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Rhyzobial Bacteria as Biofertilizer

Research Project

Submitted to the Department of (Biology) in partial fulfillment of the requirements for the degree of **BSc.** in **Biology**

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CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the **BSc.** degree in **Biology** with my approval as a supervisor.

Signature
Name:

Date: **April , 2023**

DEDICATION

This effort I dedicate to **Allah** Almighty, my lord, my powerful foundation, my source of inspiration, wisdom, knowledge, and understanding. Throughout this project, he was the source of my energy.

Student Name

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To begin with, I thank (**Allah**) for His blessing, which made me able to complete and perform this study with success, the lord of the universe, blessing, and peace be on **Muhammad** (Allah’s peace and prayers be upon him).

Finally, I want to say thanks to all those I forgot them here to mention his/her name, who assisted me even by one useful scientific word directly or indirectly.

SUMMARY

Rhizobium species are usually defined as nitrogen-fixing soil bacteria capable of inducing the formation of root or stem nodules on leguminous plants in which atmospheric nitrogen is reduced to ammonia for benefit of the plant. The many research results showed that the rhizobial bacteria as biofertilizers improved productivity of plant yield as well as the fertility status of the soil. As plant growth promoting rhizobacteria (PGPR), rhizobia can also be used in non-legumes. Due to their considerable agricultural and environmental significance, these legume symbionts have been extensively studied. The present review is an attempt to summarize the applications of rhizobial bacteria as biofertilizer. Number of research articles have been included in this review to describe rhizobial bacteria and used their as biofertilizer.

Keywords: Biofertilizer, Rhizobial bacteria

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1. INTRODUCTION

Biofertilizer is a special type of compound that enhances soil nutrient by using microorganisms that establish symbiotic relationships with the plants(Begom et al., 2021). One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth-promoting rhizobacteria (PGPR). These bacteria exert beneficial effect on plant growth and development, and many different genera have been commercialized for use in agriculture (Shameer and Prasad, 2018). Biofertilizers are directly or indirectly induce beneficial effects on plant growth and development. Direct mechanisms include production of phytohormones, phosphorous solubilization, and siderophore production. Some indirect mechanisms are that they act as biocontrol agents and induce systemic resistance in plants(Singh et al., 2013). Rhizobial bacteria are the best biofertilizers (Etesami et al., 2009). Rhizobia are bacteria that establish symbiosis with legumes, forming root or stem nodules and fixing atmospheric nitrogen(Etesami, 2022). This symbiotic relationship reduces the requirements for nitrogenous fertilizers during the growth of leguminous crops and also enrich soil with nitrogen. It is one of the most favorable bacteria to agriculture (Akhtar *et al*., 2009). *Rhizobium* the unique ability to produce substantial amounts of organic nitrogen through symbiotic biological nitrogen fixation (Begom et al., 2021). However, they have been reported as biofertilizers in non-nodulating crops like rice, wheat and maize(Hussain *et al*., 2016).Researches about the positive impacts of *Rhizobium* bacteria on non-leguminous plants are relatively new, and the most studies reveal that these bacteria may have beneficial effect on cereal crops (Silva *et al*., 2014). The positive effect on plant growth and development following inoculation with rhizobacteria in non-legume crops is related to various mechanisms other than the formation of root nodule and the biological fixation of N_2 (Saghafi *et al*., 2018). Therefore, the objectives of this study were to determine the effects of rhizobial bacteria as biofertilizer on plant growth and development.

2. LITERATURE REVIEWS

2.1 Rhizobial bacteria as biofertilizer

Rhizobium belongs to family Rhizobiaceae, symbiotic in nature, are rod-shaped cells, 0.5-0.9 μm in width and 1.2-3.0 μm in length. They do not form endospores, are gram-negative. Optimal growth of strains occurs at a temperature range of 25-30 C^o and a pH of 6.0-7.0 (Shakhawat, 2007). Rhizobium has ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like Parasponia. Rhizobium population in the soil depends on the presence of legume crops in the field. In absence of legumes, the population decreases(Mahdi *et al*., 2010). Rhizobia encompass a range of bacterial genera, including *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium,* and *Azorhizobium*. They elicit the formation of specialized organs, called nodules, on roots or stems of their hosts, in which they reduce atmospheric nitrogen and make it available to the plant(Sessitsch *et al*., 2002). In the biofertilizer technology, Rhizobium-legume is most common and widely used in different countries (Al-Shamma and Al-Shahwany, 2014). Recently, it is also found that rhizobia can make an association with graminaceous plants such as rice, wheat, maize, barley millets and other cereals without forming any nodule-like structure or causing any disease symptoms. Increasing the ability of rhizobia in biofertilizer, crop enhancing activity in non-legumes especially cereal grains would be a useful technology for increased crop yields among resource-poor farmers(Mia and Shamsuddin, 2010). Huang and Erickson (2007) treated pea seeds with *Rhizobium leguminosarum* and reported that shoot/root growth and nodulation of pea improved as a result of rhizobia inoculation. Moreover, seed yield of pea was significantly improved. Afzal and Bano (2008) showed that combination between *Rhizobium leguminismarum* and fertilizer (P_2O_5) caused significant increases in root weight, shoot weight and plant height of the wheat plants. Mehboob et al. (2012) studied the yield responses of maize to inoculation with different species of rhizobia, isolated from the root nodules of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* M.) and mung bean (*Vigna radiata* L.) in field. Results revealed that all the selected isolates improved the grain yield and 1000 grain weight in field condition as compared with un-inoculated control. Saber and Khursheed (2019) revealed that soil application of different rhizobial bacteria had a significant effect on some wheat yield components compared with the control treatment (Table1).

Table (1) Effects of different species of rhizobial bacteria on wheat yield components

Rhizobial species	Spike number.plant^{-1}	Spike length (cm)	Spike weight.plant^{-1}	Spikelet number.plant^{-1}	Grain number. plant^{-1}	Weight of 1000grain (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	
Control	2.67	8.07	5.54	17.33	68.75	47.99	1819.77	5492.33	**(Saber and Khursheed, 2019)**
B1	4.000	8.51	7.31	18.50	95.28	48.49	2550.29	7464.03	
B2	3.417	8.33	7.59	18.83	97.08	50.40	2708.82	7650.86	
B3	3.500	8.33	7.53	18.33	95.72	53.47	2823.15	7636.71	
B4	3.833	8.60	8.09	18.83	106.22	47.64	2780.06	7686.72	
B5	3.500	8.80	7.76	19.17	105.83	46.68	2661.63	7517.50	
Tukey0.05	0.91	n.s.	0.70	n.s.	6.58	n.s.	271.42	468.25	

B1: *Bradyrhizobium* sp. (Vigna), B2: *Rhizobium leguminosarum* bv. viciae, B3: *Bradyrhizobium Mungbean*, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

2.2 Mechanism of action

Rhizobium species can promote plant growth and development either directly and indirectly (Singh *et al*., 2013):

1- **Direct stimulation** includes biological nitrogen fixation, producing phytohormones like auxins, cytokinins and gibberellins, solubilizing minerals like phosphorus and iron, production of siderophores and enzymes and induction of systemic resistance.

2- **Indirect stimulation** is basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyze the fungal cell wall and competition for niches within the rhizosphere.

2.3 Role of Rhizobial bacteria in nitrogen fixation

The legume root hair secretes a chemo-attractant which causes the bacteria to accumulate. The bacteria secrete lipochito-oligosaccharides that cause more root hairs to be formed and alter root metabolism. They cause root-hair curvature and the bacteria then attach to the hair by sugar-binding proteins called lectins. An infection thread is then formed, by which the bacteria pass through the root hair to the root cortex, where they proliferate. Cell division is stimulated to form a nodule within which nitrogen fixation occurs. The nodule has good vascular connections through which carbohydrates are supplied to the nodule and nitrogen-containing compounds are exported to the plant(Etesami, 2022).

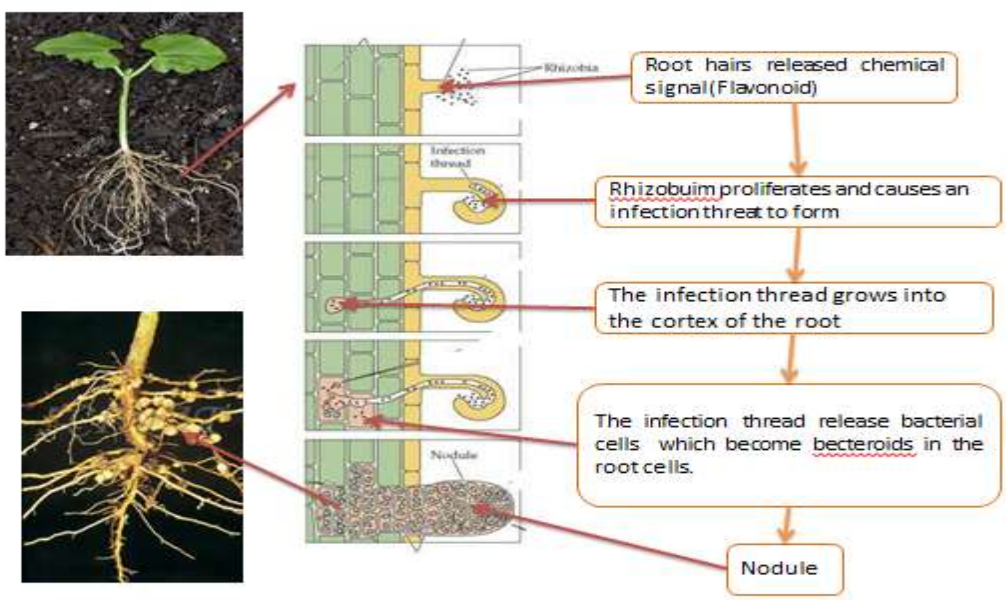

Figure 1 Nodule formation by *Rhizobium* sp.

Figure 2 Rhizobial characteristics

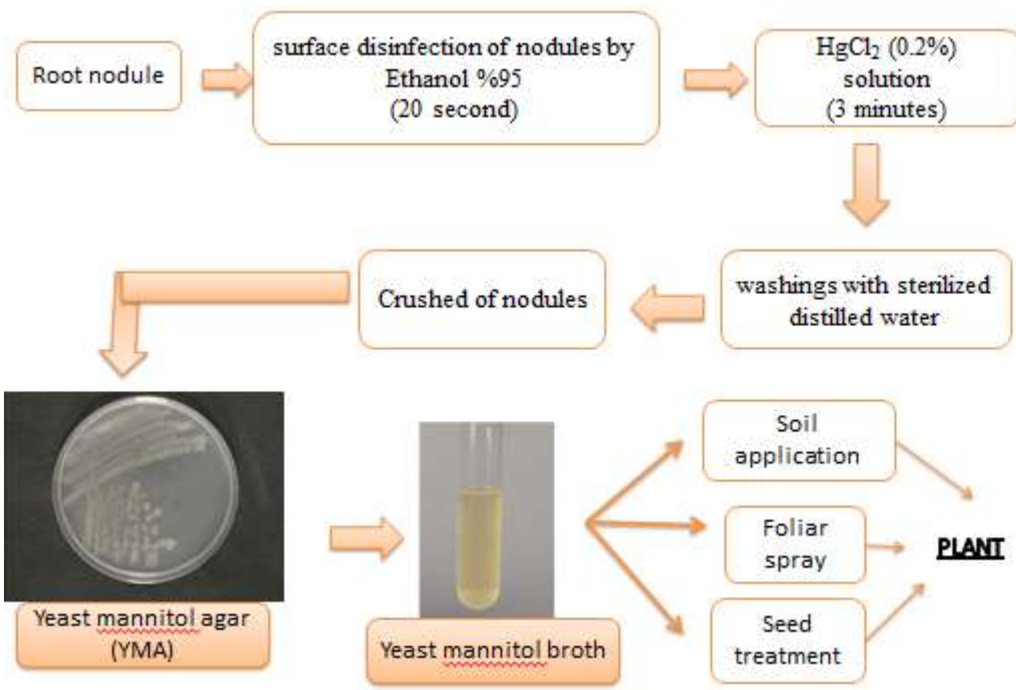

Figure 3 Isolation of Rhizobial bacteria and preparation of inoculum

3. CONCLUSIONS

Based on the reviews of the present study, rizobial bacteria as biofertilizers had the positive effect on legume and non-legume plant growth. Recently, biofertilizers have become an important means in agriculture because they use beneficial rhizobial bacteria to improve plant growth in a more economical and environmentally friendly way.

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