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The study of *Rhizobium*, *Azotobactor* bio-fertilizer effect on *Zea mays* and *Vigna radiata*

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Abstract
A biofertilizer is a material containing living microorganisms that, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Now a days, it is one of the most effective and modern agricultural tools. The present study is carried out to assess the effect of *Rhizobium* and *Azotobactor* biofertilizers on Dicot and Monocot Plantation Root and Shoot Measurement. The seeds of *Zea mays* and *Vigna radiata* were used as a Dicot and Monocot plants. Seeds treated with *Rhizobium* and *Azotobactor* biofertilizers increased the growth parameters of Mungbean (*Vigna radiata*) and Maize plants significantly (*Zea mays*). Their morphological parameters increased significantly, including shoot length, root length, and overall plant length. When Rhizobium was added at a concentration of 20% in monocot and 20% in dicot plantations, the total and beneficial number of nodules, plant height, and root and shoot length all increased significantly.

Keywords: biofertilizers, *Rhizobium*, *Azotobactor*, *Zea mays*, *Vigna radiata*, shoot length, root length

Introduction
A biofertilizer is a material containing living microorganisms that, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers supplement nutrients by using natural processes such as nitrogen fixation and phosphorus synthesis, as well as stimulating plant growth through the synthesis of growth-promoting substances. Biofertilizers microorganisms restore the soil's natural nutrient cycle and increase soil organic matter.
Healthy plants can be grown with the help of biofertilizers, while also improving the soil's sustainability and health. Biofertilizers are expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet capable of completely replacing them. Because they serve multiple functions, the scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria" (PGPR).
Biofertilizers are organic agro-input that is "eco-friendly." Biofertilizers like *Rhizobium*, *Azotobacter*, *Azospirilium*, and blue green algae (BGA) have long been used. Leguminous crops benefit from *Rhizobium* inoculant. Wheat, maize, mustard, cotton, potato, and other vegetable crops can all benefit from the use of *Azotobacter*. *Nostoc* or *Anabaena* or *Tolypothrix* or *Aulosira*, a general cyanobacteria genus, fix atmospheric nitrogen and are used as inoculants for paddy crops grown in both upland and lowland conditions. *Anabaena*, in conjunction with the water fern *Azolla*, contributes up to 60 kg/ha/season of nitrogen while also enriching soils with organic matter. Since seaweeds are high in mineral elements (potassium, phosphorus, trace elements etc.), they are commonly used as manure by people in coastal regions. Seaweed - manure also aids in the breakdown of clays. On a large scale, Irish people use *fucus* as manure.
Bottom mud from dried up ponds introducing various blue green algae is commonly used as manure in tropical Countries. The combination of seaweeds and blue green algae may be an excellent fertilizer. Legumes, which are members of the Leguminosae family, are the second most important source of protein. Legumes' mature and dried seed have a high nutritional value and good storage ability. Because legumes fix nitrogen on their roots, they increase soil fertility, so they added a lot of nitrogen to the soil after harvesting. Mungbean (*Vigna radiate* L.) is an important crop in Asia because its green pod is used fresh, and some varieties are used as cover crops and thus to produce hay. Efforts to increase production per unit area, as well as excessive and imbalanced chemical fertilizer consumption, have negative environmental consequences, in addition to increasing production costs and yields.
Rhizobium is a bacterium in soil. *Rhizobium's* symbiotic nitrogen fixation with legumes contributes significantly to total nitrogen fixation. *Rhizobium* inoculation is a well-known agronomic practice for ensuring adequate nitrogen availability. *Rhizobium* is a genus of nitrogen-fixing Gram-negative soil bacteria. *Rhizobium* species have an endosymbiotic nitrogen-fixing relationship with legume and Parasponia roots.
The bacteria colonise plant cells inside root nodules, where they use the enzyme nitrogenase to turn atmospheric nitrogen into ammonia and then supply organic nitrogenous compounds to the plant, such as glutamine or ureides. The plant, in turn, provides organic compounds generated by photosynthesis to the bacteria. This mutually beneficial relationship exists among all rhizobia, with the genus *Rhizobium* being a prime example. *Rhizobium* can also solubilize phosphorus.
The taxon has largely subsumed the genera *Agrobacterium* Conn (1942) and *Allorhizobium* as a result of phelogenetic research conducted from the late 1990s to the early 2000s, which revealed that the two genera were not related. As a

result, *Agrobacterium tumefaciens*, now *Rhizobium radiobacter*, is still the type species of *Agrobacterium*. The division of Rhizobiaceae genera is still fluid. ***Azotobacter*** is a genus of bacteria that are usually motile, oval or spherical in shape, form thick-walled cysts, and can produce large amounts of capsular slime. They are aerobic, free-living soil microbes that play an important role in the nitrogen cycle in nature by binding atmospheric nitrogen, which is inaccessible to plants, and releasing it into the soil in the form of ammonium ions (nitrogen fixation).

It is used by humans to manufacture biofertilizers, food additives, and some biopolymers, in addition to being a model organism for studying diazotrophs. Martinus Beijerinck, a Dutch microbiologist and botanist, discovered and described the first member of the genus, *Azotobacter chroococcum*, in 1901. *Azotobacter* species are Gram-negative bacteria that live in neutral and alkaline environments. Biofertilizers convert atmospheric nitrogen into a form that plants can use. Biofertilizers are low-cost, renewable sources of plant nutrients that are used in conjunction with chemical fertilizers. Biofertilizer is one of the most effective and modern agricultural tools. The use of biofertilizers is critical because they are components of integrated nutrient management, as well as cost effective and renewable. The present study is carried out to assess the effect of *Rhizobium* and *Azotobactor* biofertilizers on Dicot and Monocot Plantation Root and Shoot Measurement.

Material and Method

The seeds of *Zea mays* and *Vigna radiata* were obtained through the courtesy of Agricultural College, Pune. Make varied concentration fertilizer solutions after seed washing. And then put them in bottles and cover them properly with lead. (Fertilizer concentration percentages: -5 percent, 10%, 15%, 20%) Following the preparation of the solution, the five seeds were sowed on 5-5 Petri plates on soaked Blotting Papers and stored in a sterile environment for germination.

Two control Petri plates with untreated seeds were also maintained. Daily fertilizer solution was applied to the seeds at regular intervals, and growth metrics were analyzed and recorded. The morphological parameters of *Zea mays* and *Vigna radiata*, such as shoot length and root length, were measured after 3 and 5 days of sowing. Also, take note of the observations.

Effect on morphological parameters of Mungbean and Maize plant treated with Biofertilizers

Fig 1: Effect of *Rhizobium* (A and C) And *Azotobactor* (B and D) fertilizer on *Zea mays* and *Vigna radiata* plantation.

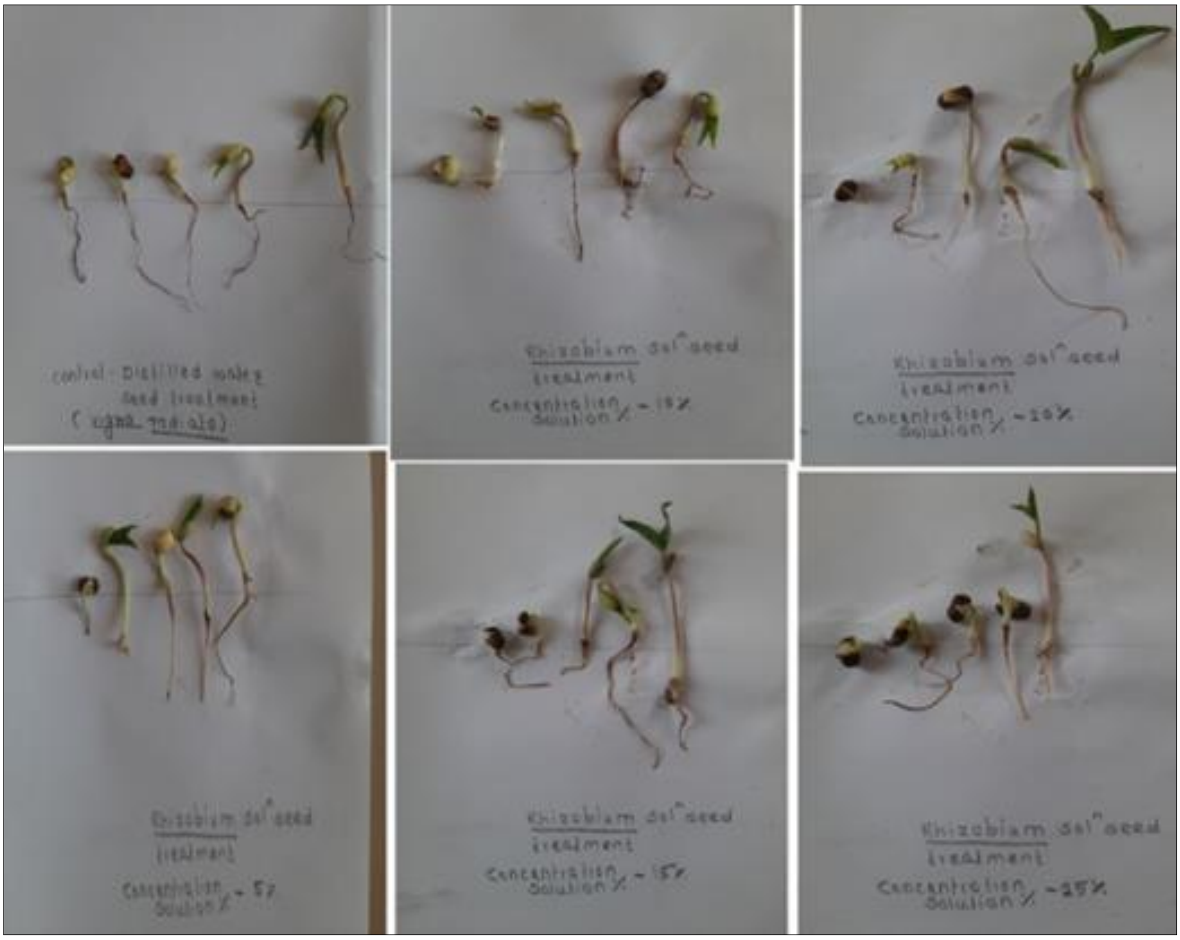

Fig 2: Effect of *Rhizobium* fertilizer solution on *Vigna radiata* plantation. A Control, B-5%, C-10%, D-15%, E-20%, F-25% Concentration solutions.

Fig 3: Effect of *Rhizobium* fertilizer solution on *Zea mays* Plantation A-Control, B-5%, C-10%, D-15%, E-20%, F-25% Concentration solutions.

Fig 4: Effect of *Azotobactor* fertilizer solution on *Zea mays* plantations A-Control, B-5%, C-10%, D-15%, E-20%, F-25% Concentration solutions.

Fig 5: Effect of *Azotobactor* fertilizer solution on *Vigna radiata* plantations. A-Control, B-5%, C-10%, D-15%, E-20%, F-25% Concentration solutions.

Table 1: Effect of *Rhizobium* fertilizer on Dicot and Monocot Plantation.

Sr. No	Concentration Solution %	Germinated Seeds		Non-germinated Seeds	
		Concentration Solution %	**Concentration Solution %**	**Monocot**	**Dicot**
1	Control	5	5	0	0
2	5	5	5	0	0
3	10	5	5	0	0
4	15	4	5	1	0
5	20	5	4	0	1
6	25	3	4	2	1
Total		27	28	3	2

Table 2: Effect of *Azotobactor* fertilizer on Dicot and Monocot Plantation.

Sr. No	Concentration Solution %	Germinated Seeds		Non-germinated Seeds	
		Monocot	**Dicot**	**Monocot**	**Dicot**
1	Control	5	5	5	5
2	5	3	5	3	5
3	10	5	4	5	4
4	15	5	4	5	4
5	20	4	3	4	3
6	25	4	3	4	3
Total		26	26	24	26

Table 3: Effect of *Rhizobium* fertilizer on Dicot and Monocot Plantation Root and Shoot Measurement.

Sr. No	Concentration Solution %		Monocot *Zea mays*						Dicot *Vigna radiata*					
			Root length cm			**Shoot length cm**			**Root length cm**			**Shoot length cm**		
	Day		**1**	**3**	**5**	**1**	**3**	**5**	**1**	**3**	**5**	**1**	**3**	**5**
1	Control	1	0	1.5	3	0	0.7	1.4	0	0.4	3.2	0	0	3.3
		2	0	1.4	2.8	0	1.2	2.5	0	0.2	4.5	0	0	2.5
		3	0	2.4	4.9	0	1.3	3.6	0	0.3	3.1	0	0	2.5
		4	0	6	12	0	1.9	3.8	0	0.2	3.3	0	0	3.6
		5	0	5.5	11	0	3	6	0	0.5	5.1	0	0	5
	Mean		0	2.2	6.7	0	1.7	3.4	0	0.3	3.84	0	0	3.38
2	5	1	0	0.6	1.2	0	0.2	0.4	0	.	0.3	0	0	2
		2	0	0.9	1.9	0	0.3	0.7	0	0.3	3.2	0	0	4.6
		3	0	3.9	7.9	0	0.3	0.7	0	0.2	2.7	0	0	2.1
		4	0	2.5	5	0	1	2	0	0.4	3.2	0	0	3.9
		5	0	3.4	6.9	0	0.7	1.5	0	0.3	3	0	0	4.3
	Mean		0	2.3	4.5	0	0.5	1.6	0	0.3	2.5	0	0	3.4
3	10	1	0	3.1	6.2	0	1.6	3.1	0	0	0.3	0	0	0.9
		2	0	2.1	4.5	0	1.5	3	0	0.3	1.1	0	0	3.4
		3	0	4	8	0	2.7	5.5	0	0.6	3.9	0	0	4
		4	0	6.6	13.3	0	3.7	7.5	0	0.8	4.1	0	0	3.2
		5	0	7	14	0	4.1	8.3	0	0.4	2	0	0	2.3
	Mean		0	4.6	9.2	0	2.7	5.5	0	0.5	2.3	0	0	2.7
4	15	1	0	.	.	0	.	.	0	.	0.3	0	0	1.1
		2	0	2.5	5	0	.	.	0	0.4	2.1	0	0	2.5
		3	0	5.4	10.9	0	2.6	5.2	0	0.2	1.4	0	0	3.9
		4	0	4.5	8.9	0	3.6	7.3	0	0.8	5	0	0	3.2
		5	0	4.9	9.8	0	3.8	7.6	0	0.4	2.9	0	0	6.2
	Mean		0	4.3	8.6	0	3.3	6.8	0	0.4	2.34	0	0	3.4
5	20	1	0	2.8	5.7	0	1.3	2.6	0	.	0.3	0	0	0.5
		2	0	6	12	0	2	4	0	0.7	3.5	0	0	1.5
		3	0	5	10	0	1.7	3.4	0	0.4	1.5	0	0	4
		4	0	7.4	14.8	0	2.6	5.2	0	1.3	7.1	0	0	3.4
		5	0	7.5	15	0	2.8	5.7	0	0.9	2.8	0	0	7.5
	Mean		0	5.7	11.3	0	2	4.1	0	0.8	3.04	0	0	3.8
6	25	1	0	.	.	0	.	.	0	.	0.2	0	0	0.9
		2	0	.	.	0	.	.	0	0.4	3.5	0	0	2.9
		3	0	3.4	6.5	0	2	4	0	0.4	3	0	0	2
		4	0	7.5	15	0	2.4	4.8	0	0.3	1.9	0	0	5
		5	0	7	14	0	4.1	8.3	0	0.4	3	0	0	2
	Mean		0	5	11.8	0	2.8	5.7	0	0.3.	2.3	0	0	2.5

Table 4: Effect of *Azotobactor* fertilizers on Dicot and Monocot Plantation root and shoot measurements.

Sr. No	Concentration Solution %		Monocot *Zea mays*						Dicot *Vigna radiata*					
			Root length cm			Shoot length cm			Root length cm			Shoot length cm		
	Day		1	3	5	1	3	5	1	3	5	1	3	5
1	Control	1	0	1.5	3	0	0.7	1.4	0	0.4	3.2	0	0	3.3
		2	0	1.4	2.8	0	1.2	2.5	0	0.2	4.5	0	0	2.5
		3	0	2.4	4.9	0	1.3	3.6	0	0.3	3.1	0	0	2.5
		4	0	6	12	0	1.9	3.8	0	0.2	3.3	0	0	3.6
		5	0	5.5	11	0	3	6	0	0.5	5.1	0	0	5
	Mean		0	2.2	6.7	0	1.7	3.4	0	0.3	3.84	0	0	3.38
2	5	1	0	0.6	1.2	0	0.2	0.4	0	.	0.3	0	0	2
		2	0	0.9	1.9	0	0.3	0.7	0	0.3	3.2	0	0	4.6
		3	0	3.9	7.9	0	0.3	0.7	0	0.2	2.7	0	0	2.1
		4	0	2.5	5	0	1	2	0	0.4	3.2	0	0	3.9
		5	0	3.4	6.9	0	0.7	1.5	0	0.3	3	0	0	4.3
	Mean		0	2.3	4.5	0	0.5	1.6	0	0.3	2.5	0	0	3.4
3	10	1	0	3.1	6.2	0	1.6	3.1	0	0	0.3	0	0	0.9
		2	0	2.1	4.5	0	1.5	3	0	0.3	1.1	0	0	3.4
		3	0	4	8	0	2.7	5.5	0	0.6	3.9	0	0	4
		4	0	6.6	13.3	0	3.7	7.5	0	0.8	4.1	0	0	3.2
		5	0	7	14	0	4.1	8.3	0	0.4	2	0	0	2.3
	Mean		0	4.6	9.2	0	2.7	5.5	0	0.5	2.3	0	0	2.7
4	15	1	0	.	.	0	.	.	0	.	0.3	0	0	1.1
		2	0	2.5	5	0	.	.	0	0.4	2.1	0	0	2.5
		3	0	5.4	10.9	0	2.6	5.2	0	0.2	1.4	0	0	3.9
		4	0	4.5	8.9	0	3.6	7.3	0	0.8	5	0	0	3.2
		5	0	4.9	9.8	0	3.8	7.6	0	0.4	2.9	0	0	6.2
	Mean		0	4.3	8.6	0	3.3	6.8	0	0.4	2.34	0	0	3.4
5	20	1	0	2.8	5.7	0	1.3	2.6	0	.	0.3	0	0	0.5
		2	0	6	12	0	2	4	0	0.7	3.5	0	0	1.5
		3	0	5	10	0	1.7	3.4	0	0.4	1.5	0	0	4
		4	0	7.4	14.8	0	2.6	5.2	0	1.3	7.1	0	0	3.4
		5	0	7.5	15	0	2.8	5.7	0	0.9	2.8	0	0	7.5
	Mean		0	5.7	11.3	0	2	4.1	0	0.8	3.04	0	0	3.8
6	25	1	0	.	.	0	.	.	0	.	0.2	0	0	0.9
		2	0	.	.	0	.	.	0	0.4	3.5	0	0	2.9
		3	0	3.4	6.5	0	2	4	0	0.4	3	0	0	2
		4	0	7.5	15	0	2.4	4.8	0	0.3	1.9	0	0	5
		5	0	7	14	0	4.1	8.3	0	0.4	3	0	0	2
	Mean		0	5	11.8	0	2.8	5.7	0	0.3.	2.3	0	0	2.5

Result and Discussion

When Rhizobium was added at a concentration of 20% in monocot and 20% in dicot plantations, the total and beneficial number of nodules, plant height, and root and shoot length all increased significantly. It promotes nodulation, root development, and growth, as well as accelerating maturity and improving crop quality. This increase in nodulation, rooting, and growth could be due to an increase in the amount of nodules, which could have supplied enough nitrogen by nitrogen fixation, increasing green grain productivity.

The presence of *Azotobacter* sp. in soils has beneficial effects on plants, but their abundance is dependent on a variety of factors, including soil physicochemical (e.g., organic matter, pH, temperature, soil moisture) and microbiological properties. The abundance varies according to the depth of the soil profile. *Azotobacteria* are much more common in plant rhizospheres than in surrounding soil, and their concentration varies depending on crop species.

Seed inoculated with *Azotobacter* aids in the absorption of N, P, and micronutrients such as Fe and Zn in monocots; these strains have the ability to boost wheat nutrition. *Azotobacter* seed inoculation significantly increases yield by providing nitrogen to crops. In a greenhouse experiment, seed inoculation with *Azotobacter* increased the carbohydrate and protein content of two corn varieties (Inra 210 and Inra260). The application of manure and *Azotobacter* results in an increase in maize biomass.

Rhizobium is a bacterial genus that is concerned with the development of root nodules on plants. These bacteria coexist with legumes. They absorb nitrogen from the atmosphere and move it on to the plant, allowing it to thrive in low-nitrogen soil.

Conclusion

Seeds treated with *Rhizobium* and *Azotobactor* biofertilizers increased the growth parameters of Mungbean (*Vigna radiata*) and Maize plants significantly (*Zea mays*). Their morphological parameters increased significantly, including shoot length, root length, and overall plant length. As a result, the Maharashtra and Indian governments should encourage the use of biofertilizers because they are both cost effective and environmentally friendly. Apart from its function in promoting plant growth, *Azotobacter* has been linked to a number of diseases.

Defending against pathogenic plant diseases There are numerous references in the literature that support the value of various Azotobacter species in disease suppression.

Azotobacter spp. are non–symbiotic, heterotrophic bacteria that can repair an average of 20kg N per year. Plant Growth

Promoting *Rhizobacteria* (PGPR) are bacteria that synthesise growth substances that promote plant growth and development and inhibit phytopathogenic growth by secreting inhibitors. It also aids in nutrient absorption and the development of biochemical substances such as protein and amino acids. Azotobacter increases seed germination and has a positive effect on crop development.

It improves in increasing nutrient availability and restoring soil fertility for improved crop response. Because of its importance in soil preservation, it is an essential component of an integrated nutrient management system. More research is required in the future to investigate the potential of *Azotobacter* in soil fertility.

The value of the *Rhizobium*-legume relationship is not limited to their symbiotic nitrogen fixation activity or other activities in the soil that may improve soil fertility and plant growth, but some rhizobia strains may be used to protect plants from pests and pathogens.

However, further research into the precise mode of action and adaptation of these microorganisms to different ecophysiological conditions may help to optimise the benefits of rhizobia for improving plant growth and health.

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