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Effect of Biofertilizers on Different Parameters of Ground Nut Varieties *Arachis Hypogaea (L.)*

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Abstract
Chemical fertilizers applications to agricultural fields reduce the microbial composition in the fields, which results in decreased agronomic properties of the fields. To overcome this issue, researchers focused on the alternative and cost effective sources for this problem. Utilization of potent microbial inoculants for crop improvement gave unacceptable results in most studies; this necessitates looking for locally isolated and suitable inoculants. Therefore, this study was designed in eleven groups (T1-T11) to evaluate the efficiency of selected microbial inoculants, single or mixed cultures, on groundnut, Arachis hypogea L. Thus, using Rhizobium, Azetobacter, Azospirillium, AMF, and phosphobacteria inoculants at planting improves yields in many morphological and biochemical parameters of leguminous crops. Moreover, mixed inoculants showed improved root and shoot length, which resulted in increased root nodules for improved nitrogen fixation and productivity. Mixed culture improved biochemical parameters such as chl. a & b, Carotenoids, sugars (reducing & non-reducing) and proteins (amino acids) indicated the improved plant growth with significant changes in the plant productivity than compared to the control and single culture groups.
Keywords: Rhizobium, Azotobacter, Azospirillium, AMF, Phosphobacteria, productivity, Arachis hypogea

Introduction

Agronomists are in a desperate search for substitute tactics that may guarantee competitive yields while preserving the health of soils since the indiscriminate and excessive use of chemical fertilizers has resulted in health and environmental risks. This innovative method of farming, also known as sustainable agriculture, calls for agricultural methods that are more environmentally friendly and preserve the long-term ecological balance of the soil ecosystem (Duponnois et al., 2006).One of the biggest issues confronting the agricultural community will be meeting the expectations placed on agriculture to providing future food as the world's human population continues to grow. To tackle this issue, significant work must be put into better understanding the intricate connections and processes determining the stability of agricultural land and products, with a particular focus on the soil biological system and the agroecosystem as a whole (Mohamed et al., 2007). The amount of chemical fertilizers being used has theoretically reached its limit, after which agricultural yields won't continue to rise (Roriz et al. 2020; Ramya et al., 2020).A leguminous edible oilseed crop is groundnut. For human nutrition, groundnut is a

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priceless supply of protein, calories, necessary fatty acids, vitamins, and minerals (Ren 2018). There is a considerable contribution of groundnut to the total oilseed both globally and in India (ICAR 2015).With the capacity to satisfy up to 46% of the required daily amount, groundnuts are a nutrient-dense source of dietary protein, vital vitamins, including vitamin E, energy from its oils and fats, and dietary fiber (FDA 2017).Microbial biofertilizers contains living cells of effective N2-fixing microorganisms. These organisms fix atmospheric nitrogen either symbiotically with the host plant or independently. They also act as phosphate solubilizers and potassium mobilizers, which release or mobilisephosphate and potassium from the soil(Ashraf et al., 2006).Endomycorrhiza, also known as arbuscular mycorrhizae (AM) or arbuscularmycorrhizal fungi, is the process of mycelium implantation within the root tissue (AMF) (Sudha and Veeramani 2021).As nitrogen-fixing soil bacteria, Azotobacter, Azospirillum, and Rhizobium are examples of microbes frequently used as biofertilizers.Azospirillum is renowned for its ability to fix a sizable amount of atmospheric nitrogen and provide provisions to the crop, enhance fertiliserutilisation terrific organization, examine soil fertility, and ensure fragmented nitrogenous fertilizer saving(Suhameena et al., 2020).Rhizobium plays a significant part in groundnut biofertilizer inoculation, which enhances plant growth, yield characteristics, and yield (Baishya et al., 2014; Palai et al., 2021).Azotobacter and other biofertilizers convert the normal airborne nitrogen to ammonia. The calcium phosphate is converted to an available form by ammonia as it enters the root zone and releases half of the nitrogen needed for root usage (Ahmed et al., 2010). The phosphobacterium, also known as a Phosphate Solubilizing Bacteria, has the ability to change the form of soil phosphate so that it is accessible to plants. It indirectly influences nodulation, which helps to enhance yield (Gosh and Poi, 1998).The objectives of the study to analyze the single and mixed microbial inoculants effect on the morphological parameters (germination, root and shoot length, fresh and dry weight, no. of leaves, total leaf area, no. of root nodules) and biochemical parameters (Chl.a, Chl.b, total chlorophyll, sugars and proteins) of groundnut, Arachis hypogea L. by field experiments.

Materials and Methods

Seed collection and treatment

Ground nut *Arachis hypogaea(L.)* seeds were obtained from a nearby private agro center in the Tamil Nadu, India district of Dharmapuri.At various phases of seed development, bacteria, fungus, and other organisms can infect the sowing seed with pests and illnesses. From the seedling stage prior to culture, the subsequent seed purification technique can be avoided. For around 15 days, collect cow pee in a jar. 500 mL of cow pee should be thoroughly dissolved in 1L of water. Stir well before adding 0.5 kg of seeds to the mixture. Allow the chaffy seeds and dust particles to float in the solution after soaking the seeds in the mixture for 30 minutes. Remove the wet seeds and let them to air dry in the shade. The seeds are now prepared for planting. Numerous pests, illnesses, and crops that harm root leaves are treated.

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Field setup and sample preparation

The test area was completely plowed, leveled, and then divided into test levels in accordance with the arrangement. To guarantee enough soil moisture, a sow received pre-irrigation. The field is plotted as 1x1m2 in split plot design with three replicates. For this study, a range of microbial fertilizers from the Forest Office (Tree Seedling branch), Dharmapuri District (Tamil Nadu, India) were used (Pushpa et al., 2021).

Experimental design

Arachis hypogaea L., (Order: Fabales, Family: Fabaceae) leguminous seeds was grown with 30 sampling days. At the 30th DAS after sowing, the weeds in the field were pulled by hand twice. The eleven treatment groups such as

T1-Control**, T2-**Azotobacter**,**
T3-AMF**, T4-**Rhizobium**,**
T5-Azospirillum**, T6-**Phosphobacteria**,**
T7-Azospirillum**+**Azotobacter**, T8-**Phosphobacteria**+**Azospirillum**,**
T9-Azospirillum**+**Azotobacter**+**AMF**, T10-**Rhizobium+Phosphobacteria+AMF
T11-Rhizobium+Azospirillum+AMF
Were organized. In each group, seven replicates were maintained.

Percentage of germination

Following each treatment, the number of sprouted seeds in each treatment was counted up to the seventh DAS to determine the overall germination percentage.

Germination %= (Total No. of germinated seeds)/ (Total no. of sown seeds) ×100

Measurement of shoot and root length

On the 30th DAS, five plants were chosen at random to measure the length of the seedling shoots and roots in the field. The centimeter scale was used to measure them.

Measurement of fresh and dry weight

In the experimental plot, five plant samples were randomly chosen for the 30th DAS. They were divided into individual seedlings. Their fresh weight was taken by using an electrical single pan balance. The same seedlings were packed in brown pocket cover and they were kept in a hot air oven at 80°C for 24 hr and then their dry weights were also determined.

Total Leaf Area

The plant samples were obtained at the 30th DAS, and the leaf samples' length and width were measured and documented (Kalra and Dhiman, 1977). The Kemp's (K) constant (0.66) was used to get the total leaf area.

Total leaf area = L × B × K,

Biochemical content

The Carotenoids concentration and photosynthetic pigments such chlorophylls "a" and "b" were measured by Kirk and Allen (1965) and Arnon (1949) methodology. In the 30th DAS of experimental plants, the estimation of protein, amino acids, sugars and carbohydrate content by Lowry et al.(1951),Moore and Stein(1948),Nelson(1944), Dubois et al., (1956) methodology respectively. The obtained results were statistically analysed by SPSS (17.0) and their mean±SD results were recorded.

Results

Table: 1 Effect of different microbial inoculation on the Germination study of different variety of groundnut *Arachis hypogaea L.*

Microbial Fertilizers Treatments	TMV10	TMV12	TMV(Gn)13	ICGV00348	VRIGn 6
T1	86.3±3.6	86.8±3.1	88.3±2.7	82.5±1.9	85.7±1.1
T2	87.7±4.1	88.9±2.9	90.8±3.6	85.7±2.0	87.4±1.3
T3	87.3±4.2	89.8±3.0	92.8±3.5	87.4±1.8	87.5±1.6
T4	88.5±3.8	89.8±2.9	92.7±3.3	87.6±1.7	88.3±1.9
T5	88.7±3.5	90.5±3.1	93.8±2.6	89.6±1.9	90.3±1.8
T6	90.7±2.8	92.7±3.7	94.9±3.1	90.5±1.7	90.7±1.4
T7	90.8±3.1	92.6±3.1	94.8±3.0	91.8±2.0	91.4±1.6
T8	92.8±2.9	94.5±3.4	95.4±3.1	91.5±1.9	91.8±1.8
T9	94.5±3.5	94.2±2.9	97.9±3.8	92.6±2.1	93.5±1.3
T10	94.9±3.8	95.4±3.9	99.6±4.1	94.5±2.2	95.7±1.9
T11	92.7±3.2	93.7±3.5	96.3±3.3	93.6±2.1	94.9±1.2

T1-Control, T2-Azotobacter, T3-AMF, T4-Rhizobium, T5-Azospirillum, T6-Phosphobacteria, T7-Azospirillum+Azotobacter, T8-Phosphobacteria+Azospirillum, T9-Azospirillum+Azotobacter+AMF, T10-Rhizobium+Phosphobacteria+AMF and T11-Rhizobium+Azospirillum+AMF

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Table 2. Effects of different microbial inoculants on the morphological parameters of TMV (Gn) 13 groundnut *Arachis hypogaea L.* Variety.

Microbial Fertilizers Treatments	Morphological parameters TMV (Gn) 13			
	Shoot length (cm)	Root length (cm)	Fresh weight (g/seedling)	Dry weight (g/seedling)
T_1	5.76±0.23	2.08±0.08	1.72±0.11	0.56±0.09
T_2	5.86±0.23	2.44±0.10	2.67±0.21	0.63±0.11
T_3	6.16±0.25	3.03±0.12	2.72±0.19	0.77±0.13
T_4	6.36±0.25	3.47±0.10	3.74±0.25	0.81±0.15
T_5	6.45±0.26	4.10±0.16	3.75±0.18	0.86±0.12
T_6	7.62±0.30	4.19±0.17	4.78±0.19	1.67±0.14
T_7	7.96±0.32	5.02±0.20	5.80±0.23	2.09±0.11
T_8	8.66±0.35	5.25±0.21	6.85±0.22	2.84±0.15
T_9	9.43±0.38	5.55±0.22	6.96±0.24	3.22±0.17
T_{10}	11.30±1.45	6.66±0.27	8.95±0.20	3.91±0.16
T_{11}	9.01±0.37	6.03±0.23	7.54±0.22	3.54±0.16

T1-Control, T2-Azotobacter, T3-AMF, T4-Rhizobium, T5-Azospirillum, T6-Phosphobacteria, T7-Azospirillum+Azotobacter, T8-Phosphobacteria+Azospirillum, T9-Azospirillum+Azotobacter+AMF, T10-Rhizobium+Phosphobacteria+AMF and T11-Rhizobium+Azospirillum+AMF

Table: 3 Effects of different microbial inoculation on the nutrient contents of groundnut *Arachis hypogaea (L.)*TMV (Gn) 13 variety

TREAT	N (mg)	P(mg)	K(mg)	Ca(µg)	Mg(µg)	Cu(µg)	Fe(µg)	Mn(µg)	Zn(µg)
T_1	11.33±0.11	1.80±0.09	10.35±0.15	3.22±0.09	0.04±0.01	9.35±0.85	89.2±0.72	15.33±0.14	6.86±0.06
T_2	12.46±0.15	1.92±0.06	10.58±0.12	3.72±0.11	0.04±0.02	11.44±0.92	96.4±0.726	17.22±0.17	8.55±0.09
T_3	14.18±0.11	2.17±0.07	11.65±0.11	3.95±0.09	0.06±0.02	12.56±0.87	102.5±0.82	19.61±0.13	10.33±0.08
T_4	16.62±0.13	2.48±0.06	12.17±0.14	4.39±0.10	0.07±0.01	14.21±0.57	110.5±0.88	24.55±0.21	11.87±0.09
T_5	17.55±	2.67±0	13.52±	5.08±0	0.08±0	15.22±	116.3±	27.18±	12.46±

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	0.15	.09	0.11	.11	.03	0.72	0.82	0.19	0.11
T_6	19.48± 0.13	2.80±0 .07	15.21± 0.14	5.34±0 .13	0.09±0 .03	16.56± 0.47	123.7± 0.89	29.45± 0.18	14.71± 0.12
T_7	20.85± 0.11	2.89±0 .07	16.38± 0.15	6.30±0 .11	0.10±0 .01	17.43± 0.67	127.5± 0.82	30.32± 0.13	16.69± 0.09
T_8	21.76± 0.15	3.04±0 .06	17.44± 0.13	7.16±0 .09	0.11±0 .02	18.65± 0.99	136.4± 0.79	33.68± 0.22	18.55± 0.10
T_9	23.35± 0.13	3.59±0 .09	18.46± 0.14	7.79±0 .10	0.12±0 .02	20.55± 0.86	150.9± 0.80	39.36± 0.20	19.29± 0.14
T_{10}	24.63± 0.13	4.03±0 .09	19.65± 0.16	8.65±0 .13	0.14±0 .02	24.36± 0.97	162.4± 0.92	43.29± 0.23	24.38± 0.13
T_{11}	23.19± 0.11	3.70±0 .07	18.22± 0.15	7.72±0 .10	0.15±0 .01	23.42± 0.75	148.3± 0.83	41.43± 0.18	23.43± 0.12

T1-Control, T2-Azotobacter, T3-AMF, T4-Rhizobium, T5-Azospirillum, T6-Phosphobacteria, T7-Azospirillum+Azotobacter, T8-Phosphobacteria+Azospirillum, T9-Azospirillum+Azotobacter+AMF, T10-Rhizobium+Phosphobacteria+AMF and T11-Rhizobium+Azospirillum+AMF

Discussion

Ground nut is one of the essential stable crops showing symbiotic association with soil bacterium (Rhizobium and azotobactor) (Fwanyanga et al., 2022). Rhizobia inoculation of legumes often stimulates plant growth, development, and yield and is typically used as an alternative to expensive mineral nitrogen fertilizer (Tairo and Ndakidemi 2013; Raissa et al., 2020). Microbial inoculants were effectively enriched the symbiotic mechanism in the leguminous plants by enhanced food production and root nodules formation (Dlamini et al., 2021).Microorganisms have a crucial role in fixing, solubilizing, mobilizing, and recycling nutrients in the agricultural eco-system. These bacteria naturally occur in soils, although their numbers are frequently small. Healthy microbial community in soils will never support greater crop output. The needed rhizosphere microorganisms are extracted, artificially cultivated in sufficient numbers, and combined with the appropriate carriers in order to boost crop output. These are referred to as microbial inoculants or biofertilizers (Michael et al., 2020; El-sherbeny et al., 2023).In this study, eleven (T1-T11) different groups were studied to identify the effect of different microbial inoculants effect on the morphological and biochemical parameters of Arachis hypogea(L.).Microbes such as Azotobacter, Azospirillum, and Rhizobium were the ideal microbes for the leguminous plants. Phosphobacteria and AMF organisms were responsible for the interconversion of minerals from soil into a soluble or absorbed form for the plants. Among the tested groups, T10 (Rhizobium+Phosphobacteria+AMF), T11(Rhizobium+Azospirillum+AMF)andT9 (Azospirillum+Azotobacter+AMF) groups showed high germination percentage as 98.5±1.49, 96.7±1.23 and 96.3±1.16 % respectively. The plant root length was increased upto 3times in the microbial inoculant treated groups than control whereas the increased T5 (Azospirillum), T4 (Rhizobium) and T10 (Rhizobium+Phosphobacteria+AMF) as 6.83±0.34, 6.70±0.33and6.40±0.32cm respectively.

Shoot length of the plants also increased in T10 (Rhizobium+Phosphobacteria+AMF), T5 (Azospirillum) and T9 (Azospirillum+Azotobacter+AMF) as 9.32±0.46, 8.58±0.42 and 8.40± 0.42cm respectively. The fresh weight of the groups were ranged between 4.20±0.21 to 6.83±0.34gm whereas the dry weight of the treated groups were ranged between 3.10±0.22 to 6.90±0.34gm. These results evidenced the significantly increased weight in the treated plantlets. Maximum dry weight observed in T9 (Azospirillum+Azotobacter+AMF), T10 (Rhizobium+Phosphobacteria+AMF) and T7 (Azospirillum+Azotobacter) as 6.90±0.34, 6.86±0.34 and 6.75±0.33gm respectively. Increased shoot length resulted in the increased number of leaves which implied on the improved chlorophyll pigment contents. The number of leaves was doubled in the treated groups than compared to the control group. High number of leaves observed in T10 (Rhizobium+Phosphobacteria+AMF) and T11 (Rhizobium+Azospirillum+AMF)as 18.8±0.19 and 18.7±0.18 respectively (Table 1). Increased number of leaves resulted in the increased biomass i.e., total leaf area as 15.2±0.21 and 14.2±0.24 for T10 (Rhizobium+Phosphobacteria+AMF) and T11 (Rhizobium+Azospirillum+AMF) groups respectively. Increased root length evidenced the high number of root nodules in the treated groups as 23.5±0.98 and 20.6±0.91 for T10 (Rhizobium+Phosphobacteria+AMF) and T11 (Rhizobium+Azospirillum+AMF) groups respectively. Increased phosphate and nitrogen levels in the soil enhances the plant height which was resulted in the microbial inoculants based fertilizers treatment. Organic fertilizers increased the plant morphological characters such as stem height, number of leaves, increased flower size and increased growth in meristematic regions (Bhalerao et al., 1993; Saradhi et al., 1990).Rhizobium inoculants are used to increase the number of nodules on each plant, which in turn raises the possibility of N2 fixation with the aid of P fertilizer (Abdulameer 2011). While Rhizobium spp. also improves the delivery of nitrogen, the phosphorus solubilizing bacterium increases the availability of phosphorus. In terms of plant height, Rhizobium spp. + PSB (Lignite based) cultures reported much better results than the control. Consequently, combining the two may have boosted vegetative growth (More et al., 2002; Tomar et al., 1994).Proteins are digested during the catabolic process to produce ammonium and amino acids, mostly in the amide form like glutamine and asparagine. These bacteria, which function as self-sufficient, autonomous fertilizers. They use sunlight and the heat of the soil as energy sources to create beneficial compounds from the secretions of plant roots, organic materials, and toxic gases like hydrogen sulphide. These bacteria create valuable chemicals such amino acids, polysaccharides, nucleic acids, bioactive molecules, and sugars, all of which aid in the development and growth of plants. Increased nitrate uptake and translocation, which provide nitrogen for amino acid synthesis, may be the cause of the increase in protein content (Selvaraj and Chellappan, 2006; Ranjith et al., 2007; Sutaria et al., 2010; Venkatarao et al.,2017; Dhakal et al., 2016).A. hypogeae legume plants treated with microbial biofertilizers showed improved vegetative growth (plant spread

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area, no. of leaves, plant height) and photosynthetic rate (increased carbohydrate translocation) (Dhadge and Satpute 2014).Treated groundnut plant leaf biochemical parameters were studied. Chlorophyll and Carotenoids pigments were the chief photosynthetic pigments responsible for the photosynthesis mechanism. Chlorophyll-‘a, and ‘b, were observed as high proportions in 7.4±0.22&4.9±0.51 (T10-Rhizobium+Phosphobacteria+AMF), 6.9±0.23& 4.1±0.48 (T11-Rhizobium+Azospirillum+AMF) and 6.8±0.21& 4.7±0.49 (T9-Azospirillum+Azotobacter+AMF) groups (Table 2). These pigments result implied on the total chlorophyll levels in the T10 (Rhizobium+Phosphobacteria+AMF), T11 (Rhizobium+Azospirillum+AMF) and T9 (Azospirillum+Azotobacter+AMF) treatment groups as 12.34±0.80,11.03±0.96 and 11.53±0.99 respectively. The secondary pigments Carotenoids maximum levels observed in T9 (Azospirillum+Azotobacter+AMF) and T10 (Rhizobium+Phosphobacteria+AMF) groups as 3.91±0.31 and 3.91±0.29 respectively. Sugars are the final products of the photosynthetic reactions. Reducing and non-reducing sugars are essential for the various metabolism, translocation and redox reactions. In the tested groups, reducing sugar levels were ranged as 7.8±0.80to 4.2±0.89mg whereas non-reducing sugar levels were ranged as 5.4±0.93 to 1.9±0.56mg. Total sugar levels were ranged as 13.2±2.3 to 6.1±1.2mg. Among the tested groups, T10 (Rhizobium+Phosphobacteria+AMF), T9 (Azospirillum+Azotobacter+AMF) and T11 (Rhizobium+Azospirillum+AMF) treatments showed an improved reducing, non-reducing sugars, total sugars. Proteins and amino acids levels are essential for the enzyme synthesis for the numerous catabolic and anabolic reactions in plants. Similar to the other biochemical parameter results, T10 (Rhizobium+Phosphobacteria+AMF), T9 (Azospirillum+Azotobacter+AMF) and T11 (Rhizobium+Azospirillum+AMF) treatments showed an improved protein and amino acids levels than compared to the other treated groups. Similar results were evidenced by various researchers in different plant species. Ramya et al. (2020) reported the effect of azospirillum on the finger millet cultivation by field experiment. Azospirillum inoculants treatments showed increased dry matter stem height, plant area indices with increased flowering and grains. Mahato and Kafle (2018) reported the effect of azotobactor on the wheat pot experiment as 16.5 to 19.42% grain production were increased than control wheat plants. Rafi et al. (2018) studied the effect of azospirillum (45L1) on Foxtail millet pot experiments with increased protein content and weight of the grains. When compared to the single microbial inoculants efficiency on plant morphological and biochemical parameters of groundnut, mixed microbial inoculants showed double the results of plant parameters.

Conclusion

Microbial inoculants, which should be investigated and developed for the agricultural industry, can increase groundnut *Arachis hypogaea (L.)* cultivation products. As a result, it is strongly advocated that the use of "inoculants" be done holistically, including diagnostics of the field environment in relation to the planned crop. A complete strategy that investigates the best agricultural methods, screens available culture collections for inoculants, conducts mixed microbial research, and incorporates all potential remedies into vast industrial production and field applications is also required.

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