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EFFECT OF PGPR AND BIOGAS SLURRY ON GROWTH AND YIELD OF PEA UNDER SALT AFFECTED CONDITIONS

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Abstract

Salt stress is a significant abiotic plant growth restrictive factor; it is becoming a severe environmental threat. The microorganism in the rhizosphere especially fungi and bacteria can increase the plant production under stress conditions both by direct and indirect mechanisms. A field experiment was conducted to evaluate the potential of biogas slurry and plant growth promoting rhizobacteria (PGPR) at different levels of salinity to improve the growth and yield of pea (*Pisum sativum*). In field experiment biogas slurry @ 600kg $ha^{-1}$and 800 kg ha^{-1} and PGPR strain "*Bacillus subtilis*" was applied along with 6dS m^{-1} and 8 dS m^{-1} levels of salt stress in addition to recommended doses of NPK fertilizer. The results revealed that the combined application of PGPR and biogas slurry under normal soil conditions increased shoot length by 30.27%, while under saline conditions it increased up-to 65.27%. Soil salinity reduced root length up-to 79.155% at 8 dS m^{-1}as compared to control. Soil salinity reduced chlorophyll content 36.54% of pea decrease under salt stress, the application of biogas slurry under the same condition improved 29.26% chlorophyll content of pea but the combined application of PGPR and biogas slurry enhanced the chlorophyll contents 4.68% as compared to solely application of biogas slurry. CRD was used to analyze the data statistically. The results clearly indicated that the combined application of PGPR and biogas slurry is the best source to enhance the growth and yield of pea under normal as well as under salinity stress.

***Key Words:** PGPR, Biogas, Salinity, Peas, Growth, Yield.*

INTRODUCTION

In worldwide 20% of total cultivated and 33% of irrigated agricultural soils are distressed by increased salts in soil (Lin *et al.,* 2020). Moreover, the salt affected part of world is increased at the rate of 10% yearly for numerous causes, comprising of weathering of native rocks, poor cultural practices, low precipitation, irrigation with saline water and high surface evaporation. Moreover, it was reported that more than 50% of the cultivated soil could be salt affected by the year 2050 (Jamil *et al.*, 2011). It is necessary to sustain the fertility and quality of our soil and land resources to combat the problems regarding food security (Belimov *et al.,* 2020). Climatic condition of Pakistan is a main reason behind the salt stress problem in the soils. Salinity is posing a serious problem towards an individual farmer livelihood, as most of the fertile land is being lost through increased salt stress (Measham, 2009).

Pea (*Pisum sativum* L.) a grain legume and belongs to the leguminoseae family is an instinctive to central Asia (Nawab *et al*., 2008). It grows well in cool weather in the presence of sufficient moisture. It is widely cultivated in temperate regions for its fresh green seed. Its worldwide production is 17.4 million tones and it is cultivated in 1.6% area of world (FAO, 2022). In Pakistan, it is cultivated on an area of 1 million hectares with a total production of 0.114 million tons (FAO, 2022). Peas are an important crop because of their diversity of utilization and extensive production area (Boros and Wawer, 2009). Peas are an excellent source of protein, fiber, minerals and vitamins (Moon and Ali 2022). Pea seed is a source of moderately good levels of lysine and vitamins A, B, C and contains 35 - 40% starch, 4-7% fiber. This makes it a suitable nutritional accompaniment to cereals (Abbasi *et al.*, 2020; Dhama *et al*., 2010). In Pakistan, Peas is an important crop of the Punjab Province, Which plays a major role in farmer's economy. The dried peas contain 24.6 percent protein as compared to wheat which contains only 9.4 % (Anonymous, 2011).

The microorganism in the rhizosphere especially fungi and bacteria can increase the plant production below stress conditions both by direct and indirect mechanisms (Dimkpa *et al.*, 2009). To cope with the salt stress different PGPR use different methodologies like some use direct application of a straight stimulation on plant progress by provision of iron, atmospheric nitrogen and phyto-hormones that was confiscated by siderophores, and soluble phosphates of microbial community (Hayat *et al.*, 2010). While the some other PGPR can indirectly guard the plants against soil-borne infections and diseases, and mostly these are produced by pathogenic fungi (Lutgtenberg and Kamilova, 2009).

In order to mitigate the toxic effects of salt stress on plant development, containing plant genetic engineering, there are several approaches have been established (Abdela *et al*., 2020), and now-a-days the usage of plant growth-promoting bacteria (PGPB) (Dimkpa *et al*., 2009; Mastoi *et al*., 2023). Earlier research have been showed that application of PGPR has become an auspicious substitute to lower the salt stress produced by salts (Yeo *et al*., 2010). Different microbes have different mechanisms to minimize salt stress i.e. accumulation of sugars (trehalose), solutes (e.g. proline and ectoine by most of the bacterium (Ahluwalia *et al.*, 2021). Microbes containing ACC deaminase activity such as *Achromobacter piechaudii* ARMV8, significantly lowered ethylene production and also increased biomass production of tomato plants when it was grown under severely saline conditions (Mayak *et al.*, 2004) Organic amendment application under salt stress condition can enhance microbial population. Thus biogas slurry can impart a good role in salinity stress (Maqbool *et al.*, 2014).

The present study was carried out to evaluate the combined effect of plant growth promoting rhizobacteria and different levels of biogas slurry under salt stress on growth and yield of Pea (*Pisum sativum*). Keeping in view the above mentioned facts and comprehensive discussion, the present study was initiated with the following objectives;

- To evaluate the effect of biogas slurry and PGPR on yield related parameters of peas under salt stress conditions.
- To estimate the combined effect of PGPR and biogas slurry under salt affected conditions on pea growth and related parameters.

MATERIALS AND METHODS

Research work reported in this manuscript was conducted to evaluate integrated use of PGPR and biogas slurry for improving growth and yield of pea (*Pisum sativum L.)* under saline conditions. Seeds of pea (*Pisum sativum L.*) were collected from Vegetable Laboratory of Institute of Horticulture, University of Agriculture Faisalabad.

Pre isolated salt resistant bacteria isolates were taken from Soil Microbiology and Biochemistry lab; Seed were inoculated with bacterial strain using autoclaved peat and clay as carrier material and 10% sugar solution as adhesive. Bio gas slurry was obtained through the outlet of biogas plant of cattle dung.

Treatment Plan:

Factors Levels:

Salinity 2 (S1= 6 d Sm^{-1}, S2 = 8 S d Sm^{-1})

Plant Growth Promoting Rhizo-bacteria 1 Strain/ Isolate

Biogas Slurry 2 (BGS_1= 600 kg ha^{-1}, BGS_2= 800kg ha^{-1})

Pot Experiment: Pot experiment was conducted in green house of Institute of Soil and Environmental sciences, University of Agriculture, Faisalabad to evaluate the effect of integrated use of biogas slurry and PGPR on growth and yield of pea under different salinity levels by using CRD technique. Pots were filled with 10 kg of homogeneously mixed air dried; sieved soil contaminated with 2 levels of salinity 6dSm^{-1} and 8 dSm^{-1} using NaCl salt. Five seeds of selected crop were sown in each pot. Recommended dose of urea and DAP was applied @ 25 kg $acre^{-1}$ and 35 Kg $Acre^{-1}$ to each pot respectively. The treatments included the use of PGPR alone and in combination with 600kg $ha^{-1}$and 800 kg ha^{-1} of biogas slurry in saline soil of EC 6dSm^{-1} and 8dSm^{-1}. Adequate moisture level was maintained in pots by applying irrigation at regular intervals. Various plant protection measures such as manual weeding, spraying of fungicides were done during the whole growth stage of plant. Physico-chemical characteristics of soil and slurry are illustrated in table 1 and 2 respectively.

Statistical analysis: Main and interaction effects of applied treatments on different parameters were analyzed by ANOVA (analysis of variance) technique according CRD three under Factorial. Least significant difference (LSD) test was applied to differentiate the treatment differences (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Growth and yield related parameters.

Shoot length (cm): Balanced plant nutrition ensures good growth and shoots length of plant. The shoot length of pea was affected by all applied treatments. The data regarding the shoot length is presented in the Fig. 1. Soil salinity reduced shoot length and 51.37% decrease was observed in shoot length at 8dsm^{-1} as compared to control. Application of biogas slurry (BGS) improved shoot length under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 61.08% shoot length under salt stress (8dsm^{-1}) as compared to respective control. The inoculation with PGPR improved shoot length under normal and saline conditions. The combined application of PGPR and BGS proved more effective than individual application for enhancing growth of pea. Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha^{-1}) increased shoot length by 30.27%. Under saline conditions (8 dsm^{-1}), the combined application of PGPR and BGS (800 kg ha^{-1}) enhanced shoot length by 65.27%.

These results are in accordance with Bhattacharyya and Jha, (2012) who reported enhancement of plant growth and development by application of PGPR. As PGPR containing ACC deaminase activity reduces ethylene level which is produced in plants under high salt stress conditions (Akhtar *et al*., 2020). The similar results were reported by Andy *et al*., (2020). They reported that the combined application of PGPR and biogas slurry was more effective for improving maize growth under saline conditions as compared to their individual us

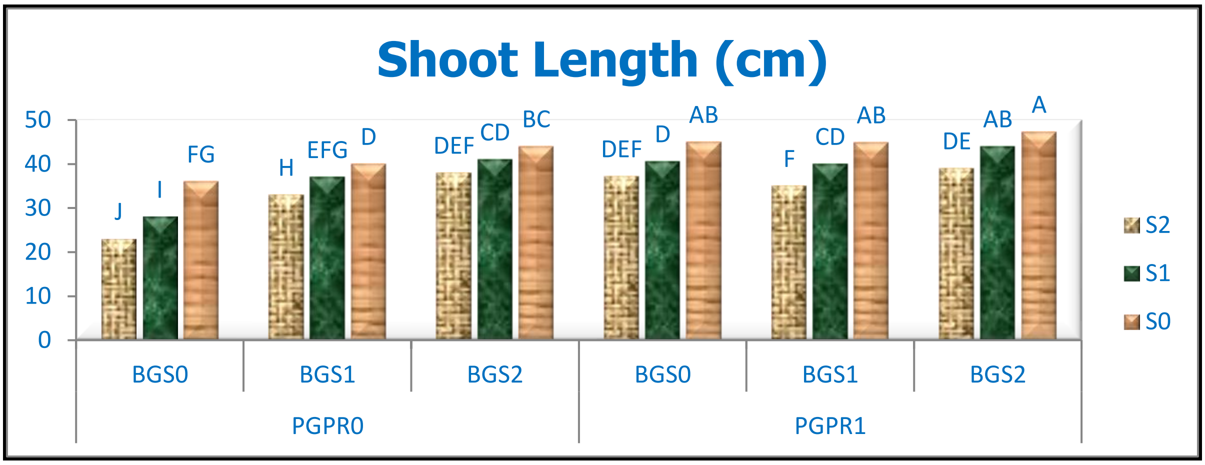

Fig.1. Effect of PGPRs and Biogas Slurry on shoot length of pea ($P \geq 0.05$).

Root length (cm): One of the most important physical parameters is root length. Plant having good root growth can penetrate easily and resulting in higher yield. The root length of pea was affected by all applied treatments. The data regarding the root length is presented in the Fig.2. The statistical analysis of the data revealed that the treatments effects were highly significant.

Soil salinity reduced root length and 79.155% decrease was observed in root length at 8dsm^{-1} as compared to control. Application of biogas slurry (BGS) improved root length under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 5.93% root length under salt stress (8dsm^{-1}) as compared to respective control. The inoculation with PGPR improved root length under normal and saline conditions. The combined application of PGPR and BGS proved more effective than individual application for enhancing growth of pea. Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha^{-1}) increased root length by 33.128%. Under saline conditions (8 dsm^{-1}), the combined application of PGPR and BGS (800 kg ha^{-1}) enhanced root length by 73.53%.

In Fig.2 results showed that the root length of pea decreased with increasing level of salinity. However PGPR containing ACC deaminase inoculation reduced the inhibitory effect of salinity. But the co-inoculation was more efficient for reducing the inhibitory effect of salt stress. And similar results were reported by Asghari *et al*., (2020) that co-inoculation increases the relative water content in plants due to the longer roots of plants co-inoculated with *Rhizobium* and PGPR containing ACC deaminase under stress which help the plants to uptake relatively more water from deeper soil under stressed conditions. Ahemad and Khan (2013) indicated PGPR can facilitate the plant growth directly by either supporting in source attainment or controlling plant hormone levels,

Number of Pods per pot: Effect of biogas slurry and PGPR strain "*Bacillus subtilis*" application and recommended doses of N, P and K fertilizers on number of pods per pot is shown in Fig. 3. The data clearly indicated that the application of biogas slurry and PGPR strain "*Bacillus subtilis*" application significantly affected the number of pods per pot. Application of PGPR increased in number of pods per pot 48.69% and 77.77% at both levels of with and without salt stress at 6 and 8 dsm^{-1} level respectively as compared to respective controlled conditions.

Due to salt stress the reduction in number of pods per pot calculated and 81.44% decrease was observed in shoot dry weight at 8dsm^{-1} as compared to control. Biogas slurry application improved number of pods per pot under both normal and salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 55.55% number of pods per pot under salt stress (8dsm^{-1}) as compared to respective control. The inoculation with PGPR improved number of pods per pot under normal and saline conditions.

Under salt stress conditions (8dsm^{-1}) the combined application of PGPR and BGS (800 kg ha^{-1}) produced higher number of pods per pot by 79.65% than control. While the combined application of PGPR and BGS (800 kg ha^{-1}) increased number of pods per pot by 140.84% under salt stress conditions. Effect on the number of pods per pot observed was more obvious in BGS application along with PGPR strain "bacillus subtilus". Carlson *et al.*, (2020) also declared that application of increased organic matter content, available phosphorus and exchangeable potassium of soil, influenced the levels of some nutrients that was measured in plant. Similar study was also founded by (Yu *et al.* 2010) that increase in fruit yield on the application of biogas slurry in combination of N inorganic fertilizer was in line with the results of my research

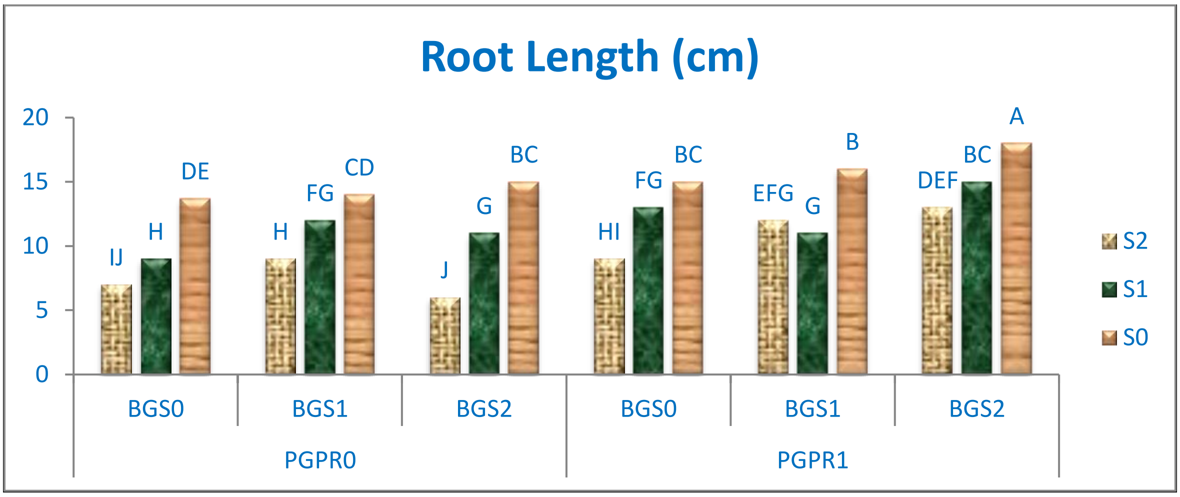

Fig.2. Effect of PGPRs and Biogas Slurry on root length of pea (P≥0.05).

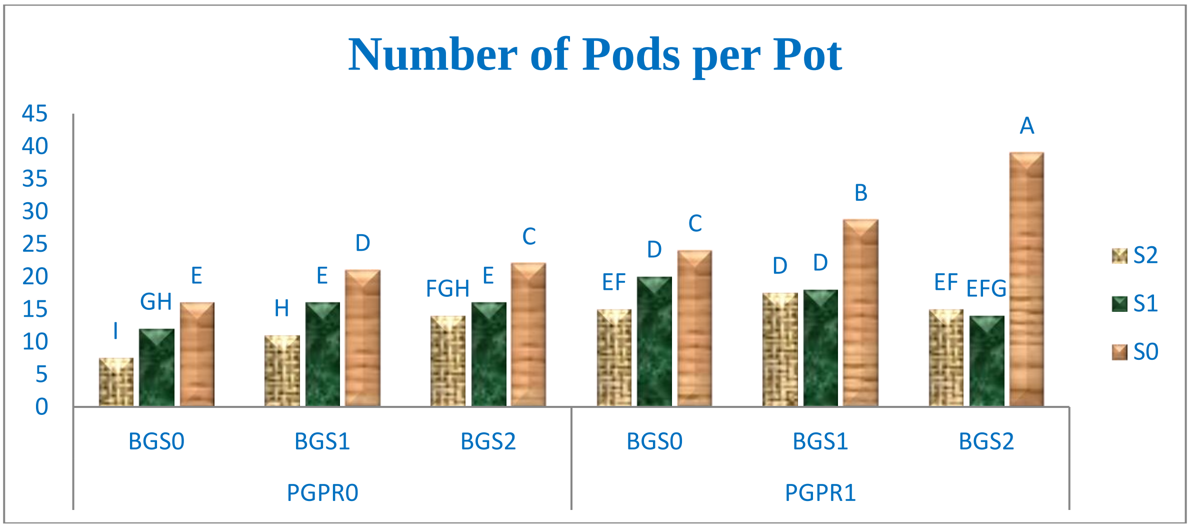

Fig.3. Effect of PGPRs and Biogas Slurry on No. of pods of pea per Pot (P≥0.05).

Fresh Weight of Pods (g): Data exhibiting the significant effects of both treatments on fresh weight of pods of pea is mentioned in below given Fig. 4. PGPR and high level of biogas slurry (BGS) (800 kg ha^{-1}) application proved an effective measure in saline soils.

Application of PGPR increased in fresh weight of pods 46.94%, 43.14% and 36.14% at all three levels of with and without salt stress (6 and 8 dsm^{-1}) level respectively as compared to respective controlled conditions. Soil salt stress conditions reduced fresh weight of pods and 86.93% decrease was observed in fresh weight of pods at 8dsm^{-1} as compared to control. Application of BGS improved fresh weight of pods under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 15.42% fresh weight of pods under salt stress (8dsm^{-1}) as compared to respective control. The inoculation with PGPR improved fresh weight of pods under normal and saline conditions.

Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha^{-1}) increased fresh weight of pods by 90.49%. Under saline conditions (8dsm^{-1}), the combined application of PGPR and BGS (800 kg ha^{-1}) enhanced fresh weight of pods by 88.43%. These results are in accordance with Danish *et al.*, (2020). Who concluded that higher yield of maize and sunflower was included in biogas slurry (BGS). This finding has resemblance with the result of Gontia *et al.*, (2020) who obtained the highest 1000-seed weight of peas with the treatment comprising of rhizobium inoculant in combination with 1.5 kg ha^{-1} MO. Similar results were found by (Grover *et al.*, 2021; Khan *et al.*, 2020 and kim *et al.*, 2020) they also reported that rhizobium inoculation significantly increased the 100-seed weight in chick pea

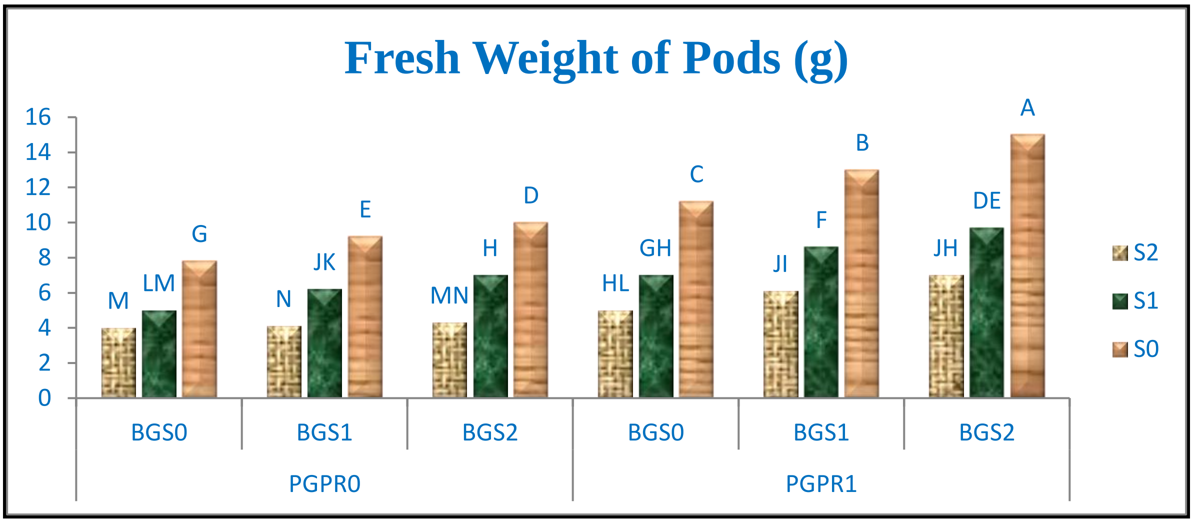

Fig.4. Effect of PGPRs and Biogas Slurry on Fresh Weight of Pods per Pot (P≥0.05).

Physico-chemical parameters:

Emergence percentage: Soil salinity decreased the emergence percentage of pea by 37.50% at salt stress (8dsm^{-1}) as compared to control (Fig. 5). Application of BGS enhanced the emergence percentage under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 84.90% emergence percentage of pea under salt stress (8dsm^{-1}) as compared to respective control. The inoculation with PGPR improved emergence percentage of pea under normal and saline conditions. Application of PGPR increased the emergence percentage of pea by 62.49%, 54.54% and 30% at all three levels of with and without salt stress at 6dsm^{-1} and 8dsm^{-1}level respectively as compared to respective controlled conditions.

The combined application of PGPR and BGS proved more effective than individual application for enhancing growth of pea. Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha^{-1}) increased emergence percentage of pea by 118.18%. Under salt stress conditions (8dsm-1), the combined application of PGPR and BGS (800 kg ha^{-1}) enhanced shoot dry weight by 120%. These results are in confirmation with (Lin *et al.*, 2020) who revealed that the early seedling stage of growth is the most salt sensitive for most crop

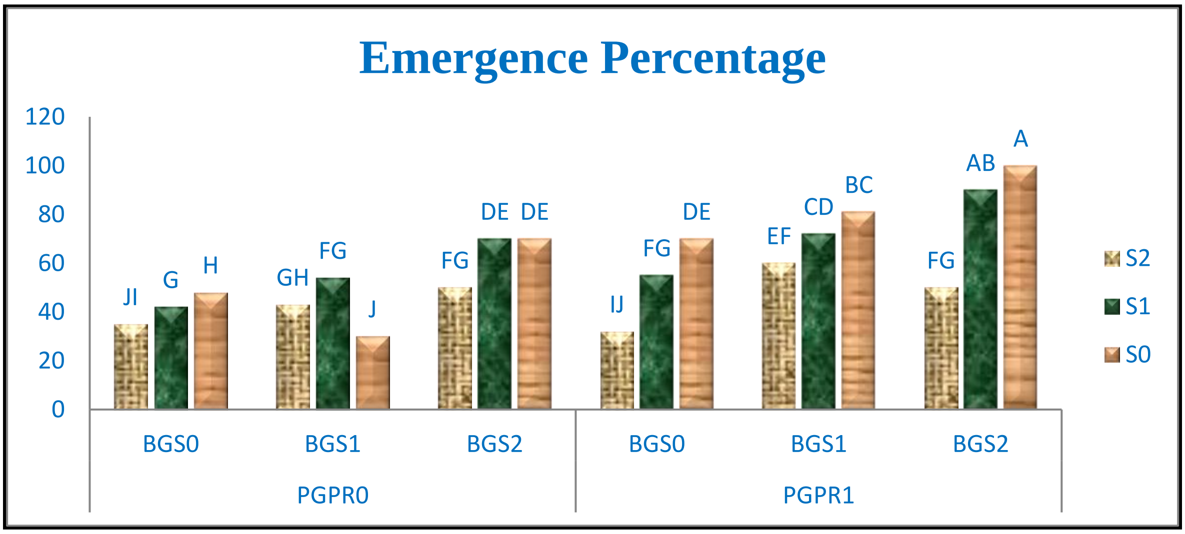

Fig.5. Effect of PGPRs and Biogas Slurry on Emergence Percentage (P≥0.05).

Chlorophyll Content (SPAD value): Soil salinity reduced chlorophyll content of pea and 36.54% decreases was observed in shoot dry weight at 8dsm^{-1} as compared to control (Fig 6). Application of biogas slurry (BGS) improved chlorophyll content of pea under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 29.26% chlorophyll content of pea under salt stress

(8dsm-1) as compared to respective control. The combined application of PGPR and BGS proved more effective than individual application for enhancing growth of pea. Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha-1) increased chlorophyll content of pea by 30.16%. Under saline conditions (8dsm-1), the combined application of PGPR and BGS (800 kg ha-1) enhanced chlorophyll content of pea by 4.68%. These results are in accordance with the Cuin *et al.*, (2008) who concluded that decrease in photochemical efficiency of PSII indicates the detrimental salinity effects on leaf photochemistry under stress conditions. These results are in line with the results of Yu *et al.*, (2010) stated that the increase in fruit yield on the application of biogas slurry in combination of N inorganic fertilizer.

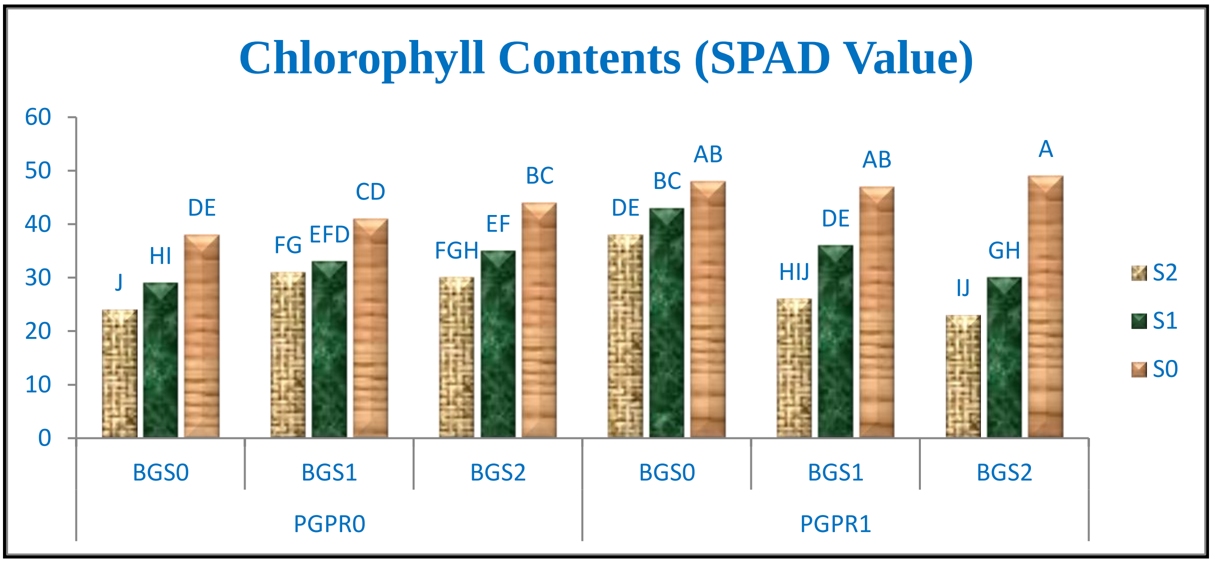

Fig.6. Effect of PGPRs and Biogas Slurry on Chlorophyll Contents (SPAD Value) (P≥0.05).

Sodium Uptake mg g-1 (dry Wt.): Soil salinity increased the sodium uptake by 209.29% in pea at 8dsm-1 as compared to control (Fig. 7). Application of biogas slurry (BGS) reduced the sodium uptake values of pea under normal condition as well as salt stress condition. Biogas slurry (800 kg ha-1) 27.64% increased the reduction of sodium uptake of pea under salt stress (8 dsm-1) as compared to respective control. The combined application of PGPR and BGS proved more effective than individual application for the reduction in sodium uptake values of pea. Under saline conditions (8 dsm-1), the combined application of PGPR and BGS (800 kg ha-1) reduced the sodium uptake values of pea, increased by 145.76%.

These results are in accordance with Munns *et al.* (2006) concluded better photosynthetic capacity resulted in greater salt tolerance while high K^+ and low Na^+ is the major attribute for higher K^+/Na^+ ratio in the cytoplasm of mesophyll cells for both wheat and barley. They also suggested Na^+ exclusion as a key selection criterion for salt tolerance in wheat.

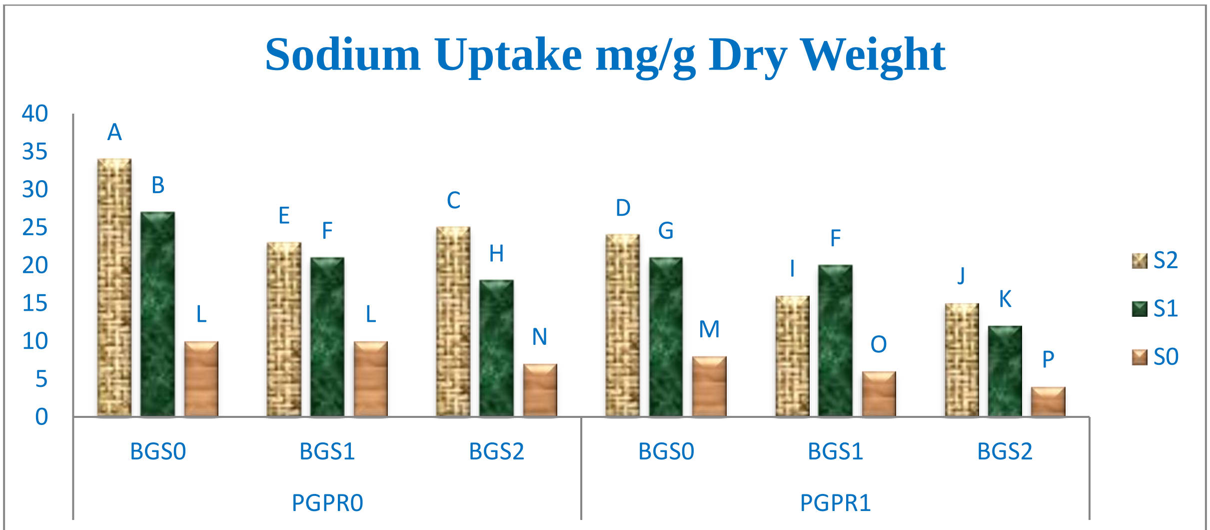

Fig.7. Effect of PGPRs and Biogas Slurry on Sodium Uptake mg g-1 of dry weight. (P≥0.05)

Potassium Uptake mg g^{-1} dry Weight: Soil salinity reduced potassium uptake values of pea and 75.05% decrease was observed in potassium uptake values of pea at 8dsm^{-1} as compared to control (Fig. 8). Application of biogas slurry (BGS) improved potassium uptake values of pea under normal condition as well as salt stress condition. Biogas slurry (800 kg ha^{-1}) improved 71.42% potassium uptake values of pea under salt stress (8dsm^{-1}) as compared to respective control. Under normal soil conditions, the combined application of PGPR and BGS (800 kg ha^{-1}) increased potassium uptake values of pea by 165.43%. Under saline conditions (8dsm^{-1}) the combined application of PGPR and BGS (800 kg ha^{-1}) enhanced potassium uptake by 214.28%.

These results are in accordance with Cuin *et al.*, (2008) suggested that a higher selectivity of such cation transport systems for K^+ over Na^+ could be an important determinant for salt tolerance in many field crops including barley and wheat. Mishra *et al.*, (2020); Nascimento *et al*., (2020) also concluded that the optimal K^+/Na^+ ratio can be maintained by either confining Na^+ accumulation in plant tissues or by preventing K^+ loss out of the cell.

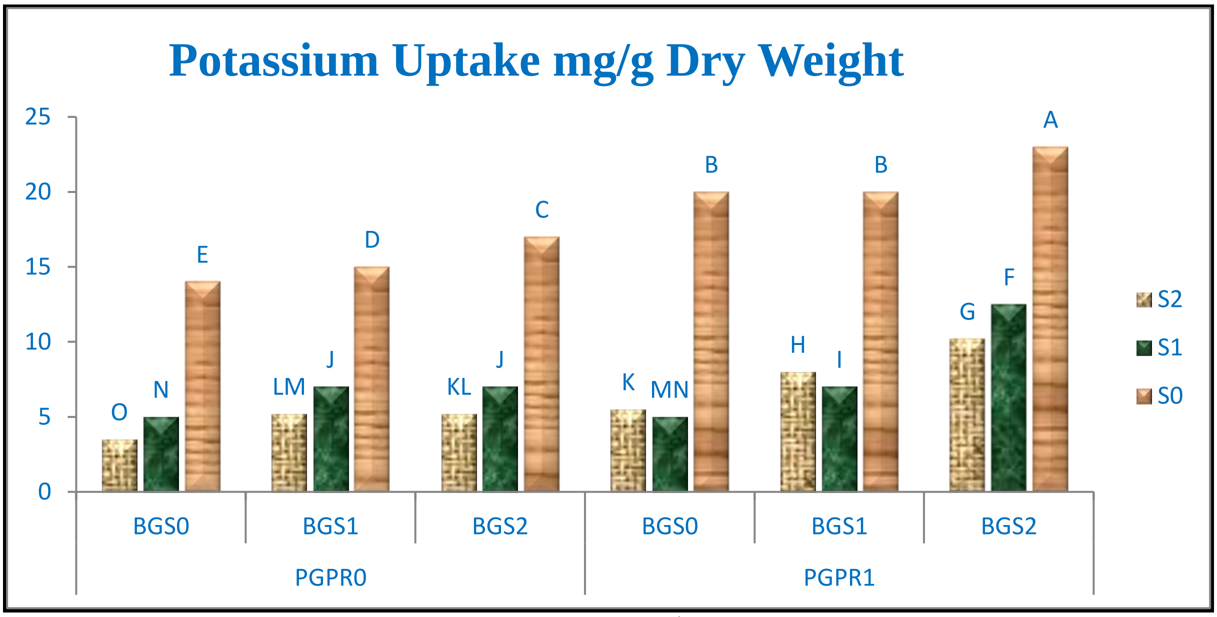

Fig.8. Effect of PGPRs and Biogas Slurry on Potassium Uptake mg g^{-1} of dry weight. ($P \geq 0.05$)

CONCLUSION

From above given results and discussion it might be concluded that integrated use of *PGPR* with *BGS* can improve the pea plant growth and ability to cope with the toxic effect of chromium. The uptake of potassium was enhanced on the other hand uptake of sodium was reduced by exploiting the combined effect of "*Bacillus subtilis*" and biogas slurry.

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