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Topic: **EVALUATING THE COMBINED EFFECT OF PGPR AND BIOCHAR ON THE GROWTH OF GROUNDNUT PLANT**

EVALUATING THE COMBINED EFFECT OF PGPR AND BIOCHAR ON THE GROWTH OF GROUNDNUT PLANT

Abstract

In the current situation, there is a growing interest in reducing the use of chemical fertilizers and pesticides for the development of organic agriculture. The use of biomass and plant growth promoting rhizobacteria (PGPR) is an environmentally friendly alternative that can improve soil conditions and increase ecosystem productivity. However, the impact of biochar and PGPR fertilization on forest plantations is not well understood. Mass production of agricultural by-products, i.e. pressed mud. These are either burned or thrown directly into landfills. Management of agricultural by-products can be managed through solid state fermentation, turning them into value-added products such as soil conditioners, compost, single-cell proteins, fungi, enzymes, organic acids, biogas, wax, feed for animals and plants. growing materials etc. This study focuses on the management of press mud by converting sugar industry by-products into various value-added. Therefore, this study focuses on the agro-industrial by-product of pressed mud for value-added products. This study is important because it covers the management and added value of press mud, a by-product of the agricultural industry. It is not only environmentally friendly, but also economical. The aim of this study is to investigate the effects of biochar, press mud and PGPR application on soil nutrients and bacterial communities. To achieve this goal, we used following treatments of only seed and seed + PGPR as controls and 1%,3%,5%,7% of biochar and 1%, 5%, 10%, 15% of press mud with PGPR and without PGPR . For each plant sample, various physical and biochemical properties (Plant height, root length, shoot length, number of leaves, number of shoots, no of roots, dry weight and fresh weight) (Sugar content, total chlorophyll, protein content and proline content) were analysed. The results showed that the simultaneous application of biochar, press mud and PGPR fertilization significantly increased soil fertility as compare to that of control. Biochar and press mud treatment also improved physical and biochemical parameters of ground nut plant as compare to control plant.

Introduction

In today's world, especially in developing countries, maintaining sustainable food security is extremely difficult. Significant threats to long-term food security are rapid population growth in developing countries, including South Asia, Southeast Asia, and Africa, and global climate change affecting business and agricultural production. According to the United Nations Food and Agriculture Organization, more than 2 billion people do not have enough food. The COVID-19 pandemic has exacerbated food security. Food and Agriculture systems have

already undergone major transformations, but much more needs to be done in light of the changing global environment. For years, agriculture has continued to use many dangerous and expensive pesticides to improve crop yields.

Chemical fertilizers are commonly used in modern agriculture to provide essential nutrients to crops and increase crop yields. However, their excessive and indiscriminate use can have harmful effects on the environment and human health. One major problem with chemical fertilizers is that they can lead to soil degradation and nutrient depletion. When chemical fertilizers are overused, they can make the soil more acidic or alkaline, which can reduce soil fertility and decrease the availability of certain nutrients for plant uptake. This can result in a decline in soil health, reduced crop yields, and increased susceptibility to pests and diseases. In addition to harming the soil, chemical fertilizers can also contribute to water pollution. When chemical fertilizers are applied to crops, they can leach into groundwater and surface water sources, causing eutrophication (an excess of nutrients) in aquatic ecosystems. This can lead to algal blooms, oxygen depletion, and the death of aquatic organisms, which can have serious implications for human health, recreation, and the economy. Moreover, the production and transportation of chemical fertilizers require a significant amount of energy, which leads to greenhouse gas emissions and contributes to climate change. The overuse of chemical fertilizers also contributes to the loss of biodiversity, as it promotes the growth of monoculture crops and reduces the diversity of plant and animal species in agricultural landscapes. Finally, there is growing evidence that exposure to chemical fertilizers can have negative impacts on human health. For example, farmers and farm workers who handle and apply chemical fertilizers may be exposed to toxic chemicals that can cause respiratory problems, skin irritation, and other health issues. Moreover, consuming food that has been grown with chemical fertilizers may expose consumers to residual levels of these chemicals, which can have long-term health effects. (5)
To support organic farming, there is currently a great deal of interest in minimizing the use of chemical fertilizers and pesticides. The global climate is experiencing a drastic depletion of soil nutrients due to various anthropogenic activities, burning of fossil fuel, and excess use of agrochemicals. The addition of organic matter to soil can enhance its nutrient content, chemistry, and most crucially, structure.

PGPR (Plant Growth Promoting Rhizobacteria) and biochar are two important agricultural technologies that are gaining increasing attention from farmers and researchers alike. PGPR are a group of bacteria that colonize the rhizosphere (the soil around the roots of plants) and promote plant growth by various mechanisms such as production of phytohormones, fixation of atmospheric nitrogen, solubilization of minerals, and protection against pathogens. PGPR can also enhance plant tolerance to abiotic stress such as drought, salinity, and heavy metal toxicity. The use of PGPR as biofertilizers has several advantages over chemical fertilizers, including improved soil health, reduced environmental pollution, and increased crop yields. Some examples of PGPR include Azospirillum, Pseudomonas, Bacillus, and Rhizobium. Biochar, on the other hand, is a type of charcoal that is produced by pyrolysis (heating in the absence of oxygen) of organic materials such as wood, agricultural waste, and animal manure. Biochar has a high surface area and high porosity, which makes it an excellent soil amendment for improving soil fertility, water retention, and nutrient availability. (4)

In low fertility soils, applying biochar as a soil amendment may be viable, especially when combined with another soil amendment and when the potential long-term C storage benefits in agricultural soils are also taken into account. Because of its high internal porosity and substantial surface area, biochar is a potential choice as a carrier material due to its capacity

to adsorb organic chemicals and bacteria. Plant development and the physical, chemical, and biological characteristics of the soil can all be improved by adding biochar to the soil. (6) Through the process of pyrolysis or dry carbonization, biomass is burned in anaerobic conditions at temperatures below 1000 °C to produce biochar, an activated carbon (C) soil conditioner. (7) Improved soil health and cation exchange capacity have drawn a lot of attention to biochar. It is often high in ash, pH, and surface area and helps rice crops produce more effectively. Because of its affordability and benefits for food security, waste biomass is now widely employed to produce biochar .(8) The increased availability of crucial nutrients in the soil, namely K+, and the reduction in Na+ absorption are the direct mechanisms of biochar. The indirect process entails enhancing the biological, physicochemical, and enzymatic activity of the soil, all of which improve the plant's water status. In dry conditions, biochar significantly boosted the soil's ability to hold water as well as its chlorophyll content. (9)

Biochar can also sequester carbon from the atmosphere and mitigate climate change. The use of biochar in agriculture can increase crop yields, reduce the need for chemical fertilizers and water, and improve soil health. (10) Biochar can also be used for wastewater treatment and as a feedstock for energy production. When PGPR and biochar are used together, they can have synergistic effects on plant growth and soil health. PGPR can improve the colonization and activity of beneficial microorganisms in biochar-amended soils, while biochar can enhance the survival and activity of PGPR by providing a stable habitat and a source of nutrients. The combination of PGPR and biochar can improve soil structure, water holding capacity, nutrient cycling, and plant growth, while reducing greenhouse gas emissions and environmental pollution. PGPR and biochar are two agricultural technologies that have great potential to enhance the sustainability and resilience of farming systems. The use of these technologies can contribute to the achievement of multiple Sustainable Development Goals, such as reducing poverty, improving food security, mitigating climate change, and promoting sustainable agriculture.

Press mud, also known as filter cake, can increase soil fertility and foster environments that make metals less hazardous. By balancing the pH of the soil, press mud enhances soil quality. Press mud is an important source of organic carbon and NPK. Many research have been conducted to determine its viability for usage in agriculture and energy production. The use of press mud as an organic amendment enhances the structure and health of the soil. The effectiveness of microbial transformation is increased by the ability of press mud to serve as a substrate for microorganisms.

Materials and Methods

Bacterial Culture, groundnut, and Biochar

B. megaterium strain (RGKP3) was collected from Atmiya university, department of biotechnology. Groundnut seeds were obtained from retail shop.

The biochar was collected from Ebee and press mud was collected from Ankleshwar (sugar industry).

reviving bacterial strain

Recover the PGPR strain from -70°C storage by plating on fresh nutrient agar and incubating the plate at 25°C. Pick one colony with a sterile inoculation loop and transfer to 100 ml of

sterile nutrient broth (Biolab) in a 250 ml Erlenmeyer flask. The culture was then grown for 2 days in a shaking incubator at 180 rpm and 25 °C. Broth containing bacteria at a concentration of 1 x 108 colony forming units/ml (CFU/ml) was used as inoculum in plant bioassays.

Surface Sterilization and Germination of seeds

Groundnut seeds were sorted to eliminate broken, small, infected seeds. Sodium hypochlorite solution was used for seed sterilization. Finally, seeds were washed thrice with ethanol (95%) followed by three washings with sterilized deionized water. Lay cotton on surface of petri plate. Spray cotton with distilled water, Place 5 seeds in a row evenly spaced 2cm from the top of the cotton. Cover the petri plate properly with the lid by tucking a filter paper underneath the bottom portion of the lid. Put for four days, or until tops of seedlings appear, in sterile environment. Throughout the course of four days, lightly mist the seed with distilled water at regular intervals, once in a day. Seeds were germinated in 85 mm × 15 mm tight-fitting Petri dishes with 10 mL of water. After plants have sprouted, remove immediately to stop the formation of fungus.

Seeds were kept for germination for 4 days and were daily watered.
Once the seeds are germinated properly on the 4th day seedling length was measured to calculate the following

seed germination percentage = ***N/N1*100***

vigour index = ***Ni/Di***

mean germination = ***Ni Di/Germination***

germination rate = ***seedling length * % germination***

Bacterization of Seeds

B. megaterium broth were used for the inoculation of germinated seeds. The PGPR strain was inoculated in a flask containing LB broth and kept for overnight incubation in an orbital shaker. Next day check the optical density to be 0.7 to 0.8 for attaining 1*10*8 CFU. Germinated seeds were first placed with sterile forceps into a flask containing bacterial suspension for 30 min before planting, were air-dried, and then planted in plastic pots containing 2Kg garden soil.

Pot Experiment

The effect of rhizobacteria on the growth of groundnut was studied in pot experiments. All the experiments were carried out in a randomized block design (RBD) with three replications. Experimental treatments included un-inoculated control (soil without biochar) and soil with four levels of biochar (1%, 3%, 5%, 7%), and seeds were co-inoculation with *B. megaterium as mentioned earlier.* The application rates of biochar were as follows; control 1, having no biochar (BC_0), control 2 having seeds co-inoculated with PGPR) and no biochar, followed by

pots with increased concentration of biochar treatment (containing seeds treated/co-inoculated with PGPR) as 1% w/w biochar ($BC_{1\%}$), 3% w/w biochar (BC_3), 5% w/w biochar (BC_5), 7% w/w biochar (BC_7) of total soil contained in the pot. The plants were grown in conditions at 24 °C during the day and 16 °C at night for 30 days.

Physical and Biochemical analysis

Physical and biochemical analysis of plants was carried out after the period of 1 month. Plants were carefully taken out from the pots on the day of completion of 1 month period.

Analysis of biochemical parameters:

Chlorophyll estimation:

Chlorophyll estimations were made according to Arnon (1949). Fresh leaves (0.1g) were mixed with 5ml of 80% (w/v) acetone. Homogenized mixture was centrifuged at 2000 rpm for 5mins to clear the suspension. The supernatant was used for chlorophyll determination. The OD of the solution was measured at 645nm (chlorophyll a), 663nm (chlorophyll b). Acetone (80%) was used as blank.

Chlorophyll a = 12.7 x A663 -2.69 x A645

Chlorophyll b = 22.9 x A645 - 4.68 x A663

Total chlorophyll = (12.7 x A663) + (22.9 x A645)

Proline content:

The leaves and the bulb proline content were determined following the method of Bates et al., (1973). 0.5g of plant tissue was grinded in 5ml of 3% aqueous sulphosalicylic acid. Filtrate (2ml) was taken in a test tube to which were added glacial acetic acid (2ml) and acidic ninhydrin reagent (2ml) and after heating at 100°C for 1h. Then cooling at room temperature. The toluene (4ml) was added to the reaction mixture and the color intensity of the toluene was measured at 520nm against toluene blank. The amount of proline was calculated from the following formula:

Proline content (mg. g-1) = K value × dilution factor ×Absorbance (O.D)/weight of the sample

K value = 19.6

Protein content:

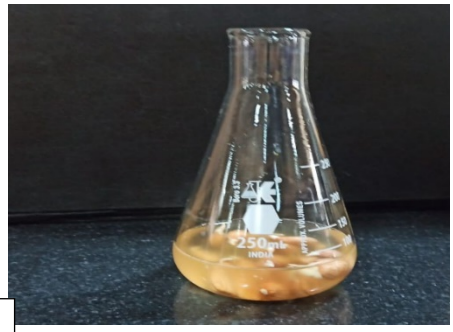
The method of Lowry et al., (1951) was followed for protein determination in leaves. Bovine Serum Albumin (BSA) was used as standard for quantification of protein content of leaves.

sugar content:

The method of nelson somogi et al., (1951) was followed for sugar determination in leaves. was used as standard for quantification of sugar content of leaves.

Results:

(a)



(b)

Figure1: a) germinated seeds (day 4),(b) bacterization of seeds

Vigour index

Parameters	Result
Germination % = N/N1*100	**97%**
Germination rate = Ni/Di	**24.25**
Mean Germination = Ni Di/Germination	**3.88**
Seed vigour index = seedling length * % germination	**349.2**

Biochar Treatment	**Plant height**	**Root length**	**Shoot length**	**Dry weight**	**Fresh weight**	**No. of roots**	**No. of shoots**	**No. of leaves**
Seed + soil	9.6 ± 5.0	1.5 ± 1.0	7 ± 2.0	0.07 ± 0.02	0.66 ± 0.38	1.6 ± 0.2	4.6 ± 0.1	18.6 ± 0.4
Seed + soil +PGPR	11.6 ± 6.0	3.1 ± 3.0	8.5 ± 3.5	0.11 ± 0.03	1.07 ± 1.13	1 ± 0.1	5 ± 0.3	20 ± 0.2
1%	13.6 ± 4.0	7 ± 2.0	10.5 ± 1.0	0.15 ± 0.06	1.31 ± 0.12	1.3 ± 0.1	5 ± 0.2	22 ± 0.8
3%	17 ± 2.0	3 ± 2.0	14 ± 4.0	0.12 ± 0.03	2.18 ± 1.7	1. 3 ± 0.1	5.6 ± 0.3	24 ± 0.4
5%	14.3 ± 8.0	2.6 ± 5.0	11.6 ± 3.0	0.17 ± 0.05	1.53 ± 1.6	1.3 ± 0.1	6.3 ± 0.1	25.3 ± 0.2
7%	17.4 ± 1.0	6.5 ± 3.0	13.3 ± 1.0	0.19 ± 0.07	1.99 ± 1.15	1.3 ± 0.1	6 ± 0.2	23.3 ± 0.11
1% + PGPR	15.1 ± 7.0	4.1 ± 2.5	11 ± 4.5	0.07 ± 0.02	2.24 ± 2.23	2.6 ± 0.1	6.3 ± 0.2	26.3 ± 0.3
3% + PGPR	15.6 ±	4.3 ±	10.8 ±	0.22 ±	1.48 ±	1 ±	6.3 ±	25.3 ±

	5.0	3.0	2.0	0.03	1.14	0.1	0.3	0.11
5% + PGPR	18.5 ± 3.0	6 ± 4.5	12.5 ± 1.5	0.17 ± 0.05	1.69 ± 0.57	6.6 ± 0.4	6.3 ± 0.1	25.3 ± 0.4
7% + PGPR	15.3 ± 3.5	3.5 ± 2.5	11.5 ± 1.5	0.15 ± 0.03	1.56 ± 0.48	2.3 ± 0.1	6 ± 0.2	24 ± 0.8

Table : 1

Press mud Treatment	**Plant height**	**Root length**	**Shoot length**	**Dry weight**	**Fresh weight**	**No. of roots**	**No. of shoots**	**No. of leaves**
Seed + soil	8.5 ± 5.5	2.8 ± 1.5	5.6 ± 5.0	0.22 ± 0.02	1.0 ± 0.3	3 ± 0.4	3.6 ± 0.1	14.6 ± 0.4
Seed + soil +PGPR	30.6 ± 6.0	3.6 ± 1.0	27 ± 7.0	0.35 ± 0.14	2.7 ± 1.7	4.3 ± 0.6	8 ± 0.1	32 ± 0.12
1%	20.2 ± 2.5	4 ± 2.0	16.2 ± 4.5	0.24 ± 0.03	1.8 ± 0.4	7.5 ± 0.1	6 ± 0.1	23 ± 0.2
5%	12.6 ±	3.5 ± 1.0	9.1 ± 6.5	0.26 ± 0.05	1.26 ± 0.5	3.6 ± 0.3	4.3 ± 0.1	17.3 ± 0.4
10%	23 ± 2.0	4 ± 1.0	19 ± 2.0	0.31 ± 0.03	2.18 ± 0.03	12 ± 0.3	5 ± 0.4	20 ± 0.2
15%	30.1 ± 5.5	5.6 ± 1.0	24.5 ± 4.5	0.40 ± 0.03	3.1 ± 1.4	9 ± 0.6	7.3 ± 0.3	29.3 ± 0.12
1% + PGPR	35.5 ± 6.0	6.5 ± 1.0	29 ± 5.0	0.43 ± 0.04	3.2 ± 1.5	6 ± 0.2	6 ± 0.1	23 ± 0.2
5% + PGPR	25.5 ± 9.0	3 ± 2.0	22.5 ± 5.0	0.21 ± 0.06	2.4 ± 1.4	7 ± 0.9	6.3 ± 0.2	25.3 ± 0.8
10% + PGPR	31.6 ± 3.0	6.3 ± 4.0	25.3 ± 7.0	0.24 ± 0.03	3.1 ± 1.0	8.3 ± 0.13	8 ± 0.2	31.6 ± 0.15
15% + PGPR	35.1 ± 9.5	5.5 ± 5.5	29.6 ± 4.0	0.20 ± 0.06	2.6 ± 0.7	4 ± 0.5	6.3 ± 0.1	25.3 ± 0.4

Table : 2

Biochar Treatment	**Sugar Content**	**Total chlorophyll**	**Protein content**	**Proline content**
Seed + soil	5.415 ± 3.3c	1.415 ± 1.3d	2.9 ± 0.5d	12.91 ± 0.12c
Seed + soil +PGPR	3.26 ± 5.1b	1.51 ± 1.3d	2.92 ± 0.8c	10.09 ± 0.13c
1%	3.851 ± 3.5c	1.533 ± 1.1d	3.21 ± 0.6d	9.301 ± 0.11d
3%	2.34 ± 2.4d	1.799 ± 1.5c	3.54 ± 0.5d	9.25 ± 0.15b
5%	6.89 ± 6.8b	2.229 ± 2.2b	3.98 ± 0.6d	10.98 ± 0.11d
7%	2.5 ± 2.3d	2.187 ± 2.2b	3.1 ± 0.7c	11.48 ± 0.14b
1% + PGPR	3.84 ± 3.6c	1.771 ± 1.5c	4.09 ± 1.4b	8.36 ± 0.11d
3% + PGPR	4.23 ± 4.1c	1.955 ± 1.6c	5.84 ± 0.5d	7.76 ± 0.12c
5% + PGPR	7.12 ± 7.1a	2.519 ± 2.2a	6.68 ± 1.9a	7.32 ± 0.19a

7% + PGPR	2.46 ± 2.1d	2.216 ± 1.9c	5.76 ± 1.8b	8.01 ± 0.15b

Table : 3

Press mud Treatment	**Sugar Content**	**Total chlorophyll**	**Protein content**	**Proline content**
Seed + soil	4.51 ± 4.4c	1.62 ± 2.4d	0.75 ± 0.4d	11.81 ± 0.11c
Seed + soil +PGPR	2.36 ± 6.2b	1.71 ± 2.4d	0.46 ± 0.7c	9.08 ± 0.14c
1%	3.85 ± 4.6c	1.63 ± 2.2d	0.35 ± 0.5b	8.20 ± 0.10c
5%	3.24 ± 3.6d	1.89 ± 2.6c	0.42 ± 0.4d	8.15 ± 0.10c
10%	7.12 ± 7.4b	2.24 ± 3.5b	0.44 ± 0.5d	9.87 ± 0.10c
15%	3.51 ± 3.2d	2.28 ± 3.8b	0.51 ± 0.5d	10.2 ± 0.13b
1% + PGPR	4.83 ± 3.7d	1.97 ± 2.6c	0.56 ± 0.5d	7.24 ± 0.10c
5% + PGPR	5.23 ± 5.2b	2.91 ± 2.7c	0.74 ± 0.4d	6.65 ± 0.10c
10% + PGPR	7.98 ± 8.2a	3.12 ± 3.7a	0.93 ± 0.9a	6.21 ± 0.18a
15% + PGPR	5.46 ± 3.3d	3.01 ± 2.9c	0.53 ± 0.8b	7.15 ± 0.13b

Table : 4

for comparative evaluation of biochar as well as pressmud as an organic carrier with PGPR, the results obtained were as follows, amongst all the groundnut plants the plants treated with 5% biochar showed maximum results for plant height, root length, shoot length, number of leaves, number of shoots and number of roots, as well as dry and fresh weights as compared to control further if biochemical parameters are considered then it showed best results with groundnut plants treated with 5% biochar + PGPR for sugar content, chlorophyll content, protein content, proline content, as mentioned in table number (table: 1, 2) however if the results for pressmud are considered then it showed the significant results in the groundnut plants treated with 10% pressmud + PGPR showed maximum results with the plant height, root length, shoot length, number of leaves, number of shoots and number of roots, as well as dry and fresh weights as compared to control. furthermore the results about the biochemical parameters that is sugar content, chlorophyll content, protein content, proline content as compared to that of other treatments as well as control mentioned in table (3,4).as here in this study the aim was to compare the biochar as well as pressmud as an organic carriers the best results were obtained in pressmud(10% + PGPR) as compared to that of biochar.

Discussion

The effect of rhizobacteria and biochar levels indicated a significant improvement in the seed germination rate and growth of the soybean plant treated with biochar and rhizobacteria over the control plant (without biochar treatment). The addition of different levels of biochar, inoculation of strains with biochar and without biochar showed variable increases in the growth parameters. Addition of 3% biochar alone enhanced the seed germination, root length, shoot length by, root dry weight, and shoot dry weight (D jabborova et.al.) .The implementation of SMS-based biochar in low and high doses of 5 g/kg and 10 g/kg, respectively, via arable soil supplementation significantly improved several traits such as pH,

and total nitrogen, However, a 10 g/Kg dose of biochar addition yielded better cauliflowers compared to those in 5 g/Kg, which might be associated with a lesser supplemented biochar dose. A 10 g/Kg dose of SMS biochar with PGPR application gave the highest crop yield and optimum biochemical response (Širić, I. et.al). The effect of rhizobacteria and biochar levels indicated a significant improvement in the seed germination rate and growth of the soybean plant treated with biochar and rhizobacteria over the control plant (without biochar treatment). The addition of different levels of biochar, inoculation of strains with biochar and without biochar showed variable increases in the growth parameters. Addition of 3% biochar alone enhanced the seed germination, root length, shoot length by, root dry weight, and shoot dry weight (D jabborova et.al.). The plant's fresh and dry weights increased up to 5% biochar application and after that decreased. According to the control, BioC2 application increased 26.9% and 45.9% in the fresh and dry weights of the plant, respectively. In the case of PGPR application to the environment, the plant fresh weight was lower with the application of PGPR; on the contrary, the plant dry weight increased slightly with the application of PGPR. The highest plant dry weight was obtained in BioC2xPGPR(+) application and increased by 61.4% compared to the control (BioC0xPGPR(-)(F.sonmez et.al).The implementation of biochar in low and high doses of 5 g/kg and 10 g/kg, respectively, via arable soil supplementation significantly improved several traits such as pH, and total nitrogen, However, a 10 g/Kg dose of biochar addition yielded better cauliflowers compared to those in 5 g/Kg, which might be associated with a lesser supplemented biochar dose. A 10 g/Kg dose of SMS biochar with PGPR application gave the highest crop yield and optimum biochemical response (Širić, I. et.al).maximum plant height was observed in the treatment 6% biochar + PGPR ,also mxximum value for root dry weight was observed in the treatment given as 6% biochar + PGPR , the soil containing treatment of 6% biochar + PGPR significantly increased amount of chlorophyll content as compared to that of tomato plant (Rasool et.al).

CONCLUSION:

Hence concluding the best carrier for PGPR strain (RGKP 3) that promotes the growth of groundnut plant is pressmud(10% pressmud with pgpr) which is most efficient and significant for plant growth promotion as inferred from analysis of all the physical and biochemical parameters of plants obtained as a result of pot experiment. Hence from this study carried out

it can concluded that 10% pressmud is the best carrier with PGPR strain (RGKP3) for promotion of groundnut plant growth.

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