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Evaluation of Growth Promotion Traits of Ridge Gourd in Vitro and In Vivo Conditions Using Soil Rhizobacteria as Green Bioinoculants

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Abstract: ***Plant growth-promoting bacteria (PGPB) are now a days an important source of biofertilizers, due to increasing trend of organic fertilizers and ill effects of chemical fertilizers. PGPBs are efficient soil microbes which enhance the nutrient utilization efficiency, growth of several crops and controlling soil borne pathogens. Ridge gourd (Luffa acutangula L.) is a potential horticulture crop due to its high economic value and belongs to the family, Cucurbitaceae. In the present study, PGPB were isolated from the rhizospheric soil of 3 different crop fields (sugarcane, mustard and wheat) from Meerut, Uttar Pradesh were used to investigate their effect on growth pattern of ridge gourd. The isolates were screened for morphological, biochemical, and plant growth-promoting characterstics. Under in-vitro condition, inoculation of Ridge gourd with the P1, P2, B2, B3, B4, B5, R2, , R5, A2, A3, A5, A6, A7 showed a significant increase in seed germination and enhancement in elongation of root and shoot compared to control. Then isolated PGPB inoculated with Ridge gourd for Pot experiment and P1, P2, B2, B3, B4, B5, R2, R5, A2, A3, A4, A6, A7 showed the enhanced growth parameters as appearance of first leaf, flower and fruit as compared to the control. Hence, it can be concluded that application of PGPB has immense potential to be used as agricultural crop inoculants as they promote plant growth as well as improve the health and yield of the plants.***
Keywords: PGPB, Growth Parameters, Ridge gourd, Bioinoculant

I. INTRODUCTION

Plant growth promoting bacteria (PGPB) are the group of beneficial bacteria that enhance plant growth and biocontrol by wide variety of mechanisms. (*Kloepper J.W. and Schroth M.N. (1978).* In the context of increasing global concern for food and environmental quality, the utilization of PGPB for minimizing chemical inputs in agriculture practices is a potentially important issue. (*Verma P. and Shahi S.K. (2015)* Ridge gourd (*Lufa acutangula)* is an important commercial crop fetching good yields and returns with proper farm management practices. Low investment and high yield make the commercial success of this crop. This vegetable has good fiber and very good for digestion. Nowadays, modern agriculture relies on excessive use of chemical fertilizers and pesticides to increase crop production which caused severe adverse effect on soil health and environment. *(Aktar W., Sengupta D. and Chowdhury A. (2009)* However, use of PGPB in agriculture in order to enhance the growth of plant via circulating the nutrients in the soil is an ecofriendly strategy to minimize the need of synthetic fertilizers as much as possible. (*Saharan B.S. and Nehra V. (2011).* PGPBs can be defined as beneficial bacterial strains that colonize the roots of plant for plant growth stimulation and biocontrol potential.(*Glick B. (1995).* They can affect plant growth by promoting plant-microbe symbiosis, competition for colonization space and nutrients and decreasing the activities of plant pathogens.*(Glick B. (1995) , Lugtenberg B.J., Chin-A-Woeng T.F. and Bloemberg G.V.(2002).* PGPB stimulates plant growth and biocontrol by various direct and indirect mechanisms. Direct mechanism of PGPB includes facilitating resource acquisition i.e. solubilisation of phosphate, nitrogen fixation, iron acquisition by siderophore and modulating proper level of plant hormones like auxins, cytokinins and gibberellins and lowering the level of ethylene by production of ACC deaminase enzyme. *(Patten C.L. and Glick B.R. (1996) , Glick B.R., Penrose D.M. and Li J. , J. Theor. Biol. (1998).* Indirect mechanisms of PGPB include suppression of fungal, bacterial and nematode pathogens by the production of various enzymes and compounds likewise chitinase, protease, cellulase, antibiotics, HCN, ammonia and volatile organic compound (VOCs) etc. Several other mechanisms of indirect growth promotion and biocontrol by PGPB include antagonistic activity, quorum sensing, signal interference, inhibition of biofilm formation, increasing mineral nutrient solubilization, systemic acquired resistance and induced systemic resistance.

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PGPB has been isolated and screened from rhizospheric soil of diverse crops to enhance growth, seed emergence, crop yield and production. PGPB can be used as agricultural inputs with plant growth promoting attributes and as biological control to reduce plant diseases in various crops. PGPB have been commercialized as microbial bioinoculants or biofertilizers to increase crop production. PGPB offers an attractive strategy for replacement and reduction of heavy application of chemical pesticides and fertilizers.

Phosphorus (P) is the second-most plant-essential macronutrient present in the soil after nitrogen and is found in organic and inorganic forms, which might be both insoluble or very poorly soluble inorganic forms. Most of the P occurs in insoluble form as iron and aluminium phosphates in acidic soils and calcium phosphates in alkaline soils. Some of them appear after the application of chemical fertilizers. Due to the formation of insoluble iron and aluminium phosphates in acidic soils and calcium phosphates in alkaline soils, the deficiency of P occurs in soil. To handle the P deficiency in different crops, chemical phosphate fertilizers are regularly added in various amounts to the soil. However, this applied P is precipitated into an insoluble and stable form soon after the application and is available to plants with limited only 5% or less of the total amount of P in the soil. Besides, excessive application of chemical phosphate fertilizers can cause both environmental and economic problems. PSB plays a significant role in the release and mobilization of insoluble and fixed forms of P available to plants in a sustainable and eco-friendly approach to environmental protection. Numerous bacterial species such as *Alcaligenes* sp., *Aerobacter aerogenes*, *Achromobacter* sp., *Actinomadura oligospora*, *Burkholderia* sp., *Pseudomonas* sp., *Bacillus* sp., and *Rhizobium* sp. are capable of solubilizing phosphate in soils. Several studies have investigated the effect of applying PSB, and examining its effect on plant growth.limited work has been done on appliation of PGPB to idge gourd and studying the growth patten.

Therefore, the present study was planned to study the effect of these isolates on ridge gourd to identify the effect of isolated bacteria as inoculants on plant growth parameters.

II. MATERIAL AND METHODS

The study was conducted in the Department of biotechnology and Microbiology MIET Meerut during the March 2023 to June 2023.

A. Collection of Samples

Soil samples were collected in the month of march 2023, from the sugarcane field of vill-Javeri, Kankerkhera, Meerut (UP) region. Samples were placed individually in plastic bags and brought to MIET, Meerut for isolation of bacteria. Rhizospheric bacteria were isolated from 1g soil tightly adhering to the root by serial dilution plating on Luria–Bertani (LB) agar plates as described (*Somasegaran and Hoben, 1994*). Endophytic bacteria were isolated by serial dilution plating of sterilized crushed root samples on LB agar plates as described *(Hameed et al., 2004*). The plates were incubated at 28 ± 2°C till the appearance of bacterial colonies. Individual colonies were picked and streaked on selective media.

B. Physicochemical Analysis of Soil

For this experiment soil samples were analyzed for soil organic carbon (SOC), nitrogen, phosphorus, potassium, and pH of the soil. SOC was evaluated by using the Walkley and Black method (*Walkley and Black, 1965*). The available nitrogen was estimated following Kjeldahl method (*Subbaiah and Asija, 1956*), available potassium by ammonium acetate method (*Hanway and Heidel, 1952*) and pH of the soil samples by a digital pH meter (Mettler-Toledo, Germany).

C. Biochemical Tests

1) *Detection of Indole acetic acid (IAA) producing bacterial isolate:* The production of IAA by bacterial isolates was screened by Salkowski reagent method *(Ehmann, 1977*). Production of IAA by the bacteria was confirmed by the development of the pink color of the broth. Quantification was done by spectrophotometric method, when bacteria grown in LB broth with tryptophan ($100 mgL^{-1}$), incubated at 30±2 °C for 5 days in the dark followed by a change in color post addition of Salkowski reagent.
2) *Catalase Tests :* The catalase test was performed by adding 3% Hydrogen Peroxide solution onto bacterial colony for the evolution of Bubbles (*Schaad,1992).*
3) *Ammonia Production:* Bacterial isolates were screened to produce ammonia in peptone water. Freshly grown culture was inoculated in 10mL peptone water in different test tubes and incubate for 48 to 72 hours after 2-3 days, Nessler's reagent (0.5mL) was added in each tube development of brown to yellow color considered as positive ammonia production (*Cappuccino and Sherman, 1992*).

4) *Hydrogen Cyanide Production:* HCN production was tested qualitatively according to the method of Bakker and Schipper (1987).
5) *Salt Tolerance Test:* Inoculate one or two colonies from an 18- to 24-hour culture into 6.5% , 10% , 12.5% broth. Incubate the tube at 35°-37°C in ambient air for 48 hours. Then examine tubes for turbidity after 24 hours and if negative again at 48 and 72 hours.
6) *PH tolerance tes :* Inoculate one or two colonies from an 18- to 24-hour culture into 9, 6, 10, 5 pH broth. Incubate the tube at 35°-37°C in ambient air for 48 hours. Then examine tubes for turbidity after 24 hours and if negative again at 48 and 72 hours.

III. BIOASSAY-BASED EVALUATION OF PLANT GROWTH PROMOTION

The bioassay on the effect of plant growth promotion of isolates was examined with Ridge gourd (Luffa acutangula) in a pot experiment. In this experiment, three treatments were carried out: (1) seeds sown without any inoculation (control), and (2) seeds treated with the bacterial mass of the various PGPB under in vitro conditions and (3) seeds treated with the bacterial biomass PGPB suspension under pot experiment.

Initially, Ridge gourd seeds were surface sterilized with a treatment of 95% ethanol for 5 min and rinsed with sterilized distilled water for four times. Then Ridge gourd seeds were inoculated with bacterial biomass 1.9×10^8 cfu/ml PGPB suspension separately at room temperature for 24hr. Control seeds were also treated in the same manner with sterilized distilled water.

A. Germination Stage

The treated and control seeds of Ridge gourd were placed on Whatman filter paper (No. 41) moistened with deionized water in a Petri plate for 24 h under room conditions. The seeds were watered twice a day using a hand sprayer. The seedling emergence (%), fresh biomass (g), growth rate (g/day), seedling length (cm), and root length (cm). Seedling emergence was calculated based on the percent germination out of total planted seeds. Fresh biomass was weighted using a digital scale (Samson HI-600K, Edapally, India) following official standardizing international nomenclature (SI: kg and g). The growth rate was expressed as a rate of biomass increase per day, while seedling and root lengths were measured using a calibrated scale (15 cm).

Germination Index = No. of germinated seeds × 100/Total no. of seeds

Relative seed germination=No. of germinated seeds in treatment×100 / No. of seeds germinated in control

B. Pot Experiment

The seeds were grown in pots filled with autoclaved sterilized soil. The experimental design was laid in a randomized block design (RBD). In each treatment, a block of 3 seeds planted in a pot (45 x 45 x 45 cm) at a distance of 4-5 inches was considered as a replicate. Pots were irrigated with sterile distilled water in seven days intervals. The temperature was recorded every day and it was 25–30 °C throughout the experiment. The vines were allowed to creep until the full net was covered. The experiments lasted for 90 days under pot conditions where average temperature and humidity were noted as 29 °C and 55% using a digital thermo-hygrometer.

The plants were watered periodically after three days. In this, the defected vine parts (yellowed leaves, flowers, fruits, etc.) were carefully removed from time to time. The plants were watered periodically after three days using the normal borewell water supply. In this, the defected vine parts (yellowed leaves, flowers, fruits, etc.) were carefully removed from time to time.

For growth evaluation, a total of 12 randomly selected plants from each treatment were uprooted after 45 days of inoculation, plant length (cm), fresh, no. of leaves, and shoot were recorded.

C. Statistical Analysis

MS excel was used to calculate Per cent of each parameter and was expressed as the mean ± SD. Statistical significance was accepted at $P<0.05$.

IV. RESULT AND DISCUSSION

In the present investigation around 105 bacteria were screened for plant growth promoting properties. Bacteria are screened morphologically, microscopically

The strain no. P1, P2, P3, R1, R2, R3, R4, R4, R5, R6, B1, B2, B3, B4, B5, A1, A2, A3, A4, A5, A6, A7 showed Gram's negative results while B1, B2, B3, B4, B5 showed Gram's positive results.

TABLE I
MORPHOLOGICAL FEATURES OF BACTERIAL ISOLATES

Strain no.	Gram's staining	Shape	Arrangement
P1	Negative	Rod	Single
P2	Negative	Rod	Single
P3	Negative	Rod	Single
B1	Positive	Rod	Chain
B2	Positive	Rod	Single
B3	Positive	Rod	Chain
B4	Positive	Rod	Single
B5	Positive	Rod	Chain
R1	Negative	Rod	Single
R2	Negative	Rod	In group
R3	Negative	Rod	In group
R4	Negative	Rod	Single
R5	Negative	Rod	Single
R6	Negative	Rod	In group
A1	Negative	Cocci	Cluster
A2	Negative	Cocci	Single
A3	Negative	Cocci	Single
A4	Negative	Cocci	Cluster
A5	Negative	Cocci	Cluster
A6	Negative	Cocci	Single
A7	Negative	Cocci	Single

The PGP Characterization of isolated PGPB based on the difference in the biochemical test of isolated PGPB. The strain no. P1, P2, P3, B1, B2, B3, B4, B5, R1, R2, R3, R4, R5, R6, A1, A3, A4, A5 showed positive results while A2, A6, A7 showed negative results for Catalase activity showed in table. The strain no. P3, B1, B2, B3, B4, B5, R1, R2, R3, R4, R5, R6, A1, A2, A3, A4, A5, A6, A7 showed positive results for Indole Acetic Acid while P1, P2 showed negative results shown in table. The strain no. P3, B1, B3, B4, R1, R3, R4, R6, A7 showed positive results for Organic Acid Production while P1, P2, B2, B5, R2, R5, A1, A2, A3, A4, A5, A6 showed negative results shown in table .
The strain no. P1, P2, P3, B1, B2, B3, B4, B5, R3, R4, R5, A1, A2, A3, A4, A5, A6, A7 showed positive results for Ammonia Production while R1, R2, R6 showed negative results shown in table

TABLE II
BIOCHEMICAL TEST

Bacterial isolates	Catalase test	Indole test	Organic acid Prod.	Ammonia Prod.
P1	+	-	-	+
P2	+	-	-	+
P3	+	+	+	+
B1	+	+	+	+
B2	+	+	-	+
B3	+	+	+	+
B4	+	+	+	+
B5	+	+	-	+

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R1	+	+	+	-
R2	+	+	-	-
R3	+	+	+	+
R4	+	+	+	+
R5	+	+	-	+
R6	+	+	+	-
A1	+	+	-	+
A2	-	+	-	+
A3	+	+	-	+
A4	+	+	-	+
A5	+	+	-	+
A6	-	+	-	+
A7	-	+	+	+

TABLE III
IDENTIFICATION OF ISOLATED PGPB ON THE BASIS OF SALT CONCENTRATION, PH, AND TEMPERATURE RANGE

STRAINS	Salt conc.			pH			Temperature Range			
	10%	15%	20%	7	4.5	12	20°c	30°c	-4°c	45°c
P1	-	-	-	+	-	-	+	+	-	-
P2	+	+	+	+	-	-	+	+	-	-
P3	+	+	-	+	-	-	+	+	-	-
B1	+	+	+	+	-	-	+	+	-	-
B2	+	+	+	+	-	-	+	+	-	-
B3	+	-	+	+	-	-	+	+	-	-
B4	+	+	+	+	-	-	+	+	-	-
B5	+	+	+	+	-	-	+	+	-	-
R1	+	+	+	+	-	-	+	+	-	-
R2	+	+	+	+	-	-	+	+	-	-
R3	+	+	+	+	-	-	+	+	-	-
R4	+	+	+	+	-	-	+	+	-	-
R5	+	+	+	+	-	-	+	+	-	-
R6	+	+	+	+	-	-	+	+	-	-
A1	+	+	+	+	-	-	+	+	-	-
A2	+	+	+	+	-	-	+	+	-	-
A3	+	+	+	+	-	-	+	+	-	-
A4	+	+	+	+	-	-	+	+	-	-
A5	+	+	+	+	-	-	+	+	-	-
A6	+	+	+	+	-	-	+	+	-	-
A7	+	+	+	+	-	-	+	+	-	-

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Twenty-one bacterial strains based on their morphological and biochemical characters, abiotic stress tolerance *were* used for inoculation of Ridge gourd. The organisms were tentatively identified as Rhizobium (R), Pseudomonas (P), Azotobacter (A), Bacillus(B). Following are the results after inoculation of ridge gourd at seedling stage and in pot experiment.

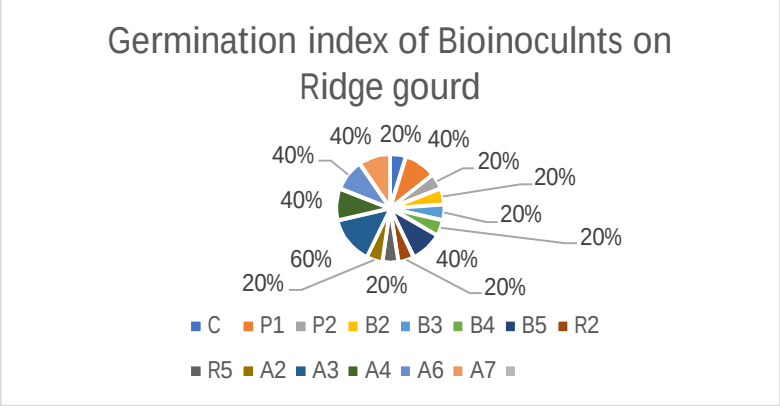
A. Physicochemical Analysis of Soil

The soil used in pot experiment was analyzed for their physicochemical properties and the results revealed pH (7.18 ± 0.05), NO_3-N (2.07 ± 1.380 g/kg), and PO_4-P (1.22 ± 1.10 g/kg) SOC 42 % indicating the soil deficient in nitrogen and phosphorus and moderate in carbon level. The result obtained in this study demonstrated higher SOC contents in the soil Which decides the Cation exchange capacity of the soil. It is source of various nutrients in the soil e.g. more than 95 per cent of nitrogen and sulphur to plants is provided by the soil organic matter. Soil Organic Matter (SOM) is the central indicator of soil quality and health, which is strongly affected by agricultural management (Farquharson et al 2003).

B. Effect of PGPB on Germination and Seedling Growth

The treatment of PGPB on seed inoculation showed significant increase in germination percentage, seedling biomass, seedling length and root length as depicted in the table and pie chart.

It was observed that germination of the seeds started after 10 days of sowing. In-vitro condition, inoculation of Ridge gourd with the P1, B1, R, R2, R3, R5, A2, A4, A5, A6, A7 showed a significant increase in seed germination. It was seen that 40% in P1, 20% in P2, 20% in B2, 20% in B3, 20% in B4, 40% in B5, 20% in R2, 20% in R5, 20% in A2, 60% in A3, 40% in A4, 40% in A6 and 40% in A7 seeds were germinated(as shown in pie chart). It was also found that mean length of P1, B5, A3, A4, A6, A7 is also highest than that of other treatments. The mean germination percentage is obviously higher in A3(60%) than other treatment. The result revealed that seeds of strains P1, B5, A3, A4, A6, A7 recorded higher germination (40-60%) after 30 days. It is very interesting that there was no germination in control, and water soaked lots of seeds during the first reading.



The results of fresh biomass (g), growth rate (g/day), seedling length (cm), and root length (cm) by various PGPB treatments are shown in Fig 1. The results showed maximum increase with A3 treatment. The highest seedling biomass, growth rate, seedling length, and root length were recorded as 8.6 ± 0.3 g, 2.1 ± 1.0 g/day, 12.5 ± 0.3 cm, and 10.4 ± 0.3 cm, respectively.

Table IV
Evaluation of relative effect of pgpb on the shoot length and shoot weight on day 20 and day 45

STRAINS	DAY 20			DAY 45		
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
C	21.2 ±1.2	1.2± 0.9	1.3± 0.1	48.8 ±2.1	2.8±1.3	2.3±1.0
P1	110.1±3.6	12.9±0.9	1.3±0.4	201.1±6.2	18.7±3.1	2.3±1.4
P2	33.0±2.1	3.8±1.3	0.5±0.2	99.2±3.9	10.1±0.3	1.2±1.0

P3	-	-	-	-	-	-
B1		-	-	-	-	-
B2	48.6±2.2	3.9±0.9	0.6±0.0	80.8±3.8	5.8±2.3	2.2±0.9
B3	58.6±2.6	2.8±1.1	0.9±1.2	78.4±3.1	6.9±0.8	2.5±2.2
B4	29.6±3.1	3.9±1.2	1.3±1.5	90.2±4.8	8.8±3.2	1.9±1.1
B5	56.2±2.9	6.0±2.1	1.5±1.6	111.2±5.1	19.7±3.5	2.0±1.9
R1	-	-	-	-	-	-
R2	38.2±2.3	4.2±1.9	0.5±0.3	89.3±2.9	16.5±3.9	1.2±0.8
R3	-	-	-	-	-	-
R4	-	-	-	-	-	-
R5	41.2±2.1	7.8±4.1	0.9±0.5	98.7±3.1	17.9±4.6	1.5±1.0
R6	-	-	-	-	-	-
A1	-	-	-	-	-	-
A2	68.9±3.1	21.4±3.2	1.9±1.8	190.1±4.5	71.4±4.9	2.6±2.0
A3	112.0±3.4	17.5±3.1	2.1±1.9	221.1±5.1	59.2±3.9	2.8±2.1
A4	115.1±3.1	19.6±3.9	2.2±1.4	229.6±6.3	61.5±4.1	3.1±1.8
A5	-	-	-	-	-	-
A6	98.8±2.9	18.8±3.8	1.8±1.1	211.1±6.1	52.4±4.7	2.1±1.5
A7	109±3.1	22.4±3.4	2.2±1.6	234.9±6.8	44.4±4.4	3.1±1.8

V. FIGURES

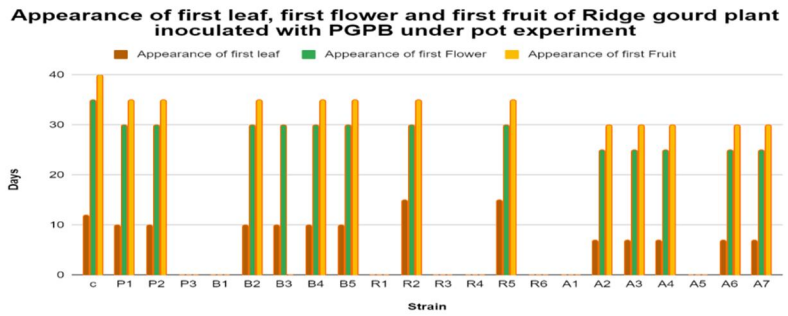


FIGURE 1. The *Azotobacter* strains showed best results in appearance of first leaf and first fruit followed by *Pseudomonas strains.*

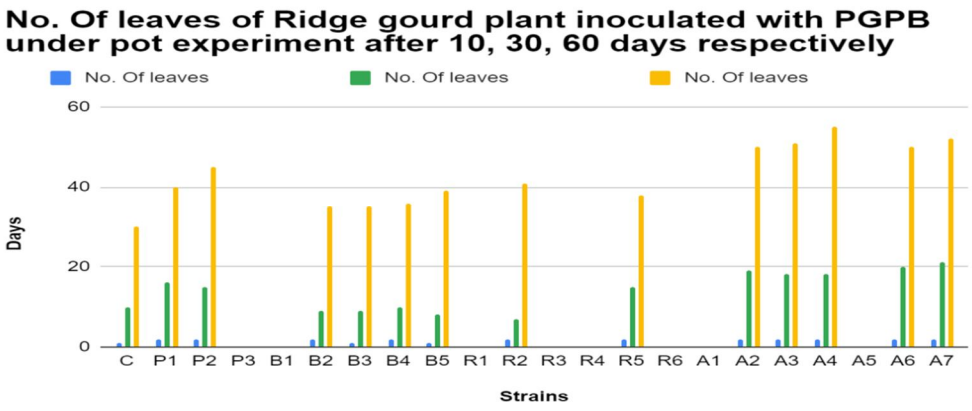


FIGURE 2. The best results for the No. Of leaves after 10, 30, 60, days respectively have been shown by P1, P2, B2, B3, B4, B5, R2, R5, A2, A3, A4, A6, and A7.

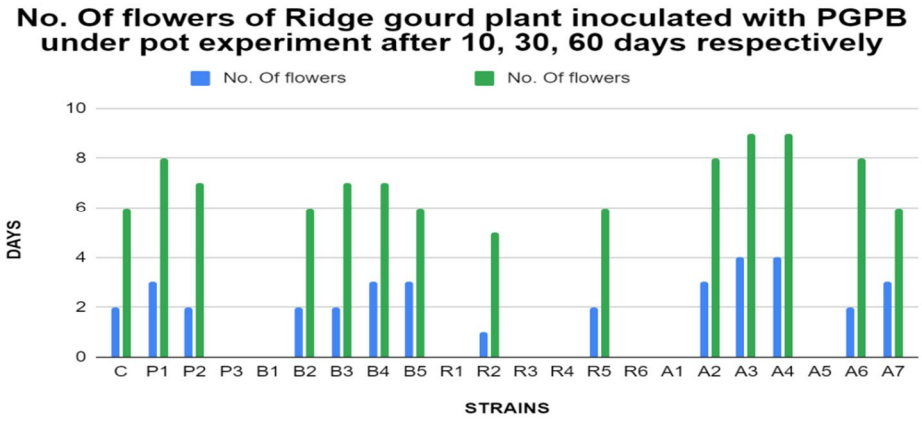


FIGURE 3. The best results for the No. Of Flowers after 10, 30, 60, days respectively have been shown by P1, P2, B2, B3, B4, B5, R2, R5, A2, A3, A4, A6, and A7.

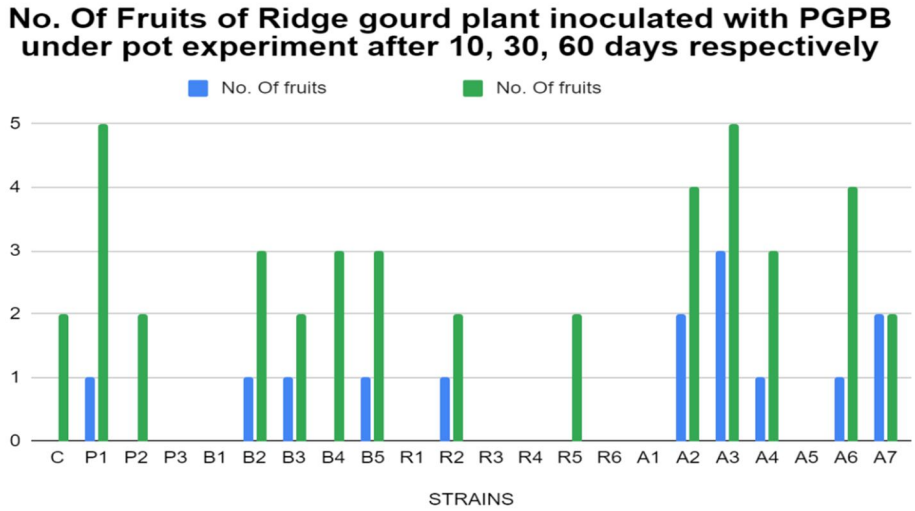


FIGURE 4. The best results for the No. Of Fruits after 10, 30, 60, days respectively have been shown by P1, P2, B2, B3, B4, B5, R2, R5, A2, A3, A4, A6 and A7.

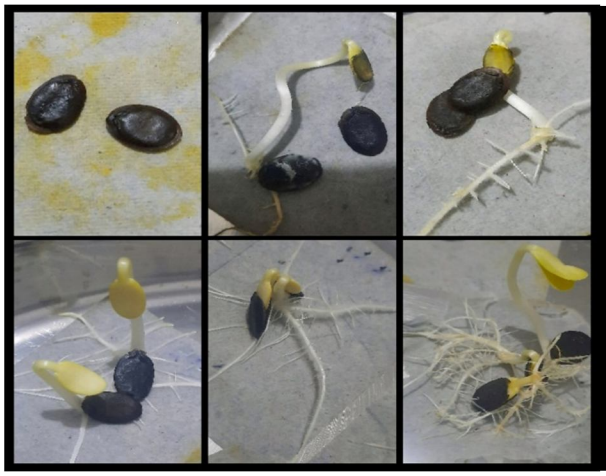


Figure 5. Effect of Bioinoculants in-vivo condition



Figure 6. Effect of bioinoculants in-vitro condition

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Abiotic stress tolerance was exhibited by several isolates in the current investigation, suggesting that bacteria are adjusting to the changing environment and creating particular secondary metabolites that will function as factors for stimulating plant development, as evidenced by enhanced growth metrics. Numerous studies have demonstrated the positive effects of PGPB treatment on plant growth and yield (Pandey and Gupta, 2020; Fahsi et al., 2021). Beneficial bacteria can improve plant health and growth through both direct and indirect mechanisms of action (Saleemi et al., 2017). Phytohormones and enhanced nutrient input from the environment are examples of direct processes that have an immediate impact on plants (Backer et al., 2018; Basu et al., 2021). Through solubilization or mineralization, some bacteria may convert insoluble inorganic nutrients and their organic counterparts into soluble ones that plants can absorb. Numerous crops, including cotton and wheat, have been found to benefit from strains that can solubilize zinc from sources such as zinc oxide and other sources (Kamran et al., 2017; Ahmad et al., 2021). By promoting root and shoot development, several biopriming chemicals or bio stimulants also improve plant resistance. According to Van Oosten et al. (2017), this enables plants to investigate deeper soil layers during the dry season, promote the synthesis of suitable solutes to reestablish favourable water potential gradients, and boost water intake when soil water levels decrease. Additionally, some bacteria may indirectly promote plant development by suppressing pathogens or improving abiotic stress tolerance (Grover et al., 2021). By causing positive changes in physical, chemical, and biological responses, PGPB can increase plant tolerance to abiotic stress (Fatima et al., 2020; Fatima and Arora, 2021; Grover et al., 2021).

VI. CONCLUSION

Chemical fertilizers, herbicides, and pesticides must still be used today for an agricultural practice to be effective. They initially promote plant growth but eventually have a detrimental impact. This standard has impacted the earth and its inhabitants, but it has also put human life in danger through the food chain. Due to a lack of government policy and inadequate knowledge, PGPR has not been widely adopted in India in Ridge gourd (vegetable crops) despite decades of advocacy. The PGPB in the present investigation applied to the seeds of Ridge gourd positively affected the seedling emergence, plant growth and measurements, as well as the positively influence the production traits. The performance of each of the examined PGPB, could be attributed to the soil and climatic conditions of the experimental field. Further research is required to determine, the strain and the species of the PGPB, the quantity and the time of application for different crops to improve the eco environmental safety of our community.

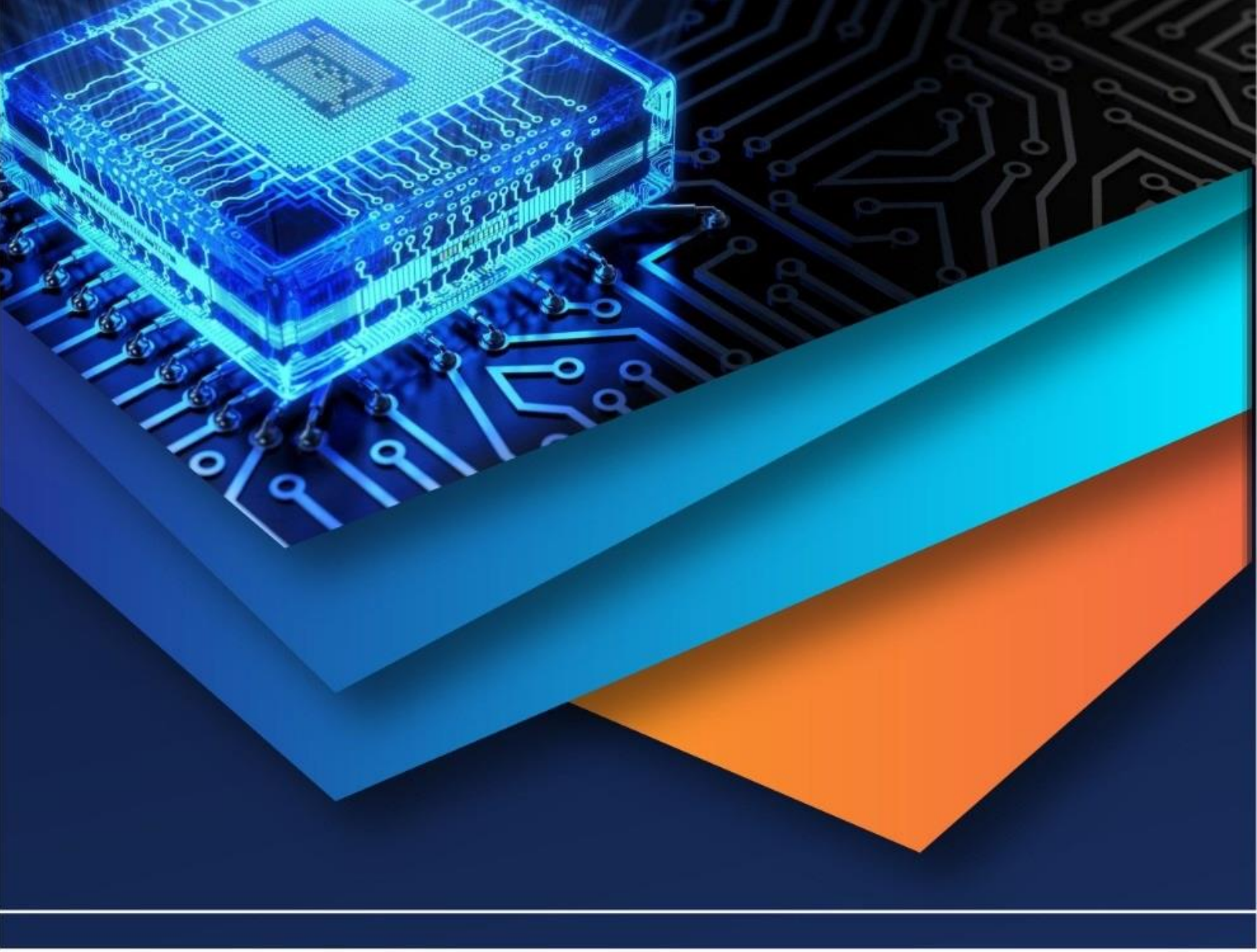
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