Analysis of biofertilizers activities on growth and biochemical parameters of two varieties, Azad P3 and PB89 of Pea (*Pisum sativum*).

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KEYWORDS

Plants growth promoting rhizobacteria (PGPR), *Bacillus subtilis*, *Rhizobium pusense*, protein content, total phenolic content, Days after sowing(DAS).

ABSTRACT

Pisum sativum establish symbiotic relationship with nitrogen-fixing bacteria and fix atmospheric nitrogen in the soil. Plant growth promoting rhizobacteria (PGPR) are a group of soil harbouring large diversity bacteria colonies interact with the plant tissue to provide the growth and development to the plants. Bacteria, *Bacillus subtilits* and *Rhizobium pusense* are PGPRs in addition with or without NPK were pre-mixed with soil before sowing the seeds of the varieties, Azad P3 and PB89 of *Pisum sativa*. The grown pea plants were checked for the root and shoot length, organic content and moisture content, and number of nodules after 30, 45, and 60 DAS. Further, the protein and phenolic contents were estimated in the Azad P3 and PB89 varieties of *Pisum sativa*. The use of PGPRs increased the overall plant growth and development with or without NPK, although use of the PGPRs increased the plant growth and protein and total phenolic contents. Further, it is important to notice the supplementation of both the bacteria for the growth of the pea plants.

1. INTRODUCTION

Agriculture is an important factor for the evolution of the human civilization (Zeder, 2009). The ecology of soil is determinant of plant growth and development. Agriculture soil is a dynamic and valuable natural resource for beneficial microbes. The microbes are vital for the production of nutritional food and fibre (Bishnoi, 2015).

Legume plants have ability to establish symbiotic relationship with nitrogen-fixing bacteria. The fixation of atmospheric nitrogen in the soil reduces need of synthetic fertilizers and provide nitrogen rich proteins to the legume crops in addition to dietary fibre, and significant vitamins and minerals (Tosh et al., 2013). *Pisum sativum* L. is an essential vegetable needed for their important physiological and morphological features that also possess a variety of bioactive compounds with antioxidant properties. (Cieslarova et al., 2012).

Plant growth promoting rhizobacteria (PGPR) are a group of soil harbouring large diversity of bacteria that have innate ability to fix atmospheric nitrogen in soil. The PGPRs are known to have an intense network of interactions in the rhizosphere. Soil bacteria of rhizosphere interact with the plant tissue to provide the growth and development to the plants due to plethora of mechanisms (Vessey, 2003).

Soil bacteria are preserved as a spore because of which they can survive for a long time even under unfavourable conditions. Ability of *Bacillus* species to form spores can be used for promoting legume plant growth as Plant growth promoting rhizobacteria (PGPR). The *B. subtilis* can involve in the solubilization of phosphates in addition to the fixation of atmospheric nitrogen and formation of siderophores. In addition to the improving the growth of plants, the *B. subtilis* increases the defence mechanism, stress tolerance and influence plant hormones (Hasem et al., 2019). *Rhizobium pusense* is a symbiotic rhizobium having symbiotic association with leguminous crops. As a result of nitrogen fixation, *Rhizobium sp.* primarily performs the nodulation process, which further significantly enhance the root growth and essential nutrient uptake, results in increased growth, yield and biochemical parameters of crops. Similarly, *R. pusense* bacteria strain MB-17A has also been shown to improvise the growth of *Vigna radiata* in the terms of fresh and dry weight, and nodule number, and nodule fresh weight (Chaudhary et al., 2021).

Pisum sativum has many known varieties like PB-89, Azad Pea -3, Azad Pea -1, VL-7, Arkel, Bonneville, Arka Kartik, JM-2 and Palam Priya (Sharma et al., 2020). In the present study, two varieties, Azad P3 and PB89 were grown in the soil conditions enriched by different PGPRs with or without NPK in order to check the plant height, fresh and dry weight, protein content and total phenolic content.

2. MATERIALS AND METHODS

2.1. Procurement of PGPR and Biofertilizer formulations:

The *Rhizobium pusense* was procured from the Hisar Agriculture University, Hisar, Haryana. The bacteria, *Bacillus subtilis* was procured from Microbial Type Culture Collection (MTCC). The formulations of the bacteria as biofertilizers were formulated as described in Devi P et al. (2024).

2.2. Pot experimentation:

A pot experiment was carried out to analysis the impact of PGPRs alone and with integration of chemical fertilizers on various parameters of Pea varieties at field of Sampla, Haryana (Latitude: 28.784815 and Longitude: 76.785142). The experiment was performed in triplicates using complete randomized design (CRD) as described in Devi P et al. (2024).

Seven treatments used in the study included:

01 Control,

02 Nitrogen phosphorus potassium (NPK),

03 NPK + *Rhizobium pusense,*

04 NPK + *Bacillus subtilis,*

05 NPK + *Rhizobium pusense* + *Bacillus subtilis,*

06 *Rhizobium pusense* + *Bacillus subtilis,*

07 Biofertilizer formulation.

Each set of experiment were harvested after 30, 45 and 60 days of sowing. The sowing was done in triplicates (n=3).

2.3. Morphological parameters

Plant height:

The plant height was measured using a 30 inches' ruler from the upmost end of the shoot to the crown and lowest end of the root from the crown. The root and shoot height of 30, 45 and 60 days old 9 plants (3 plants each from 3 pots) were uprooted and the mean and standard deviation was calculated.

Dry and Fresh weight of tissues:

The weight of the 30-, 45- and 60-days old stem, root and leaf tissues were measured fresh or upon drying. For the leaf tissue, the average weight of 9 (3x3) leaves were measured. For the shoot and root tissues, the plants were uprooted, washed and the shoot and root were cut. Shoots/ roots of 3 plants of each pot were weighed separately and the mean and SD was calculated.

2.4. Biochemical parameters

Total protein content:

Total protein content of the pea plant leaf was estimated using the Lowry method. For the purpose, ethanoic extract was prepared using Soxhlet extract as described by Awele Okolie et al. (2016). The protein extract to be quantitated was taken in 5 mL of 2% Na_2CO_3 solution and was incubated at 25 ^oC for 15 minutes. Half a mL of Folin-Ciocalteu's phenol reagent was added to the sample and incubated at room temperature for 30 minutes. The absorbance was measured at 660 nm using a spectrophotometer. A protein standard curve was prepared using bovine serum albumin (BSA) to plot the curve and quantify the protein content.

Total phenolic content (Swain and Hills, 1959):

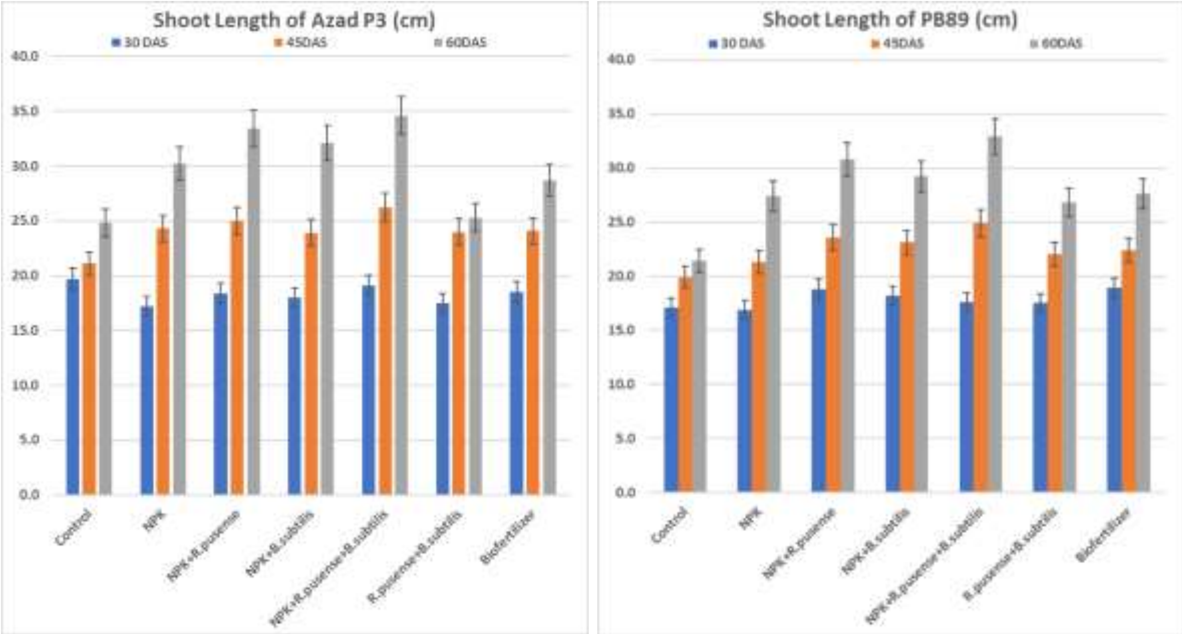
The phenolic content estimation based upon the Folin-Ciocalteu assay (Swain and Hills, 1959). 0.5 mL of tissue extract sample was added to 0.2 mL of F-C reagent and a substrate solution of 0.5 ml of Na_2CO_3 solution. The final volume was raised by added to 10 ml with distilled water and incubated at 27˚C for half an hour. The absorption was read at 765 nm using a spectrophotometer. The concentration was calculated using gallic acid as a standard and the results were expressed as gallic acid equivalents/100g of sample.

3. RESULTS

3.1. Shoot and Root Length of the Pea Plant:

The shoot length of Azad P3 was observed till 60 Days After Sowing (DAS) (Figure 1a). The increased growth of the shoot length of the Azad P3 in the range of 30 to 35 cm when pre-mixed with NPK alone or in addition with *R. pusense* and/or *B. subtilis* as compared with the control (~25 cm). In the case of the variety PB89, the increase in the shoot length was noticed in the soil exposed with all the treatments used higher than that of the control. Among all these treatments, the combination of NPK with either or both of the two bacteria, *R. pusense* and *B. subtilis* between 29 to 33 cm of the shoot length. The roots of the Azad P3 and PB89 was induced when soil sowed with all the treatments used in the study. The changes in the length of the roots in response to the soil treatments was higher as compared with the shoot length (Figure 1a, b). The root length of the both the varieties Azad P3 and PB89 was increased in the range of 22 to 26 cm upon treatment with NPK in addition to *R. pusense* and/or *B. subtilis* as compared with the control varieties (Figure 1b).

(a)



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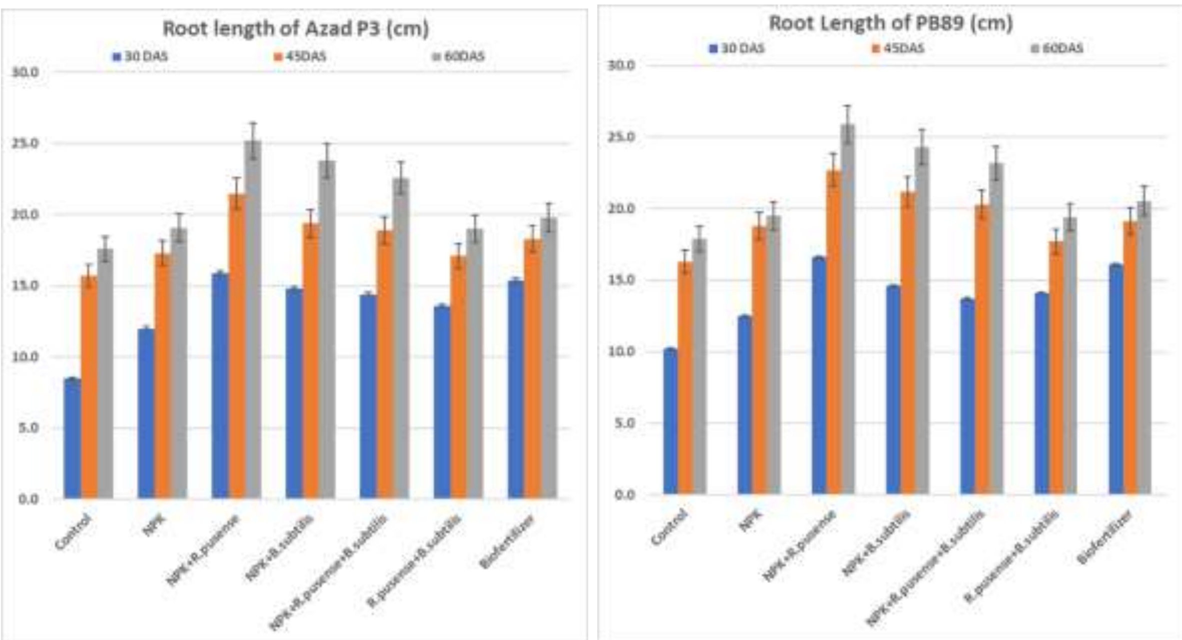
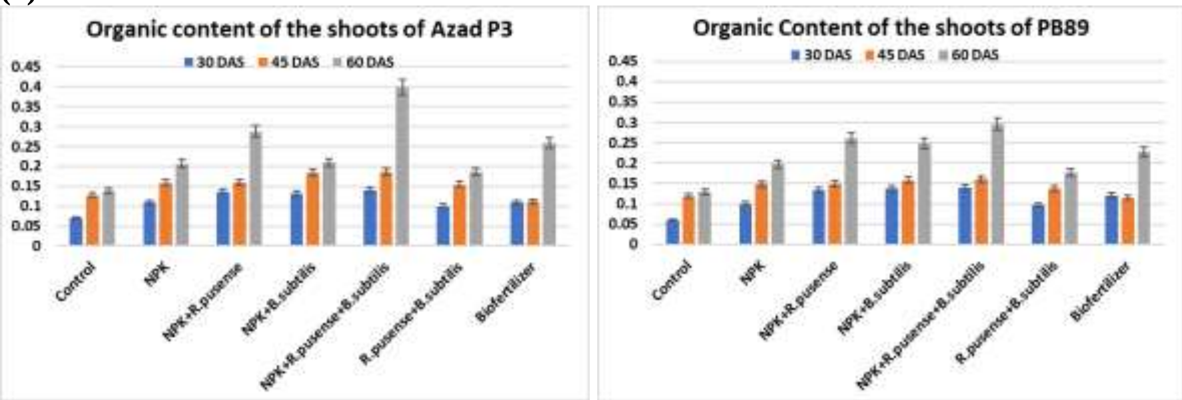


Figure 1. Shoot (a) and root (b) length of the Azad P3 and PB89 varieties of Pea Plant

3.2. Organic content of the Pea Plants:

The dry weight of the pea plant was expressed as organic content. The organic content of the shoot of Azad P3 was increased upon incorporation of the Biofertilizer or the NPK with or without PGPRs. Maximum changes in the total organic content of the shoot of the pea plant had been found in the NPK+*R. pusense*+ *B. subtilis* (~0.4 g) as compared with the control Azad P3 (~0.14) at 60 DAS (Figure 2a). Similar results were also observed in the PB89, total organic content of the shoot of the pea plant has been found in the NPK+ *R. pusense*+ *B. subtilis* (~0.3 g) as compared with the control (0.129g) at 60 DAS (Figure 2a). The organic content of the roots of Pea plant, like the shoots enhanced upon the pre-treatment of the soils. Maximum enhanced in the organic content of the roots of the varieties of Azad P3 and PB89 of the pea plant when the soil treated with the PGPR NPK+ *R. pusense*+ *B. subtilis* (~0.3 g for both the varieties compared with the ~0.1 of the control Azad P3 and ~0.125 of the control PB89 variety) (Figure 2b). The organic content of the leaves of the varieties Azad P3 and PB89 of the pea plants was also estimated. The maximum value of the organic content of the leaves of the pea plant was observed after 45 DAS and the content was found to be less at 60 DAS. The effect of the NPK+ *R. pusense*+ *B. subtilis* (~0.028 g of Azad P3 variety as varieties compared with the ~0.012 g of the control and 0.026g the of the PB89 variety compared with the ~0.011 g control) (Figure 2c)

(a)



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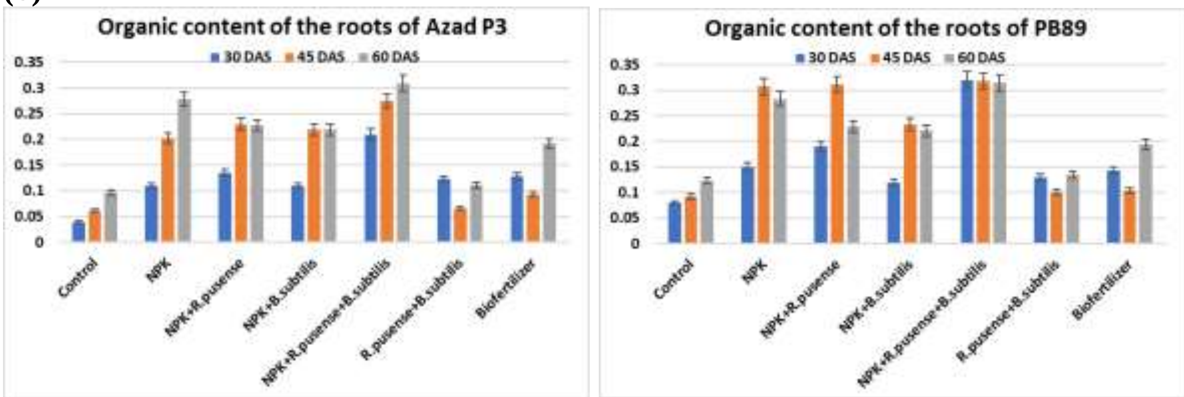

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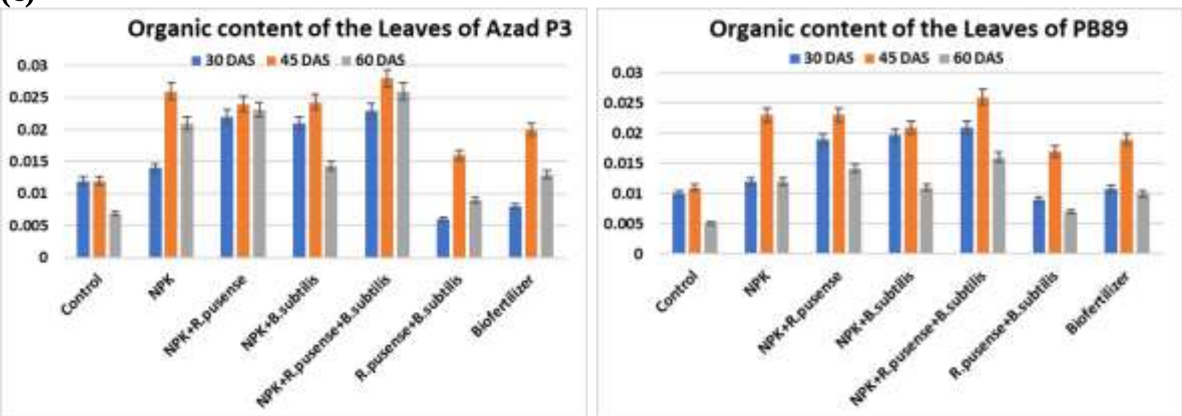


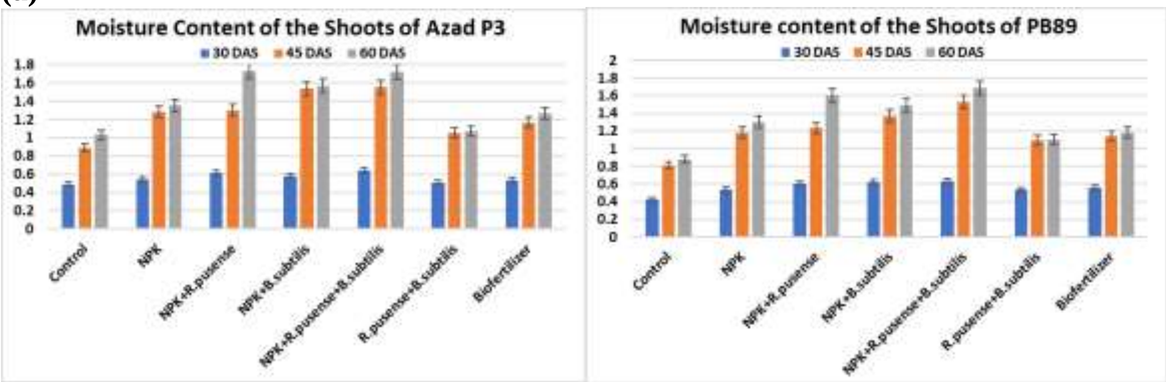
Figure 2. The organic content of the shoot (a), root (b) and leaves (c) of the Azad P3 and PB89 varieties of Pea Plant

3.3. Moisture content of the Pea Plants:

The moisture content of the plants was estimated as a difference between the wet weight and dry weight of the plant organs. The moisture content of the shoots of Azad P3 and PB89 varieties increased with time. Among all the pre-treatments of the soil, use of NPK in addition *R. pusense* and/or *B. subtilis* (Figure 3a). The moisture content of the roots of Azad P3 and PB89 was also induced when the soil was pretreated with the NPK with or without PGPRs.

Among all the pre-treatments, NPK+*R. pusense*+ *B. subtilis* pre-treatment to the soil increased the moisture content of the root of Azad P3 to ~1.8 as compared with ~0.8 g in control and ~1.875 as compared with the ~0.1 g in the control root of PB89 (Figure 3b). The increased moisture content of the leaves of the pea plants was maximum at 45 DAS. The moisture content of the Pea plant was found to be increased with due to the pre-treatment of the soil with NPK and other PGPRs. The increase in the moisture content of the leaves of both the varieties Azad P3 and PB89 seems to are important for the pre-treatment with the NPK. The pre-treatment of Biofertilizer in the soil increased the leaves of both the varieties Azad P3 and PB89, though the increase was lesser than that of the NPK + PGPRs (Figure 3c).

(a)



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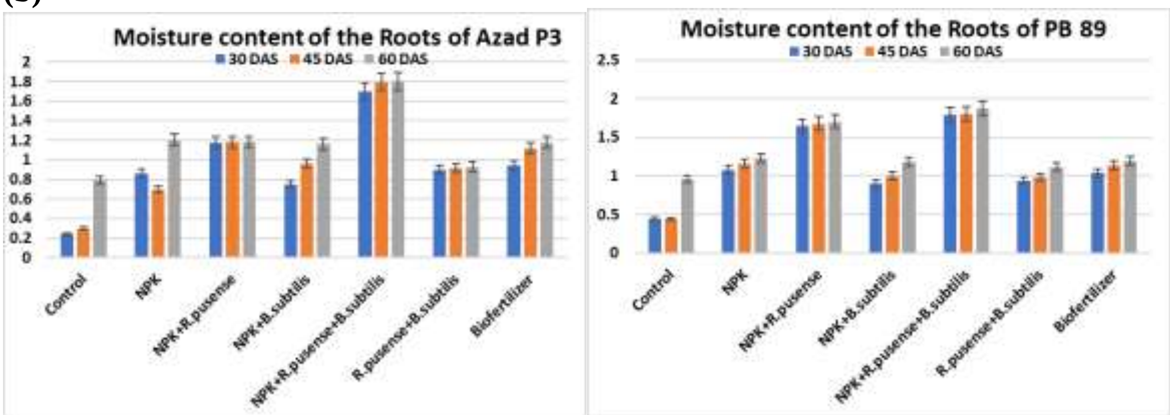

(c)

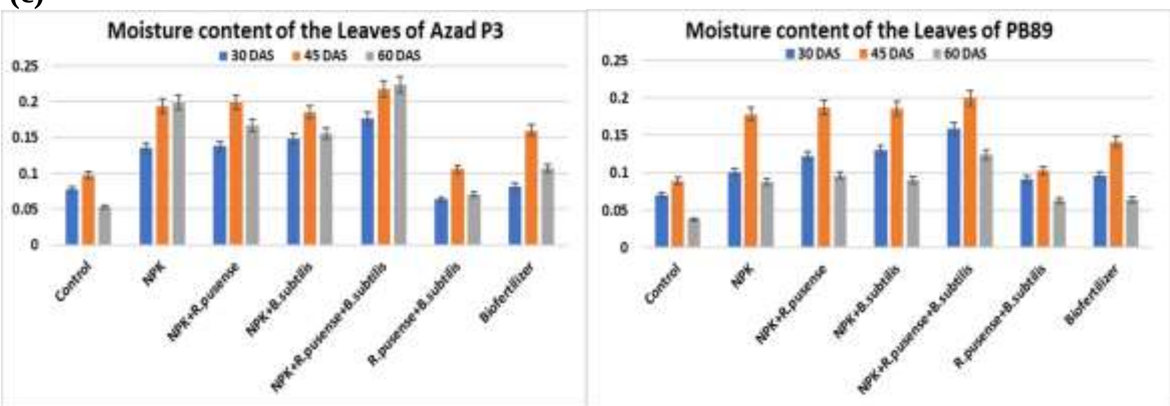

Figure 3. The moisture content of the shoot (a), root (b) and leaves (c) of the Azad P3 and PB89 varieties of Pea Plant

3.4. Root Nodule numbers of Pea Plant

Legume plants have root nodules that are important protein supply for nitrogen fixing bacteria. The nodulating pea plant was found to have high growth of the root better than the shoot. It seemed important to study the number of nodules of the root of the varieties, Azad P3 and

PB89. The number of root nodules were counted in each of the variety sown in the soil pre-treated with the various combinations of PGPRs with or without NPKs and compared with the control. The pre-treatment of soil with PGPRs increased the number of nodules of both the varieties, Azad P3 and PB89. Maximum increase in the number of root nodule of the Azad P3 as well as the PB89 was found to be associated with the pre-treatment with NPK+*R. pusense*+ *B. subtilis* (~50 root nodules against 33 in the specific control). Similar observation was observed in the variety where also maximum number of root nodules were observed when the soil was pre-treated with the NPK+*R. pusense*+ *B. subtilis* (~50 root nodules against 33 in the specific control) (Figure 4).

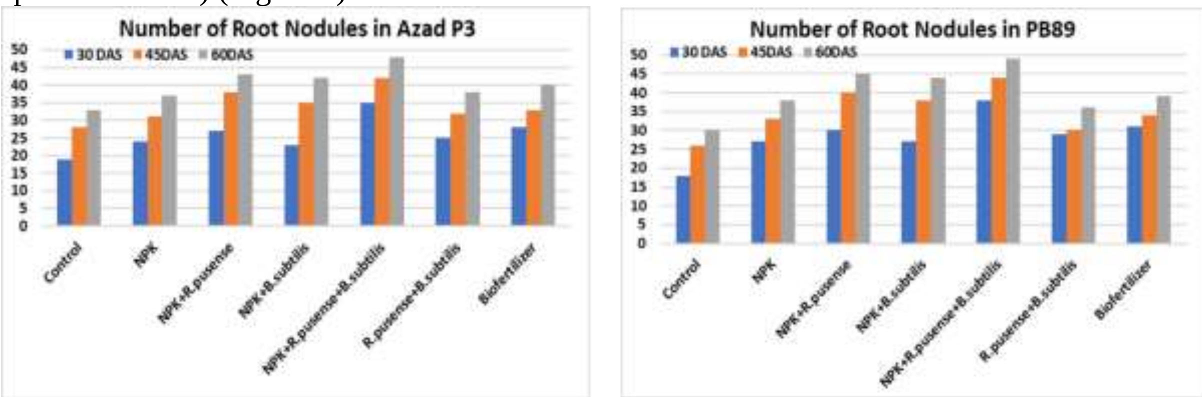

Figure 4. The root nodule of the Azad P3 (a) and PB89 (b) varieties of Pea Plant

3.5. Protein content of Pea:

The protein content of the varieties, Azad P3 and PB89 pea plants increased with time from 30 to 60 DAS. The protein content of pea also increased in the order of its number of root nodules. The protein of the Azad P3 was ~63.5 in the control it increased with the pre-treatment of the soil with various PGPRs. Among all the pre-treatments the PGPR, NPK+*R. pusense*+ *B. subtilis* was highest in Azad P3 with protein content equals to 82.61±0.058 against 63.61±0.451 in the control and was highest in PB89 with protein content 81.65±0.451 against 60.58±0.400 in the control (Figure 5).

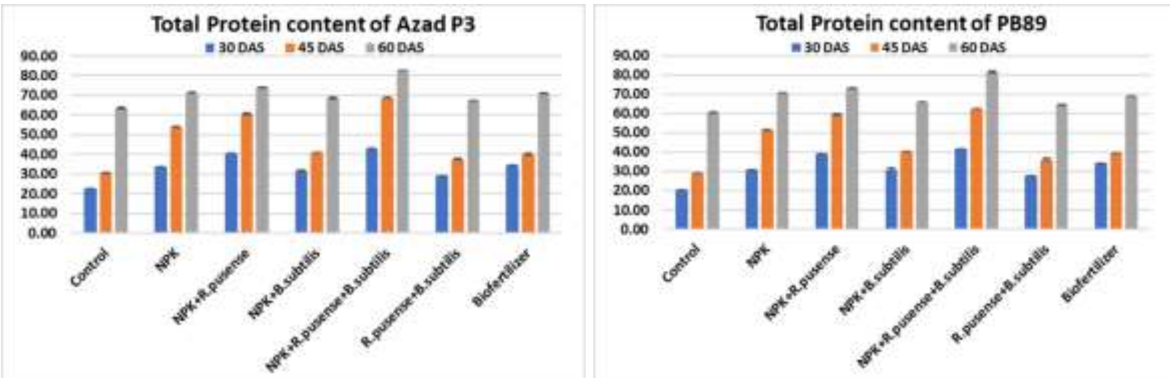

Figure 5. The protein content of the Azad P3 (a) and PB89 (b) varieties of Pea Plant

3.6. Total Phenolic Content of Pea Plant:

The phenolic compounds are a large group of naturally occurring bioactive substances found in plants. The phenolic compounds are known to be involved in both ecologically and in human health such as, defense, antioxidant, plant growth and development, stress response etc. The total phenolic content of the pea plant was increased with the pre-treatments of the PGPRs in the soil. An increase in the total phenolic contents of pea varieties, Azad P3 and PB89 were increased when the soil was pre-treated with the PGPRs in the addition of NPK. The NPK alone pre-treated with soil doubled the total phenolic content of both the varieties while pre-treatment with the NPK+*R. pusense*, NPK +*B. subtilis* or NPK+*R. pusense*+ *B. subtilis* the total phenolic content was increased up to thrice than that of the control (Figure 6).

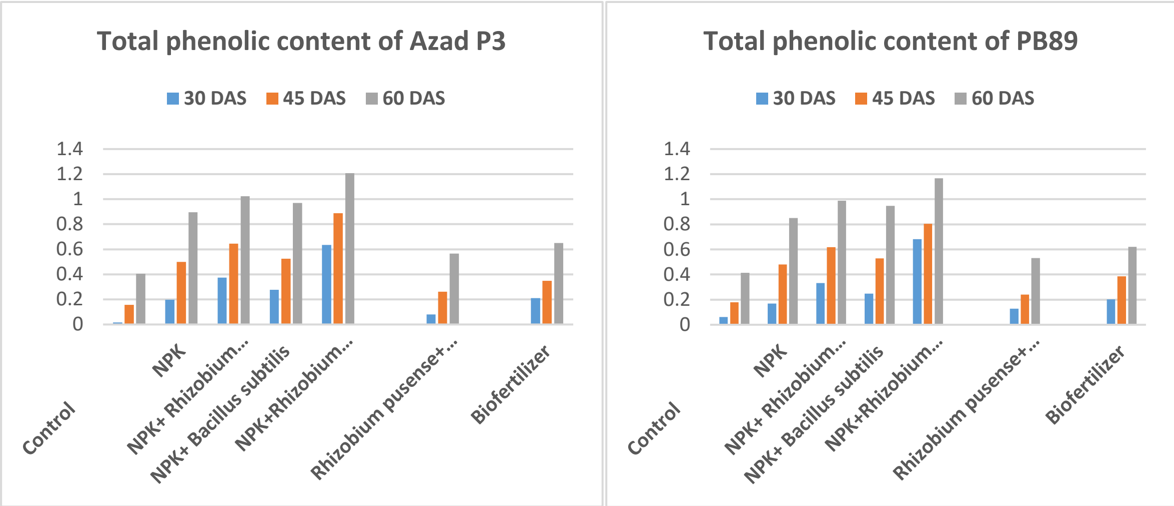

Figure 6. The phenolic content of the Azad P3 (a) and PB89 (b) varieties of Pea Plant

4. DISCUSSION

The height of plants depends upon the genetic variability and nutritional contents of the soil. The height of the variety, Azad P3 was longer than that of the PB89. Different varieties of *P. sativa* are known to have difference in the genetic diversity (Santos et al., 2019). In addition to the genetic variability, the soil nutritional conditions are also helping in increasing the growth of the plant. Supplementation of chemical and biological fertilizer increased the shoot and root length. NPK supplement increases the pea plant length, however, bacteria *R. pusense* and/or *B. subtilis* supplemented the overall growth of the plant. High levels of phytohormones, such as, auxin level are known to be higher in the rhizosphere due to its secondary metabolites (Dilfuza, 2011).

The secondary metabolite of the rhizosphere with PGPRs have various categories including bio fertilizers, rhizoremediators, biopesticides, and phytohormones (Antoun and Prevost, 2005). The direct role of phytohormones such as auxins can increase the plant growth and development. In addition, the nitrogen fixation by the rhizobacteria is another reason adding to the growth of the plants. The overall energy obtained by the roots from the soil is used either to increase the vegetation growth of the plant or the seed production of the pea. As was observed for the length of the pea plant the organic and moisture content was also influenced by the genetic composition of the varieties and nutritional constituents of soil.

The microorganisms in soil can supply nutrients to plants, positively influence plant growth, and negatively influence the plant pathogens besides improving the soil structure, bioaccumulation, microbial leaching and bioremediation (Hayat et al., 2010, Burd et al., 2000, Zhuang et al., 2007, Zaidi et al., 2008). Rhizospheres, which are diverse communities of beneficial soil microorganisms associated with the root systems of all higher plants (Khalid et al., 2006). In addition to the microbes, the plant roots are also known to exude diverse compounds as well that may alter the properties of the soil to influence the microbes in the immediate vicinity of the roots, esp., root hairs (Dakora and Phillips, 2002). Plant growth promoting rhizobacteria are the microbes that positively interacting with the roots.

P. sativum, including other legume plants are known as a good protein source. Peas contribute to soil nitrogen levels through their symbiotic relationship with nitrogen-fixing bacteria, enhancing soil fertility and benefiting subsequent crops in rotation. The number of nodules in the *P. sativa* roots was increased due to pre-mixing of the NPK in addition to and either or both

of the rhizobacteria, R. *pusense* and *B. subtilis*. The number of root nodules of the pea plant is directly linked with the protein content of the plant. The rhizobacteria are known to involve in the synthesis of protein via the phytohormone, IAA (Dullaart 1970, Ghosh and Basu 2006)
Total phenolic content of the plants are important constituents of the cells. The phenolics of the cell includes various phenolic acids, such as caffeic acid, p-coumaric acid, and ferulic acid. The total phenolic content of both the varieties, Azad P3 and PB89 was comparable. Pre-incorporation of chemical and/or biological fertilizers improved the total phenolic content of both the Azad P3 and PB89 varieties. Phenolic content of plants is known to perform various functions such as, antioxidant activity, environmental stress response, defence against herbivores, pathogens and UV radiation, and, plant growth and development.

CONCLUSION

The present study revealed the impact of both the PGPRs and chemical fertilizers within the integration leads to improved and enhanced growth and biochemical parameters in both the pea varieties at 30, 45 and 60DAS. Pea plant have diverse applications in agriculture underscore their significance for food security and sustainable farming practices. Use of a combination of PGPRs and NPK in the soil for the sowing pea plants. The use of both these PGPRs would supplement the overall growth of the pea plant and their health. Understanding these parameters in pea plants can lead to improvements in crop management aimed at enhancing yield and nutritional quality. So, the exploration of PGPR could be the effective alternative technique for sustainable agriculture practice and proved to be more eco-friendly and cost effective for crops productivity enhancement.

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Author contribution:

Pinky Devi: Conceptualization, Methodology, Investigation, Data analysis, Writing–Original Draft, Writing – Review & Editing; Ishu Khangwal: Writing – Review & Editing, Conceptualization, Validation, Supervision.

Conflict of interest: None

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