Evaluating the Combined Effect of Organic Carriers and PGPR on the Growth of Groundnut Plant

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This is to certify that the work for this manuscript entitled "Evaluating the combined effect of organic carriers and PGPR on the growth of groundnut plant" was successfully carried out by Miss Avni Ruturaj Dhamdhere towards the partial fulfilment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of her own work, carried out by her under the guidance of Dr. Shweta bhatt for a period of three months during the academic year 2022–23. The content of this manuscript, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other university.

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D E C L A R A T I O N

I hereby declare that the work incorporated in the present dissertation report entitled "Evaluating the combined effect of organic carriers and PGPR on the growth of groundnut plant**"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date: Avni Ruturaj Dhamdhere

EVALUATING THE COMBINED EFFECT OF ORGANIC CARRIERS AND PGPR ON THE GROWTH OF GROUNDNUT PLANT

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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 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. pressmud, these are either burned or thrown directly into landfills. Management of agricultural by-products can be managed by turning them into value-added products such as soil conditioners, compost, single-cell proteins, enzymes, organic acids, biogas, wax, growing materials etc. This study focuses on the management of press mud by converting sugar industry by-products and biochar the by-product of pyrolysis of mainly plant waste material into various value-added solutions. Therefore, this study focuses on the agro-industrial by-products of pressed mud and biochar for value-added products. 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% and 7% of biochar and 1%, 5%, 10% and 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.

Keywords: PGPR- plant growth promoting rhizobacteria, Bacterial Community, pressmud, biochar, pot experiment, organic carriers.

1. 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. 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. (10),(11)
So, the goal of our research is to create long-term solutions to stop using chemical fertilisers and show how doing so can protect humans from their negative consequences. The organic carriers are

employed as soil amendments because they increase PGPR activity, which in turn promotes plant growth. In this experiment, we will utilise a groundnut plant to perform pot experiment. To analyse as to what concentration of biochar and pressmud will be giving best results with that of PGPR for the groundnut plant growth promotion.

2. Materials and Methods

2.1 PGPR strain and reviving the PGPR strain

Bacillus megaterium strain which is now known as *Priestia megaterium* (RGKP3) was collected from Atmiya University, department of biotechnology. The strain used has NCBI accession number OM736148. The colony characteristics of the strain appeared to be as follows, elevation was observed to be concave, flat, surface of the colony was smooth, the colony was orange pigmented, and the shape of colony was rod shaped observed. The strain RGKP3 shows all the PGP traits positive as mentioned in the table 1 given below.

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 in a 250 ml 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. (11)

Table 1: PGP traits which are shown positive by the strain RGKP3

PGP traits	Results
IAA production	Positive
ammonia production	Positive
HCN production	Positive
Gibberellins production	Positive
zinc solubilisation	Positive
chitin hydrolysis	Positive
nitrogen fixation	Positive
potassium solubilisation	Positive

2.2 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. Place cotton on the petri dish's surface. Spray distilled water on cotton, 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 filter paper underneath the bottom portion of the lid. Seeds were germinated in 85 mm × 15 mm tight-fitting Petri dishes with 10 mL

of water. Seeds were kept for germination in dark condition for 4 days and were daily watered, lightly mist the seed with distilled water at regular intervals, once in a day. *(13)* Once the seeds are germinated properly on the 4^{th} day seedling length was measured to calculate the following

Seed germination percentage (%) = **N/N1 × 100**

Seed germination rate = **Ni/Di**

Mean germination = **Ni × Di/Germination %**

Vigour index = **seedling length × germination percentage**

2.3 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.(13)

2.4 Pot Experiment

The effect of rhizobacteria on the growth of groundnut was studied in pot experiments. All the experiments were carried out in triplicates. Experimental treatments for biochar included un-inoculated control (soil without biochar) and soil with four concentration of biochar (1%, 3%, 5%, 7%), and seeds were co-inoculation with *B. megaterium* as mentioned earlier. control 1, having no biochar , 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, 3% w/w biochar, 5% w/w biochar, 7% w/w biochar of total soil contained in the pot. Experimental treatments for pressmud included un-inoculated control (soil without pressmud) and soil with four concentration of pressmud (1%, 5%, 10%, 15%), and seeds were co-inoculation with *B. megaterium* as mentioned earlier. control 1, having no pressmud, control 2 having seeds co-inoculated with PGPR) and no pressmud, followed by pots with increased concentration of pressmud treatments (containing seeds treated/co-inoculated with PGPR) as 1% w/w biochar , 3% w/w biochar, 5% w/w biochar, 7% w/w biochar 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. (13)

2.5 Analysis of Physical and Biochemical parameters

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.

2.5.1 Analysis of physical parameters:

Physical parameters of the plants obtained after the period of one month to that of pot experiment. The physical parameters analysed are as follows 1) plant height 2) root length 3) shoot length 4) fresh weight 5) dry weight 6) number of leaves 7) number of roots 8) number of shoots.

2.5.2 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. The 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. (15)

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 colour intensity of the toluene was measured at 520nm against toluene blank. (16) 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 plant samples that is leaves were weighed 1g and protein content was analysed by the method of Lowry et al., (1951)(17). Folin lowry method was followed for protein determination in leaves. Bovine Serum Albumin (BSA) was used as standard for quantification of protein content of leaves with the concentration of standard as 1 to 10 µg/ml.

Sugar content:

The plant samples that is leaves were weighed 1g and sugar content was analysed by the method of nelson somogyi et.al., (1951). (18)Nelson somogyi was followed for sugar determination in leaves. Glucose was used as standard for quantification of sugar content of leaves with the concentration of standard as 1 to 10 µg/ml.

3. Results:

The seeds which are germinated after the period of 4 days as shown in the figure 1(a). The total seeds taken for germination were in total 100 seeds out of which the 97 of them were totally germinated after providing the treatment as mentioned in the above materials and methods in detail, once the seeds have been germinated, the total germinated seeds were calculated which were 97 in total after which the total seedling length was measured which was further used to measure the following parameters as mentioned in the table number 2 germination percentage which was found to be 97%, germination rate which was 24.25, mean germination 3.88 and finally the vigour index which was obtained 349.2.

The seed which were germinated properly after 4 days of period were carefuuly measured for seed length after that seeds were treated with PGPR culture as mentioed in materials and method in detail.the seeds were carefully taken with help of forcep and transferred to the culture flask and treated for half hour in incubator, treated seeds were as shown in the figure;

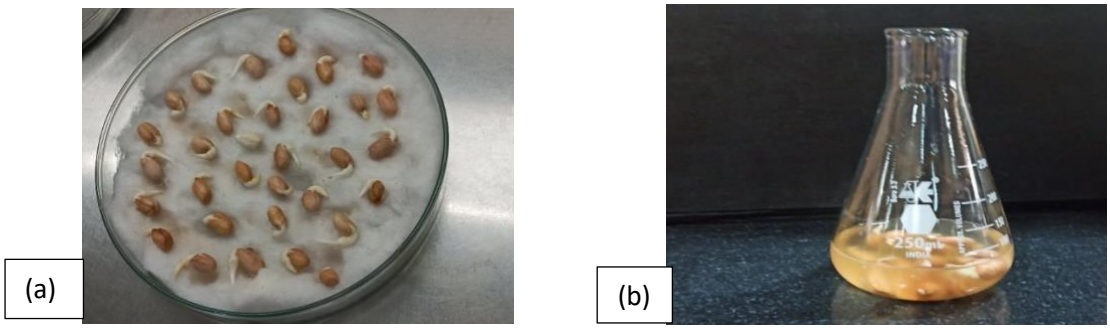

Figure1: a) germinated seeds (day 4), (b) bacterization of seeds

Table 2; calculation of various parameters for germinated seeds obtained after 4 days pf incubation period. Following parameters were calculated after the seed germination and measuring the length of germinated seeds; 1. germination percentage-97%, 2. Germination rate-24.25, 3. Mean germination-3.88, 4. Seed vigour index-349.2.

Parameters	Results
Germination percentage (%) = 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**

After the seeds were treated with the culture all the seeds were successfully sowed in the respective pots preapred according to the treatments as mentioned in the method above. The plants were obtained as follows. The best results of groundnut plant growth promotion by the help of various organic carriers to improve and enchance the activity of plant growth promoting rhizobacteria in a more better way were observed in the both organic carriers but firstly talking about the biochar, treatments of biochar that shoued best results with plant growth promoting rhizobacteria was 5% biochar and PGPR , secondly talking about that of pressmud as a carrier with plant growth promoting rhizobacteria the pressmud treatment with 10% pressmud and PGPR as compared to that of controls which were also applied together for comparing the growth between that of plants with and withou treatment that is control (only seed) , control 1 (seed + PGPR) as shown in the figure 2. a)Control, b)control1seed with PGPR c)seed with 5% biochar and PGPR, d)seed with 10% pressmud and PGPR the grownut plant growth obtained were as shown in the figure 2 below;

a) Control

b) control 1

c) Seed + PGPR + 5% biochar

d) seed + PGPR + 10% pressmud

Figure 2 : a) control (seed + soil) b) control 1 (seed + PGPR soil) c) seed + PGPR + 5% biochar d) seed + PGPR + 10% pressmud.

The plants once obtained from pot experiment were then analysed for various physical and biochemical parameters, the physical parameters below: plant height, root length, shoot length, dry weight, fresh weight, no. of roots, no. of shoots, no. of leaves. The results of physical parameters for each treatments of respective organic carriers that is biochar and pressmud are mentioned in the table no. 3 and table no. 4

Table: 3 The table given below demonstrates the data obtained after analyzing the various physical parameters for the groundnut plants obtained by taking the average for triplicate plants of each biochar treatments and then calculating the standard deviations where $p < 0.05$ which states the results are significant.

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 d	1.5 ± 1.0 d	7 ± 2.0 d	0.07 ± 0.02 d	0.66 ± 0.38 d	1.6 ± 0.2 c	4.6 ± 0.1 d	18.6 ± 0.4 d
Seed + soil +PGPR	11.6 ± 6.0 d	3.1 ± 3.0 c	8.5 ± 3.5 d	0.11 ± 0.03 d	1.07 ± 1.13 d	1 ± 0.1 d	5 ± 0.3 c	20 ± 0.2 c
1% biochar	13.6 ± 4.0 d	6 ± 2.0 b	10.5 ± 1.0 c	0.15 ± 0.06 c	1.31 ± 0.12 c	1.3 ± 0.1 c	5 ± 0.2 c	22 ± 0.8 c

3% biochar	17 ± 2.0 b	3 ± 2.0 c	11 ± 4.0 b	0.12 ± 0.03 d	2.18 ± 1.7 a	1. 3 ± 0.1 c	5.6 ± 0.3 c	24 ± 0.4 b
5% biochar	14.3 ± 8.0 c	2.6 ± 5.0 d	11.6 ± 3.0 b	0.17 ± 0.05 c	1.53 ± 1.6 d	1.3 ± 0.1 c	6.3 ± 0.1 a	25.3 ± 0.2
7% biochar	17.4 ± 1.0 b	6.5 ± 3.0 b	11.3 ± 1.0 b	0.19 ± 0.07 b	1.99 ± 1.15 b	1.3 ± 0.1 c	6 ± 0.2 b	23.3 ± 0.11
1% biochar + PGPR	15.1 ± 7.0 c	4.1 ± 2.5 c	11 ± 4.5 b	0.07 ± 0.02 d	1.14 ± 2.23 c	2.6 ± 0.1 b	6.3 ± 0.2 a	26.3 ± 0.3
3% biochar + PGPR	15.6 ± 5.0 c	4.3 ± 3.0 c	10.8 ± 2.0 c	0.22 ± 0.03 a	1.48 ± 1.14 c	1 ± 0.1 d	6.3 ± 0.3 a	25.3 ± 0.11
5% biochar + PGPR	18.5 ± 3.0 a	7 ± 4.5 a	12.5 ± 1.5 a	0.17 ± 0.05 c	2.69 ± 0.57 a	6.6 ± 0.4 a	6.3 ± 0.1 a	25.3 ± 0.4
7% biochar + PGPR	15.3 ± 3.5 c	3.5 ± 2.5 c	11.5 ± 1.5 b	0.15 ± 0.03 c	1.56 ± 0.48 c	2.3 ± 0.1 b	6 ± 0.2 b	24 ± 0.8

Table: 4 the table given below demonstrates the data obtained after analyzing the various physical parameters for the groundnut plants obtained by taking the average for triplicate plants of each pressmud treatments and then calculating the standard deviations where $p < 0.05$ which states the results are significant.

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 d	2.8 ± 1.5 d	5.6 ± 5.0 d	0.22 ± 0.02 d	1.0 ± 0.3 d	3 ± 0.4 c	3.6 ± 0.1 d	14.6 ± 0.4 d
Seed + soil +PGPR	30.6 ± 6.0 b	3.6 ± 1.0 d	27 ± 7.0 b	0.24 ± 0.14 c	2.7 ± 1.7 b	4.3 ± 0.6 c	8 ± 0.1 a	32 ± 0.12 a
1% pressmud	20.2 ± 2.5 d	4 ± 2.0 c	16.2 ± 4.5 d	0.24 ± 0.03 d	1.8 ± 0.4 d	7.5 ± 0.1 b	6 ± 0.1 c	23 ± 0.2 c
5% pressmud	12.6 ± 2.3 d	3.5 ± 1.0 d	9.1 ± 6.5 d	0.26 ± 0.05 d	1.26 ± 0.5 c	3.6 ± 0.3 c	4.3 ± 0.1 d	17.3 ± 0.4 d
10% pressmud	23 ± 2.0 c	4 ± 1.0 c	19 ± 2.0 d	0.31 ± 0.03 c	2.18 ± 0.03 c	12 ± 0.3 d	5 ± 0.4 d	20 ± 0.2 d
15% pressmud	30.1 ± 5.5 b	5.6 ± 1.0 c	24.5 ± 4.5 c	0.30 ± 0.03 c	3.0 ± 1.4 a	9 ± 0.6 a	7.3 ± 0.3 b	29.3 ± 0.12 b
1% pressmud + PGPR	30.5 ± 6.0 b	6.3 ± 1.0 b	29 ± 5.0 a	0.33 ± 0.04 c	3.2 ± 1.5 a	6 ± 0.2 b	6 ± 0.1 c	23 ± 0.2 c
5% pressmud+ PGPR	25.5 ± 9.0 c	3 ± 2.0 d	22.5 ± 5.0 c	0.41 ± 0.06 b	2.4 ± 1.4 b	7 ± 0.9 b	6.3 ± 0.2 c	25.3 ± 0.8 c
10% pressmud + PGPR	31.6 ± 3.0 a	6.5 ± 4.0 a	25.3 ± 7.0 b	0.44 ± 0.03 a	3.2 ± 1.0 a	8.3 ± 0.13 a	8 ± 0.2 a	31.6 ± 0.15 a
15% pressmud + PGPR	30.1 ± 9.5 b	5.5 ± 5.5 c	29.6 ± 4.0 a	0.40 ± 0.06 b	2.6 ± 0.7 b	4 ± 0.5 c	6.3 ± 0.1 c	25.3 ± 0.4 c

The plants once obtained from pot experiment were then analysed for various biochemical parameters, the biochemical parameters are mentioned in the table no.5 and 6 given below: sugar content, total chlorophyll, protein content and proline content.

As mentioned in the respective tables of pressmud and biochar separately. The parameters were analysed to know abou the effects of pressmud and biochar on groundnut plant growth

Table: 5 the table given below shows the results obtained for various biochemical parameters like sugar content, total chlorophyll, protein content, proline content the data contained standard deviation values where $p < 0.05$ which means the results are significant

Biochar Treatment	**Sugar Content**	**Total chlorophyll**	**Protein content**	**Proline content**
Seed + soil	5.41 ± 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% biochar	3.851 ± 3.5c	1.533 ± 1.1d	3.21 ± 0.6d	9.301 ± 0.11d
3% biochar	2.34 ± 2.4d	1.799 ± 1.5c	3.54 ± 0.5d	9.25 ± 0.15b
5% biochar	6.89 ± 6.8b	2.229 ± 2.2b	3.98 ± 0.6d	10.98 ± 0.11d
7% biochar	2.5 ± 2.3d	2.187 ± 2.2b	3.1 ± 0.7c	11.48 ± 0.14b
1% biochar+ PGPR	3.84 ± 3.6c	1.771 ± 1.5c	4.09 ± 1.4b	8.36 ± 0.11d
3% biochar + PGPR	4.23 ± 4.1c	1.955 ± 1.6c	5.84 ± 0.5d	7.76 ± 0.12c
5% biochar + PGPR	7.12 ± 7.1a	2.519 ± 2.2a	6.68 ± 1.9a	7.32 ± 0.19a
7% biochar + PGPR	2.46 ± 2.1d	2.216 ± 1.9c	5.76 ± 1.8b	8.01 ± 0.15b

Table: 6 the table given below shows the results obtained for various biochemical parameters like sugar content, total chlorophyll, protein content, proline content the data contained standard deviation values where $p < 0.05$ which means the results are significant.

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% + pressmud	3.85 ± 4.6c	1.63 ± 2.2d	0.35 ± 0.5b	8.20 ± 0.10c
5% + pressmud	3.24 ± 3.6d	1.89 ± 2.6c	0.42 ± 0.4d	8.15 ± 0.10c
10% + pressmud	7.12 ± 7.4b	2.24 ± 3.5b	0.44 ± 0.5d	9.87 ± 0.10c
15% + pressmud	3.51 ± 3.2d	2.28 ± 3.8b	0.51 ± 0.5d	10.2 ± 0.13b
1% pressmud + PGPR	4.83 ± 3.7d	1.97 ± 2.6c	0.56 ± 0.5d	7.24 ± 0.10c
5% pressmud+ PGPR	5.23 ± 5.2b	2.91 ± 2.7c	0.74 ± 0.4d	6.65 ± 0.10c
10% pressmud+ PGPR	7.98 ± 8.2a	3.12 ± 3.7a	0.93 ± 0.9a	6.21 ± 0.18a
15% pressmud+ PGPR	5.46 ± 3.3d	3.01 ± 2.9c	0.53 ± 0.8b	7.15 ± 0.13b

The results obtained from both the pot experiments were then compared for the best results of each treatments, that is comparision of physical and biochemical parameters of best treatments that promotes the growth of groundnut plant: in case of biochar treatment the best results obtained were with the treatments of 5% biochar + PGPR and in case of pressmud treatments the best results obtained were with the treatment of 10% pressmud + PGPR .The comparision of both the parameters are mentioned in the table 7 and table 8 given below;

Table 7: the table given below shows comparative analysis of physical parameters of best results obtained from all the treatments given during the pot experiment of pressmud and biochar with PGPR to promote the groundnut plant growth.

Physical Parameters	Seed + PGPR + 5% biochar	seed + PGPR + 10% pressmud
Plant height	8.5 ± 3.0	31.6 ± 3.0
Root length	6 ± 4.5	6.3 ± 4.0

Shoot length	12.5 ± 1.5	25.3 ± 7.0
Dry weight	0.17 ± 0.05	0.24 ± 0.03
Fresh weight	1.69 ± 0.57	3.1 ± 1.0
No. of roots	6.6 ± 0.4	8.3 ± 0.13
No. of shoots	6.3 ± 0.1	8 ± 0.2
No. of leaves	25.3 ± 0.4	31.6 ± 0.15

Table 8: the tbale given below shows comparative analysis of biochemical parameters of best results obtained from all the treatments given during the pot experiment of pressmud and biochar with PGPR to promote the groundnut plant growth.

Biochemical parameters	Seed + PGPR + 5% biochar	Seed + PGPR + 10% pressmud
Sugar content	7.12 ± 7.1a	7.98 ± 8.2a
Total chlorophyll	2.519 ± 2.2a	3.12 ± 3.7a
Protein content	6.68 ± 1.9a	0.93 ± 0.9a
Proline content	7.32 ± 0.19a	6.21 ± 0.18a

In the study carried out 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, 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. Further-more 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. Here in this study we also compared 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.

4. Discussion:

(D jabborova et.al.)(12) 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.(Širić, I. et.al)(21) 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. (F.sonmez et.al)(22) 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(-). (Rasool et.al)(18) for management of tomato production from rarly bright diseases the biochar and pgpr were used and different treatments were applied, maximum plant height was observed in the treatment 6% biochar + PGPR,also maximum 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. (Muhammad Jamil et.al)(23) further more about pressmud pot experiments carried out the study evaluated the impact of applying pressmud (PM). When wheat seeds were planted in containers with salty soil that had been modified with PM (0, 2.5, 10 and 15 g kg1), the biomass of the 30-day-old seedlings increased by 44%, 86%, and 90%, respectively, in comparison to the unamended soil. . (Ebrahem M Eid et.al)(24) This study examined the performance of farmed cabbage in terms of growth and yield on soil that had been treated with sugar mill pressmud. Several pressmud amendment rates (0, 50, 100, and 150 g/kg soil) were used in pot trials. The 100 g/kg pressmud treatment produced the highest yield, size, and dry weight of cabbage inflorescence with the highest level of significance.

5. Conclusion:

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. When PGPR and organic carriers like biochar and pressmud were administered together, groundnut plant physical and biochemical metrics were higher than

they were for control plants that were either not treated at all or just received PGPR.As a result, the co-inoculation with PGPR and application of biochar as well as pressmud gives the greatest chemical-free and environmentally friendly method for the long-term increase in production, replenishment of nutrients in groundnut and soil, and improvement of soil biochemical properties. The current study shows that application of PGPR alone has the potential to enhance groundnut plant growth, nutrient contents, and soil biochemical properties; however, co-inoculation has a more favourable impact on plant growth and soil biochemicals, and co-inoculation of these rhizobia in combination with biochar and pressmud has the potential to enhance groundnut plant growth and soil biochemical properties.

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