mannitol salt agar plate. Plates were incubated for 48-72 h. Pink-colored, large, gummy isolated colonies were purified further and confirmed by morphological and biochemical tests. The pure culture was maintained on sterile congo red yeast extract mannitol salt agar slant and stored in the refrigerator.

Preparation of liquid biofertilizer and slurry

The preparation of liquid biofertilizers was carried out by slight modification of the method described by Santhosh (2015). Pure slant cultures were inoculated in the respective selected liquid medium. A Starter pure culture of *Azotobacter* (1 X 10^5 cell/ml) was inoculated in an Erlenmeyer flask containing 100 ml sterile nitrogen-free Ashby's mannitol salt broth and pure culture of *Rhizobium* (1 X 10^5 cell/ml) was inoculated in 100 ml sterile yeast extract mannitol broth. Both flasks were incubated at RT on a rotary shaker for 7 days, followed by centrifugation of 50 ml broth at 5,000rpm (Remi) for 10 min. The supernatant was discarded and cell biomass with 0.5% sugary solution was used as a thick paste for coating the seeds and the remaining medium with organism was stored in the refrigerator for use as liquid biofertilizer preparation.

Seeds treatment

The seed treatment for the present investigation was carried out in triplicates and the experiment was conducted in small pots with tyndallized uniform garden soil having pH 7.0 at R.T. Direct seed coating method (Rai *et al*., 2019) with sugary syrup was applied in seed treatment. 20 healthy seeds of groundnut (approx. 50 g) were coated with the thick paste of *Azotobacter* and *Rhizobium* biofertilizers separately, and 5gm paste of each with the combination. All the seeds were thoroughly mixed with the paste and dried under the shade in the laboratory and sown on the next day. Seed treatments (Abbasniayzare *et al*., 2012) were carried out in the manner like T_1 Control, T_2 *Rhizobium,* T_3 *Azotobacter* T_4 *Rhizobium + Azotobacter,* T_5 *Rhizobium* + Urea (0.5g), T_6 *Azotobacter* + Urea (0.5g), T_7 Only Urea (0.5g). Control was maintained by one set of seeds in sterile distilled water. All the experimental pots were allowed to grow up to 20-30 days at room temperature and watered daily. During incubation, after 10 days the pots containing seeds coated with biofertilizers were again inoculated with 20 ml of each liquid biofertilizer containing the organism. After incubation, the number of seeds germinated in each fertilizer treated and control pots was observed and germination percentage was calculated. Morphological properties like shoot length, root length, the number of leaves per plant, and plant height along with chlorophyll content were measured in seeds treated with bio-fertilizer, chemical fertilizer, and in control. Data were analyzed statistically by the ANOVA method, means were compared to investigate the overall significant difference between the treatment of each fertilizer, and results were expressed accordingly.

RESULTS AND DISCUSSION

Bio-fertilizers are the preparations of living organisms that add nutrients from the soil through their natural processes such as nitrogen fixation, phosphorous solubilization, and favors plant growth by synthesizing growth-promoting materials. Because these are living organisms, they can form mutual associations with plant roots and stimulate plants growth by providing simpler compounds. Biofertilizers *Azotobacter* and *Rhizobium* were prepared in the present investigation and applied separately and in combination with chemical fertilizer to healthy seeds for investigation of their effect on morphological properties and yield of groundnut plants.

Experimental data **(Table 1)** revealed that the coating of seeds with *Azotobacter* and *Rhizobium* biofertilizers in combination had a great influence on the yield of groundnut. Significant variations were observed among treatments of biofertilizer and chemical fertilizer. The inoculation of biofertilizers had a significant effect on plant yield, shoot length, root length, number of leaves, plant height, and chlorophyll content of plants. All characters observed were differed significantly under the treatments of seeds coated with biofertilizers as compared to chemical fertilizer and control. Biofertilizer plants absorbed more nutrients from the soil, the increase in the growth parameters may be due to the favorable actions of biofertilizers which resulted in more availability of nitrogen, certain growth-promoting hormones like auxins, gibberellins, vitamins, and organic acid secreted by bio-inoculants.

Table 1. Effect of different fertilizers on the morphological properties of groundnut.

Treatment	Root length (cm)	Shoot length (cm)	Number of leaves	Plant height (cm)	Chlorophyll	Seed germination
T_1 (Control)	2.2 ± 0.2	0.3 ± 0.4	2	2.2 ± 0.2	0.40 ± 0.2	10%
T_2 (*Rhizobium*)	13.5 ± 0.3	2.4 ± 0.2	19	10.3± 0.4	0.82 ± 2.2	90%
T_3 (*Azotobacter*)	6.3 ± 0.1	0.7±0.3	14	10.5 ± 0.5	0.63 ±2.3	60%
T_4 (*Rhizobium + Azotobacter*)	14 ± 1.2	2.6 ± 0.14	25	11.3 ± 2.1	0.106 ± 0.2	100%
T_5 (*Rhizobium* + Urea)	12.6 ± 0.5	1.3 ± 0.2	20	11.5 ± 0.23	0.73 ± 0.31	80%
T_6 (*Azotobacter* + Urea)	6.4 ± 0.2	0.58 ± 1.1	13	9.3 ± 0.5	0.61 ± 0.4	60%
T_7 Urea	5.8 ± 0.1	0.5 ± 0.2	10	8.5 ± 0.2	0.73 ± 0.3	20%

Values are the means of triplicates ± S.D.

Root length and shoot length

When root length and shoot lengths were measured in centimeters results **(Table 1)** revealed that elongated roots and shoots were observed in seeds treated with Treatment T_4, i. e. with the combined action of both biofertilizers followed by treatment T_2 *Rhizobium* separately. Maximum root length 14 ± 1.2 cm and shoot length 2.6 ± 0.14 cm were observed in seeds treated with treatment T_4 as compared to control or treatment T_5. While minimum root length, as well as shoot length, was observed in control and seeds treated with only chemical fertilizers T_7

Plant height

Large variations were observed in the height of the plants due to the treatment of biofertilizers separately and in combination as shown in **Figure 1**. The maximum height was recorded in seeds treated with rhizobium biofertilizer and seeds treated with combined treatment of *Azo-Rhizo* biofertilizer. Minimum plant height was observed in control seeds as compared to seeds coated with chemical fertilizers. Similarly, a maximum number of leaves 25 and chlorophyll content 106 were observed in plants treated with treatment T_4, *Azo-Rhizo* biofertilizers as compared to control. Results in **Table 1** revealed that significant variation in the number of leaves, as well as all other morphological parameters of plants, were observed due to the treatment of biofertilizers. Microbial cells present in biofertilizers through their metabolic activities supply essential nutrients as well as growth substances for enhanced growth and morphological properties of plants.

Germination percentage

Results obtained in **Table 1** revealed that seeds coating and treatment with bio-fertilizers showed a higher germination percentage as compared to control (10%) and seeds coated with chemical fertilizer (20%). Maximum germination percentage was observed as 100% in seeds treated with *Azo-Rhizo* biofertilizers followed by seeds treated with *Rhizobium* biofertilizer (90%).

Table 2. ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-Value*	*F Crit*
Between Groups	290.84 17	6	48. 473 62	1.103229	0.385217	2.44 5259
Within Groups	1230.263	28	43.937 95			
	1521.104	34				

Results of analysis of variance **(Table 2)** revealed that the calculated F value is less than the Tabular F value which indicated that Significant differences were observed among the group means which showed that treatment of different biofertilizers was statistically significant.

a)

b)

c)

d)

e)

f)

Figure 1. Growth and yield of Groundnut seeds in different fertilizers.

CONCLUSION

Biofertilizers are microbial inoculants added to the soil to provide essential nutrients like nitrogen, phosphorous, and potassium to plants growth. In this study biofertilizers *Rhizobium* and *Azotobacter* were prepared and inoculated separately and in combination to determine their effect on the growth of groundnut seeds. Results of this experimental study revealed that biofertilizer-treated seeds showed an increase in root length as well as shoot length and more number of leaves as compared to control and chemical fertilizer. The application of biofertilizers is safe, non-pollutant, and environmentally friendly fertilizers for the growth of the plant.

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