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Biofertilizers Activities On Physiological And Yield Parameters Of Two Varieties, Azad P3 And PB89 Of Pea (*Pisum sativum*)

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Abstract

Pisum sativum is a legume crop an important protein choice for vegetarian's diet. The production of the pea crop is important for the chlorophyll constituents and various chemical and organic nutrients of the soil. The two important varieties of Pea plant viz., Azad P3 and PB89 were cultivated setting up as a pot experiment using complete randomized design by sowing seeds in soil pre-mixed with various NPKs and PGPRs in triplicates. The pea plants were observed for the presence of mean number of leaves, relative water content of leaves, quantitation of chlorophyll content, and carbohydrate content. In addition, the yield production of the two varieties was also studied in the terms of the dimensions of the pods, weight of pod/plant, number of pods/plant, number of seeds/pod, and weight of the seeds/pod. NPK, NPK+*R. pusense*, NPK+*B. subtilis,* NPK+*R. pusense*+*B. subtilis* and Biofertilizer positively increased the chlorophyll contents to as compared to that of the control of both the varieties of *P. sativum.* The yield production of the Azad P3 was more than that of the PB89. The Pods of the cultivar Azad P3 was longer than that of PB89 by nearly 1 cm length. Among all the PGPRs the consortia are the best of all of these for the yield production of the varieties. The effect of NPK+*B. subtilis*+ *R. pusense* in the soil increased the weight of pods per plant in the case of variety Azad P3 to nearly 6 gram while the weight of the pods/ plant was nearly 3.6 gram in case of PB89. Maximum output of the pea crop was found when the soil was manured with the NPK + consortia of the PGPRs.

Keywords: Plant growth promoting rhizobacteria (PGPR), Biofertilizers, *Bacillus subtilis*, *Rhizobium pusense.*

1. INTRODUCTION

Pea plant, *Pisum sativum* is a legume crop of increasingly importance grown throughout the world, with a total cultivation area of 8 million hectares and a total annual harvest of 16 million tonnes (Santos et al., 2019; FAOSTAT, 2019). Total pea plant production in India was ~6.2 million tons following only China with ~11.6 million tons in the world (FAOSTAT, 2024). Pea legume crop seeds are very nutritious food articles rich in high protein content, minerals, carbohydrates and fibers. Among the carbohydrate foods, starch constitutes the majority and has a low glycemic index in the case of the pea plants. The net availability of pulses per capita in India has been reported as lowest from 51.1 gm/day to 41.9 gm/day in the last 43 years i.e., in the period 1971 to 2013. However, the WHO recommendation of pulses in the India per capita was 80 grams per day (Statista, 2024). This raises question about the nutritional aspect as pulses are considered to be 'poor man's protein'. It is estimated that pulses contain 20-25% protein by weight, twice as much as wheat and three times as much as rice. In addition to their nutritional value, pulses also have a low carbon and water footprint, making them an important part of permaculture systems. Pea seeds contain 60-65% carbohydrates. It is estimated that the water footprint of producing one kilogram of meat is five times that of beans. In addition, one kilogram of beans emits 0.5 kilograms of CO2e, while one kilogram of meat emits 9.5 kilograms of CO_2 equivalent. The primary production of a plant is related to the photosynthetic activity of chlorophyll pigments. The primary function of chlorophyll is to facilitate the light-dependent reactions of photosynthesis. It absorbs light energy, which excites electrons and causes a series of electron transfer reactions. These reactions ultimately produce ATP and NADPH molecules, which are necessary for plant growth and development. Modern and conventional agriculture farming needs several organic and inorganic fertilizers/manures. The soil improvement needs nutrients, Nitrogen, Phosphorus, and Potassium for the plant that are required during their growth and development. In addition to the chemicals several organic materials or microorganism are also used for the soil enrichment. Various plant growth bacteria are known to provide the improvement of the overall growth of the plants and thus the crops. The bacteria, *Rhizobium pusense* and *Bacillus subtilis* are known to be associated with plants to boost the crop yields. The *R. pusense* symbiotically associate with leguminous crops including pea plants and lead to fix nitrogen, phosphate solubilizing, role in ACC deamination, production of siderophores, phytohormones and secondary metabolites (Pravin et al., 2016). While *B. subtilis* provide plant growth promoting factors to boost crop immunity against disease besides carrying out other functions such as nitrogen fixation, phosphate solubilization, and phytohormone production (Umer, M. et al., 2021). Bacteria *Rhizobium pusense* and *Bacillus subtilis* were used to evaluate individual or both these bacteria as biofertilizers in soil with or without the NPKs for the production of the two varieties, viz., Azad P3 and PB89 of pea.

2. MATERIALS AND METHODOLOGY

2.1. Procurement of PGPR and Biofertilizer formulations:

The *Rhizobium pusense* was procured from the Hissar Agriculture University, Hissar, Haryana. The bacteria, *Bacillus subtilis* was procured from Microbial Type Culture Collection (MTCC). The formulations of the bacteria as biofertilizers were inoculated along with the agro-industrial wastes (Rice bran husk) via solid-state fermentation. The organic waste was dried, ground and sieved to make formulations.

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). The seeds of two pea varieties, Azad P3 and PB89 were grown in grow bags with diameter of 16X16 inches. Sandy loam soil was taken from the field of Sampla and 7kg soil in each pot was taken. The NPK were added at ratio of 16:16:16/kg/ha before sowing the seeds. Seeds were surface sterilized by dipping in 95% ethanol for 5 minutes and washed about eight times with distilled water. Six seeds were sown in each pot. Seeds were pre-treated with PGPRs (Biofertilizer) for half an hour and kept in dark for proper application of culture on seeds surface before sowing. Then seeds were sown to a depth of 5mm in each pot.

Pot experiments carried out using complete randomized design included these seven treatments filled with rich soil:

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

After sowing the seeds each pot was placed in sun light and irrigation according to their field capacity. Each set of experiment were harvested after 30, 45 and 60 days. The sowing was done in triplicates (n=3).

2.3. Relative water content of leaves:

The fresh leaf tissues were plucked during near noon and the kept in a zip lock polythene bag. The polythene was kept in ice-bucket at 4 to 10^{o}C and transferred to laboratory. The tissues were cut in fine pieces. The fresh leaves were weighed and recorded. The sample was transferred in petri-dish plate and dipped with distilled water and capped. The leaf pieces were incubated at room temperature for 4 hours. The excess water was removed and the turgid leaf was weighed and recorded. The leaf was then dried by keeping under an oven at 80^{o}C for 24 hours. The dried leaf was also weighed and recorded.

The relative water content was calculated using the formulae

$$RWC = \frac{Leaf\ fresh\ weight - Leaf\ dry\ weight}{Leaf\ turgid\ weight - Leaf\ dry\ weight}\ x\ 100$$

2.4. Chlorophyll content:

The chlorophyll content of the 1g each of fresh leaves of Azad P3 and PB89 of *Pisum sativum* was estimated essentially following the Arnon's method (1949). Briefly, the leaf tissue was homogenized in cold 80% acetone and centrifuge at 5000 rpm for 5 minutes. The supernatant was measured at wave length 645 nm and 663 nm using a spectrophotometer. The chlorophyll-a was calculated using formula equal to (12.7 x A663) - (2.59 x A645) x V / 1000 x W; chlorophyll b equals to (20.2 x A645) - (8.02 x A663) x V / 1000 x W; and Total chlorophyll was calculated using the formula equals to (20.2 x A645) + (8.02 x A663) x V / 1000 x W

2.5. Water soluble carbohydrates (Yemm and Willis, 1954):

The water-soluble carbohydrate was estimated following the method essentially following Yemm and Wills (1953). Briefly, the tissue extract was diluted 1:10 with distilled water and treated with the Anthrone reagent. The contents were incubated at 80^{0}C for 10 minutes and upon letting them cooled down at room temperature, the absorbency was taken at 630 nm.

2.6. Yield produce:

The yield data produce was measured in dimensions of the pods, weight of pod/plant, number of pods/plant, number of seeds/pod, and weight of the seeds/pod. The mean and SD value of the data was calculated in triplet.

3.RESULTS

Two varieties of pea plants viz., Azad P3 and PB89 were analyzed for the leaf number, chlorophyll contents, total carbohydrates, and the yield content of the crops was studied to evaluate the comparison of the leaf analysis with yield comparison of the two varieties after 30 to 60 days after sowing (DAS) upon treated with the different treatments of the PGPRs.

3.1. Number of Leaves of Azad P3 and PB89:

The number of leaves of the plants was maximum in the soil connected with various PGPRs. The differences in the number of leaves were almost same in the control of the Azad P3 and PB89. However, the treatment with NPK+*R. pusense*, NPK+*B. subtilis* or NPK+*R. pusense*+ *B. subtilis* led to comparatively more leaf numbers (~60-65 leaves) that that of the control group (~50 leaves) (figure 1).

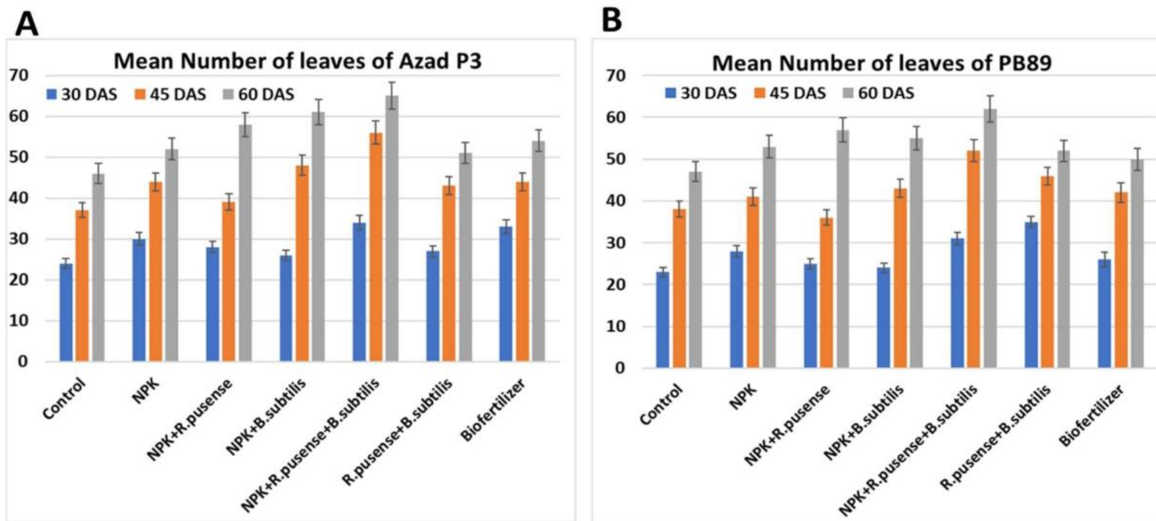

Figure 1. Mean number of leaves of the varieties, Azad P3 (A) and PB89 (B) of *Pisum sativum* upon cultivated under the influence of PGPRs with or without NPK.

3.2. Relative water content of Azad P3 and PB89:

The relative water content of the leaves of both the varieties was maximum at 45 DAS among all the three days studied. The relative water content however, was comparable in the case of the various types of PGPRs used (Figure 2).

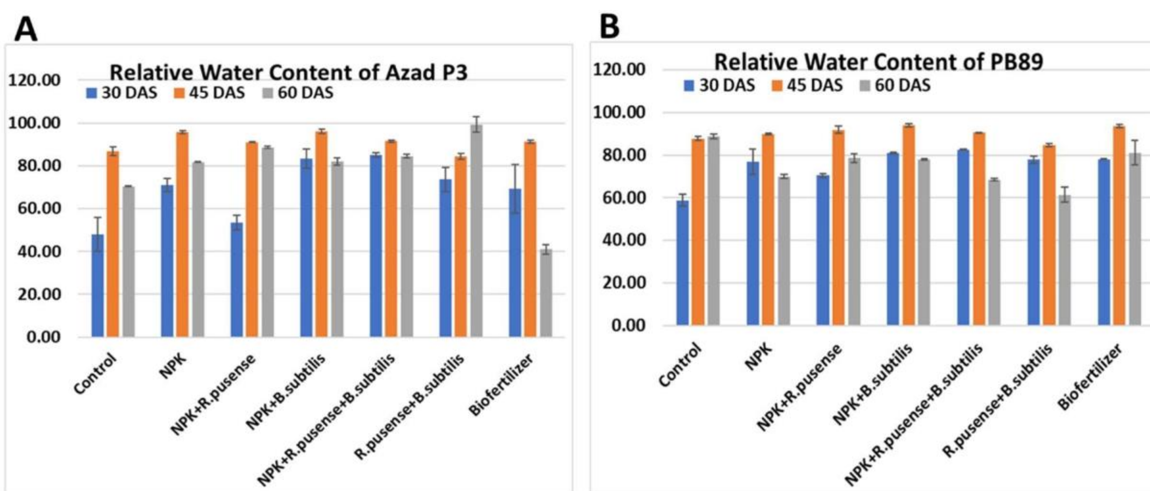

Figure 2. Relative water content of the varieties, Azad P3 (A) and PB89 (B) of *Pisum sativum* upon cultivated under the influence of PGPRs with or without NPK.

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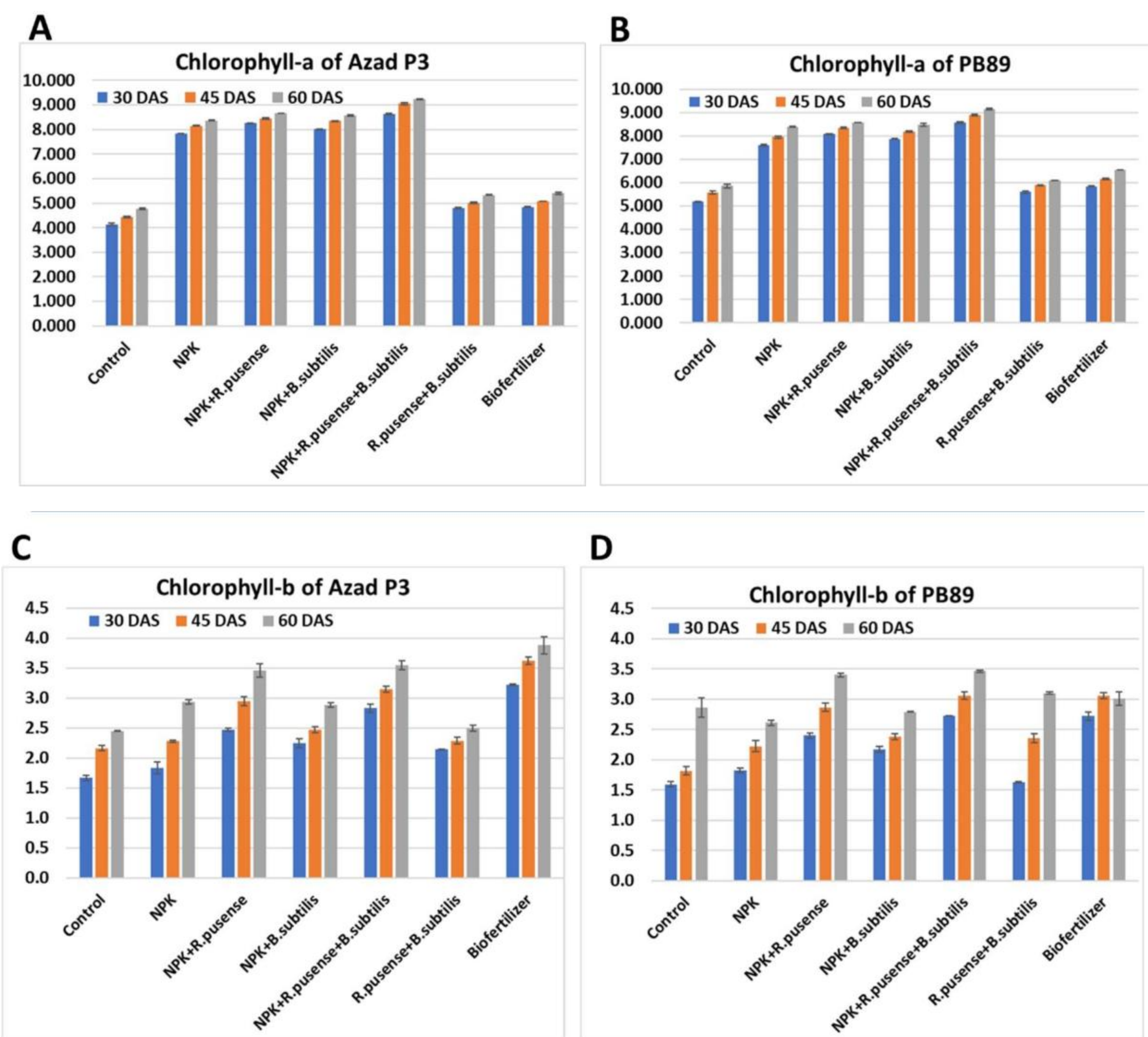
3.3. Chlorophyll of the Azad P3 and PB89:

Minor variety-specific differences in the chlorophyll, different fertilizers also have different chlorophyll contents (Figure 3). NPK, NPK+*R. pusense*, NPK+*B. subtilis*, and NPK+*R. pusense*+ *B. subtilis* positively increased the chlorophyll-a contents to 8 to 9 mg/g fresh weight as compared to that of the 4-5 mg/g fr.wt. of control of both the varieties of *P. sativum* (Figure 3A, B). The incorporation of the bacteria and formulated bio-fertilizer in the soil did not change the chlorophyll-a content of the plant much (Figure 3A, B). Similarly, the NPK (2.5 mg/g fresh wt.), NPK+*B. subtilis* (2.5 mg/g fresh wt.), NPK+*R. pusense* (3.5 mg/g fresh wt.), NPK+*R. pusense*+ *B. subtilis* (3.5 mg/g fresh wt.), and Biofertilizer (4.0 mg/g fresh wt.) of the chlorophyll b content in variety Azad P3, and NPK+*R. pusense* (3.5 mg/g fresh wt.) and NPK+*R. pusense*+ *B. subtilis* (2.5 mg/g fresh wt.) in the variety PB89, were increased in the control of both the varieties after 60 days after the sown (Figure 3 C, D). Upon treatment with NPK (~11.5 mg/g fresh wt.), NPK+*B. subtilis* (~11.5 mg/g fresh wt.), NPK+*R. pusense* (~12.25 mg/g fresh wt.), NPK+*R. pusense*+ *B. subtilis* (~13.0 mg/g fresh wt.), and Biofertilizer (~9.0 mg/g fresh wt.) to the soil led to increased total chlorophyll content of control of variety Azad P3 (~7.0 mg/g fresh wt.) and variety (~9.0 mg/g fresh wt.) after 60 DAS (Figure 3 E, F).



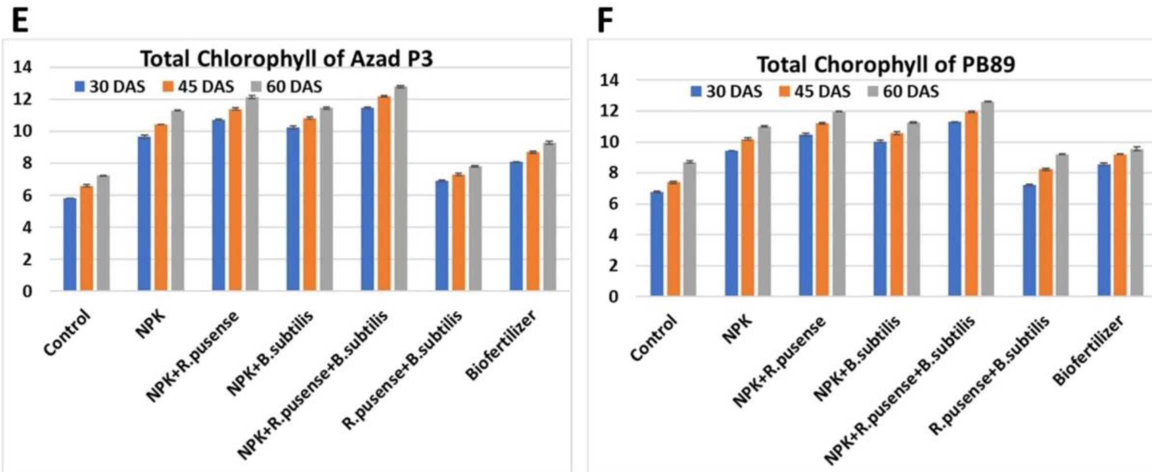


Figure 3. Chlorophyll-a (A, B), Chlorophyll-b (C, D), and total Chlorophyll (E, F) of the varieties, Azad P3 and PB89 of *Pisum sativum* upon cultivated under the influence of PGPRs with or without NPK.

3.4. Water-soluble carbohydrates:

The photosynthesis of plants results in the production of water-soluble carbohydrates. Irrespective of the differences in the chlorophyll of the two varieties viz., Azad P3 and PB89, the water-soluble carbohydrates of the two varieties were comparable. The differences of the PGPRs revealed that NPK and NPK+*B. subtilis* supplemented in the soil increased the soluble carbohydrate concentration to nearly 80 mg/g fresh wt., and NPK+*R. pusense* supplement increased it to nearly 85 mg/g fresh wt., and NPK+*B. subtilis*+ *R. pusense* supplemented increased the water-soluble carbohydrate of the two varieties, Azad P3 and PB89 to 95 mg/g fresh wt. compared with the nearly 60 mg/g fresh wt. at 60 DAS (Figure 4).

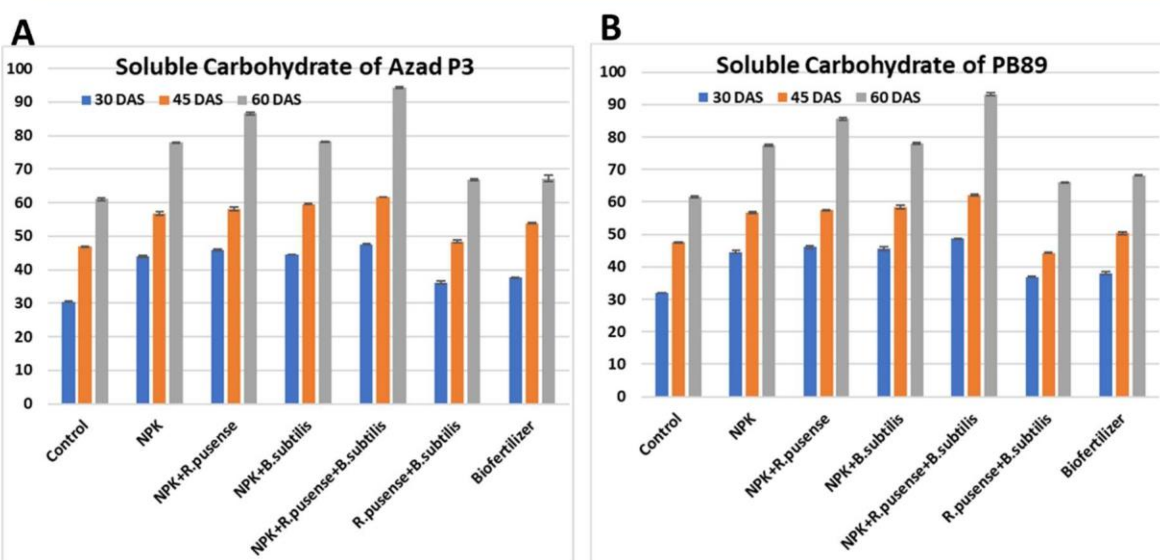
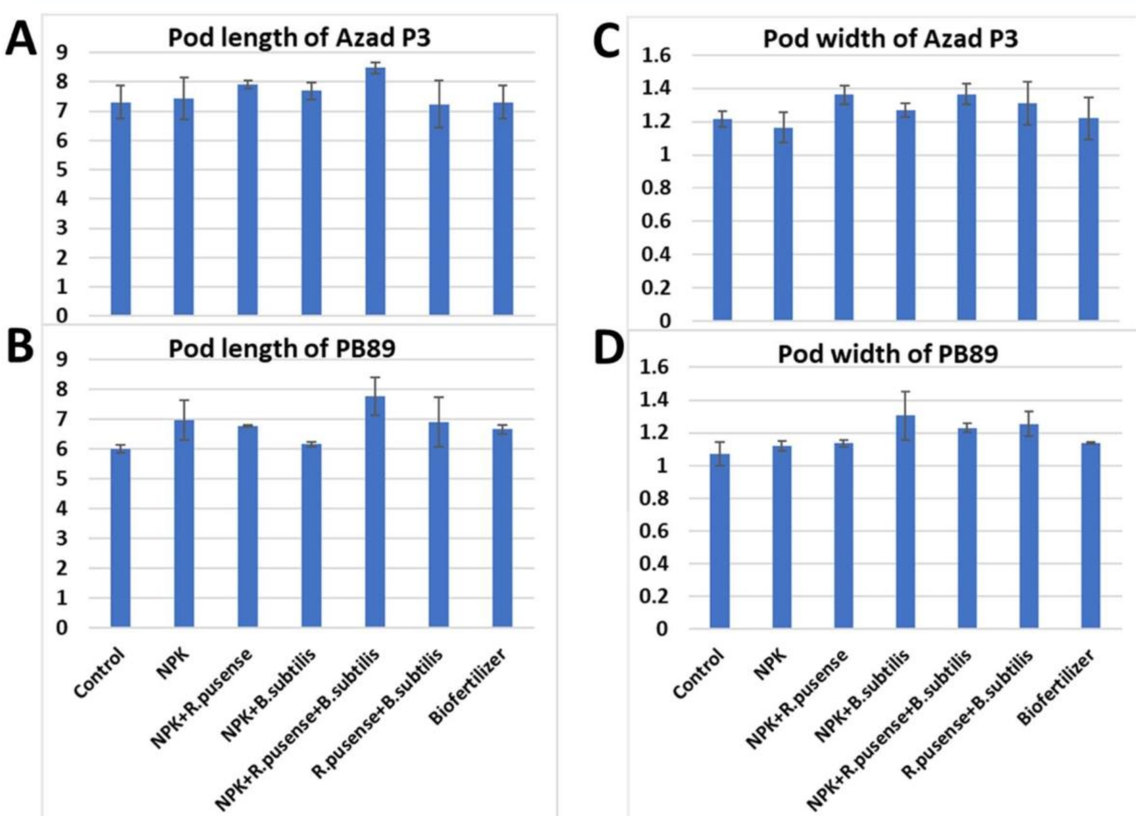


Figure 4. Soluble carbohydrate content of the varieties, Azad P3 (A) and PB89 (B) of *Pisum sativum* upon cultivated under the influence of PGPRs with or without NPK.

3.5. Yield of the varieties Azad P3 and PB89:

The yield is expressed in the terms of the dimensions of the pods, weight of pod/plant, number of pods/plant, number of seeds/pod, and weight of the seeds/pod. The Pods of the cultivar Azad P3 was longer than that of PB89 by nearly 1 cm length; however, the width was similar in the two varieties (Figure 5 A - D). The use of fertilizers in the soil has enriched the numbers of pods/plant in both the varieties, Azad P3 and PB89 of *Pisum sativum*. The number of the pods per plant were similar in the two varieties, however the yield of the weights of pods per plant were comparatively more in the Azad P3 than that of the PB89. The effect of NPK+*B. subtilis*+ *R. pusense* in the soil increased the weight of pods per plant in the case of variety Azad P3 to nearly 6 gram while the weight of the pods/ plant was nearly 3.6 gram (Figure 5 E - H). The pods of the Azad P3 was longer than that of the PB89, the more length of the pods added on the yield of both the varieties of the *Pisum sativm*. NPK+*B. subtilis*+ *R. pusense* resulted in best yields in term of the pods/plant. Between the two varieties, the production of Azad P3 was more than that of the PB89. The seeds are ready output of a crop yield. The seeds output per pod of Azad P3 was comparatively higher than that of the PB89 cultivar of *Pisum sativum* (Figure 5 I –L). Maximum output was found when the soil was manured with the NPK + consortia of the PGPRs.



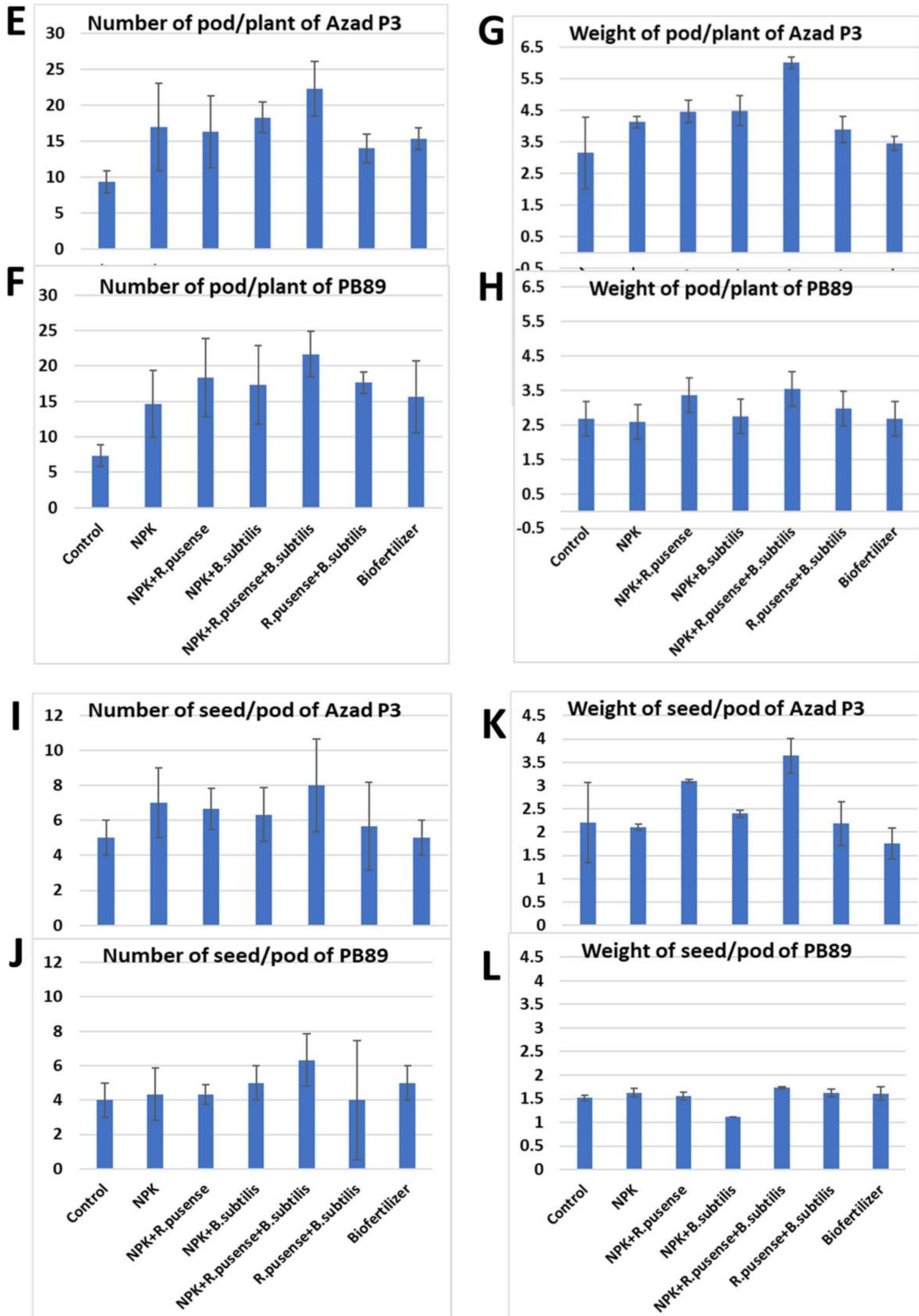

E
Number of pod/plant of Azad P3
G
Weight of pod/plant of Azad P3
F
Number of pod/plant of PB89
H
Weight of pod/plant of PB89
I
Number of seed/pod of Azad P3
K
Weight of seed/pod of Azad P3
J
Number of seed/pod of PB89
L
Weight of seed/pod of PB89
Control
NPK
NPK+R.pusense
NPK+B.subtilis
NPK+R.pusense+B.subtilis
R.pusense+B.subtilis
Biofertilizer

Figure 5. Yield of the varieties, Azad P3 and PB89 of *Pisum sativum* upon cultivated under the influence of PGPRs with or without NPK in the terms of Pod length (Figure A, B), Pod width (Figure C, D), Number of pods/plants (E, F), and Number of seeds/pods (G, H).

DISCUSSION

Crop production is dependent upon the chlorophyll content in leaves of the plant (Baker and Rosenqvist, 2004). The chlorophyll content of the plants is dependent upon various conditions including, environmental stresses, day-length, light intensity, temperature, and CO2 concentration. The land of the Haryana state of India was arable (Newas et al., 2006). Salinity stress poses a major challenge to good agricultural produce in the arid and semiarid regions. Appearance of stress factors during the growing season causes physiological changes in plants.

The results clearly established that the chlorophyll content is affected by the type of PGPR used. The composition of the PGPRs influences the types of different chlorophyll namely, chlorophyll-a. chlorophyll-b, and total chlorophyll. NPK treatment to the plants led to increased chlorophyll-a as compared to that of the control of both the varieties. The PGPRs has its relation with chlorophyll content and relative water content which is an important determinant of leaf stomata (Szpunar-Krok, E. 2022). The relative water content of the leaves reached to its maximum at 45 DAS and was found to be reduced at 60 DAS.

A difference in the yields of different varieties is well-known throughout the crops. Pea is not an exception. Several varieties had different yield (Sharma et al., 2020, Szpunar-Krok, E. 2022). The chlorophyll content of different varieties was also different. The chlorophyll content of the PB89 was higher than that of the Azad P3 of *Pisum sativum*. In accordance to the chlorophyll contents the yield of the varieties was also different. The pods of the Azad P3 was longer than that of the PB89.

Application of PGPRs in the soil enriched the yields of Pea crop, however the yield was comparatively more in the Azad P3 than that of the PB89. More length of the pods added on the yield of both the variety of the *Pisum sativm*. The results of a study of Pea plants in Himachal Pradesh (Sharma et al., 2020) reported relation between the pods/ plants and height. The study in the foot-hills of Himalaya by Sharma et al., 2020 carried out a study of the yield production of different varieties of *P. sativum*. Two varieties Azad P3 and PB89 were also carried in the study however, the results of the two studies were different. Azad P3 is known as tall and straight, while PB-89 varieties are medium dwarf but more vigorous plants.

Yield of different crops are decided by the genetic makeup of varieties (Khan et al., 2013, Singh et al., 2017). The yield of the crops is dependent upon the nutrients provided to the plants via soil. The plant growth regulators of different types of crops provide us required nutrients for full growth of the plants (Khan et al., 2013 and Kumawat et al., 2018). Amendment of soil riches the micro-nutrients as well as the macro-nutrients of degraded ecosystems (Karapouloutidou et al., 2019). Use of fertilizers and/ bio stimulants have shown improvement of crop yield in various crops (Szparaga et al., 2018; Kocira et al. 2020; Sulewska et al., 2020).

Our studies showed that the combination of these two bacteria along with the NPK increased the yield of both the bacteria. Use of these two bacteria, *Rhizobium pusense* and *Bacillus subtilis* had supplementary effect on the growth of Pea plants. It is important to notice that the growth of pea plant in presence of both the *R. pusense* and *B. subtilis* bacteria showed positive improvement of the variety, Azad P3 as compared than that of the PB89.

CONCLUSION

Pisum sativum is an important crop of India. Different varieties are known to grow in the area. Among the two varieties, Azad P3 showed increased chlorophyll content than that of the PB89 of *P. sativum*. Likewise, chlorophyll content, the yield of the Azad P3 variety was found to be higher as compared with PB89. Different PGPRs enhanced the growth and development of the two varieties resulting in the improved yield. The use of the bacteria, *R. pusense* and/or *B.subtilis* in addition with NPK were found to improve of the soil fertility. The combinations of NPK plus both these bacteria were the best for the development of Azad P3 variety than that of the PB89.

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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; Anil Sharma: Data analysis, Writing – Review & Editing.

Conflict of interest: None

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