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ROLE OF PLANT GROWTH PROMOTING RHIZOBACTERIA APPLIED IN COMBINATION WITH COMPOST AND MINERAL FERTILIZERS TO IMPROVE GROWTH AND YIELD OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

This study was conducted to assess the possible role of the integrated use of seed inoculation with plant growth promoting rhizobacteria (PGPR), compost and mineral fertilizers for improving growth and yield of wheat sown at different plant spacing. PGPR were isolated from rhizosphere soil of wheat plants. Four treatments were applied in the main plots viz., T_1 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} as Control), T_2 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Compost @ 250 kg ha^{-1}), T_3 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Compost @ 250 kg ha^{-1} + Inoculation with PGPR), T_4 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Inoculation with PGPR). Basal dose of P and K @ 100 and 60 kg ha^{-1} as Diammonium phosphate and murate of potash respectively was applied to all treatments at sowing time. Maximum increase in plant height, number of tillers m^{-2}, and number of spikelets $spike^{-1}$, grain and straw yield were recorded with the use of PGPR inoculated seeds in combination with compost and chemical fertilizers. Maximum grain yield and 1000 grain weight were observed where PGPR inoculated seeds were used in combination with recommended chemical fertilizers. Higher N content in grain and straw were recorded with the application of seed inoculation with PGPR along with compost and recommended chemical fertilizers. Planting space had a significant effect and maximum growth and yield was recorded at 25cm plant to plant spacing.

Introduction

Plant growth promoting rhizobacteria (PGPR), compost and chemical fertilizers significantly affect the growth and yield of different crops. A novel approach could be that composted material may be converted into a value added product such as an effective biofertlizer by blending with plant growth promoting rhizobacteria which are free living soil bacteria that can either directly or indirectly facilitate rooting (Mayak *et al*., 1999) and growth of plants (Glick, *et al.*,1995; Mayak *et al*., 1999).

There are several mechanisms by which PGPR affect plant growth such as their ability to produce various compounds (such as phytohormones, organic acids, siderophores), fix atmospheric nitrogen, solubilize phosphate and produce antibiotics that suppress deleterious rhizobacteria, and production of biologically active substances or plant growth regulators (PGRs). Production of biologically active substances or plant growth regulators (PGRs) is one of the major mechanisms through which PGPR influence the plant growth and development (Arshad & Frankenberger, 1998). Therefore the use of PGPR to enhance plant growth and crop yield is predicted to become an emerging trend in contemporary agriculture in the near future (Pal *et al*., 2000).

Wheat (*Triticum aestivum* L.) is the source of almost 20% of the total necessary calories of the world's population. It is the most valuable staple food, which occupies a central position in forming agricultural policies because of it's per hectare yield is far below the actual potential of the existing varieties.

The planting spacing has a great bearing on the productivity of wheat by determining proper crop stand of individual plants through balancing competition between plants. In contrast to high yield levels, at moderate levels, widening the row space appears not to decrease grain yield (Hiltbrunner *et al*., 2005). Wide rows are more profitable than narrow rows due to savings in seed and machinery cost. Plants that perform in wide rows tend to grow tall due to favorable weather. They also have a non-erect growth habit that allows them to fill in the wide row middles which can also compensate for skips in the row or low population. High rates of tillering also favor higher yields in wide rows **(**Beuerlein, 2001). Among the various determining factors in improving crop yield, soil fertility status and plant nutrition is also of a great importance. Hence under these circumstances, there is a need to improve plant nutrition and increase crop yield by using non-conventional approaches.

Keeping this in view, a field trial was conducted in the experimental area at the Institute of Soil and Environment Sciences, University of Agriculture, Faisalabad, Pakistan, to assess the effectiveness of the integrated application of seed inoculation with PGPR, compost and mineral fertilizers for improving growth and yield of wheat sown at different plant spacing.

Materials and Methods

Isolation of plant growth promoting rhizobacteria: Plant growth promoting rhizobacteria were isolated from rhizosphere soil of wheat crop by using a dilution plate technique with a salt minimal medium (Dworkin & Foster, 1958) containing ACC as the sole nitrogen source (enrichment technique). Wheat plants were uprooted along with good amount of non-rhizosphere soil, brought immediately to the laboratory in polythene bags (2 kg size: 23 × 320 mm) and were subjected to air dry within 2h. The non-rhizosphere soil was removed by gentle shaking leaving behind the rhizosphere soil only. The rhizosphere soil was collected from roots by dipping and gentle shaking in sterilized water under aseptic conditions. The isolated rhizobacteria were purified by streaking on fresh plates containing DF minimal salt medium and preserved in 20% glycerol at – 30°C for subsequent inoculation.

Inoculum preparation: The inoculum was prepared by growing the selected rhizobacterial strain in 250 ml flasks containing DF minimal salt medium (Dworkin & Foster, 1958). The medium was incubated at 28 ± 1°C for 48 h in an orbital shaking incubator at 100 rpm. The optical density of inoculum was measured and a uniform population of rhizobacteria (10^7-10^8 colony forming units) maintained at the time of inoculation. Peat was ground and autoclaved at 121°C for 20 minutes. A 100 ml inoculum of the selected rhizobacteria was mixed with 1 kg of peat and incubated for 24 h at 28 ± 1°C before using for seed coating, with a seed peat ratio 1:1 (w/w).

Composting: Organic waste material containing fruit and vegetable wastes was collected from the various fruit and vegetable markets of Faisalabad, Pakistan. This organic waste material was allowed to decompose in open air for two months. The heaps of the organic material were reshuffled and moistened at different intervals to promote microbial activity for composting. Thereafter the composted material was allowed to air dry and processed in a locally fabricated composter, consisting of drier, crusher and grinder for preparing the compost. Air dried organic waste materials were graded to sort out all unwanted substances from the organic wastes and oven dried at 65 ± 5°C for 24 h before crushing. Oven dried material was crushed into a 4 mm crusher unit of the composter.

Field trial: A field trial was conducted in the research area of the Institute of Soil and Environmental Science, University of Agriculture, Faisalabad, Pakistan. The field was ploughed, prepared and laid out according to the split split randomized complete block design with four replications with a net plot size of 5m x 4m. Before conducting the experiment one composite soil sample taken from a plough layer (0-15 cm depth) was air dried, sieved through a 2 mm screen and analyzed for various physieochemical properties. The analysis of the composite soil sample revealed a pH of 7.4, EC 1.4 dS m^{-1}, cation exchange capacity (CEC) 6.3 cmol (+) kg^{-1}, organic matter 0.74%, Olson P 6.35 mg kg^{-1}. Four treatments applied in main plots were as T_1 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} as Control), T_2 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Compost @ 250 kg ha^{-1}), T_3 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Compost @ 250 kg ha^{-1} + Inoculation with PGPR), T_4 (Recommended N as Chemical Fertilizer @ 120 kg ha^{-1} + Inoculation with PGPR). The wheat seeds (cv. Inqlab-91) to be sown in inoculation treatment plots were surface sterilized by dipping in 95% ethanol solution for 5 minutes and 0.2% $HgCl_2$ solution for 3 minutes, and then washed thoroughly with sterilized water (Khalid *et al*., 2004). For inoculation, the seeds were dressed using the inoculated peat and clay mixed with 30 ml of 10% sugar solution and 60 ml of PGPR broth culture. For the uninoculated treatments, the seeds were coated with the sterilized peat treated with sterilized broth and 25 ml of sugar solution. In each treatment wheat seeds were sown by using the dibbler method keeping a row to row distance of 25 cm in main plots and plant to plant distance S1 = 10 cm, S2 =15 cm, S3 = 20 cm and S4 = 25 cm, in sub plots under each treatment.

A basal dose of phosphorous (100 kg ha^{-1}) as diammonium phosphate (DAP) and potassium (60 kg ha^{-1}) as murate of potash (MOP), respectively were applied in the main treatment plots before sowing of wheat. N fertilizer as urea was applied in two split doses i.e., half at sowing time and half at the grain formation stage. Five irrigations of fresh canal water {electrical conductivity, 0.04 dSm^{-1}; sodium adsorption ratio, 0.28 (mmol $l^{-1})^{1/2}$; and residual sodium carbonate, 0} with an average depth of 10 cm were applied to the wheat crop after germination. Growth and yield parameters i.e., plant height at maturity, number of tillers m^{-2}, number of spikelets $spike^{-1}$, 1000-grain weight, straw and grain yield were recorded. N contents in grain and straw were determined according to the procedure described by Jackson (1962). Error bars in each figure depict the standard error mean of the data.

Results

The application of PGPR and compost in combination with chemical fertilizer significantly improved the growth and yield of wheat sown at different plant to plant spacing. Maximum plant height (96 cm) was observed with treatment T_3 where recommended N fertilizer was applied in combination with compost and seed inoculation which showed 6.67% increase over control (Fig. 1). T_4 was second best treatment where recommended NPK fertilizers were applied in combination with seed inoculation; it showed of 3.33% increase over control. Regarding plant spacing, in T_1, S_4 (25 cm plant to plant spacing); T_2, S_3 (20 cm plant spacing) and S_4 (25 cm plant spacing) showed similar and maximum response on plant height. In T_3, S_2 (15 cm plant spacing) and S_3 (20 cm plant spacing) were similar in their effect on plant height and showed maximum response whereas in T_4, maximal response was observed with S_3 (20 cm plant spacing) and S_4 (25 cm plant spacing).

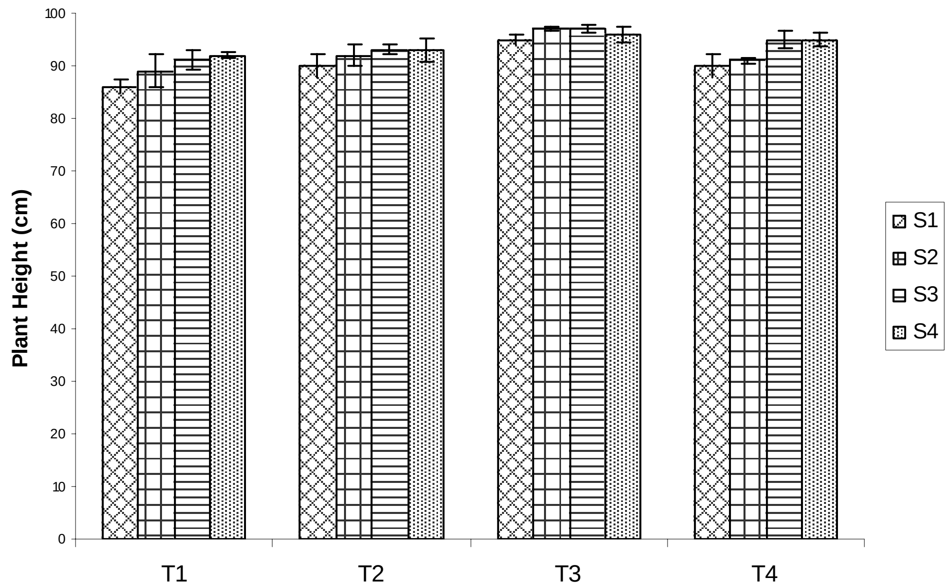

Fig. 1. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on plant height of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

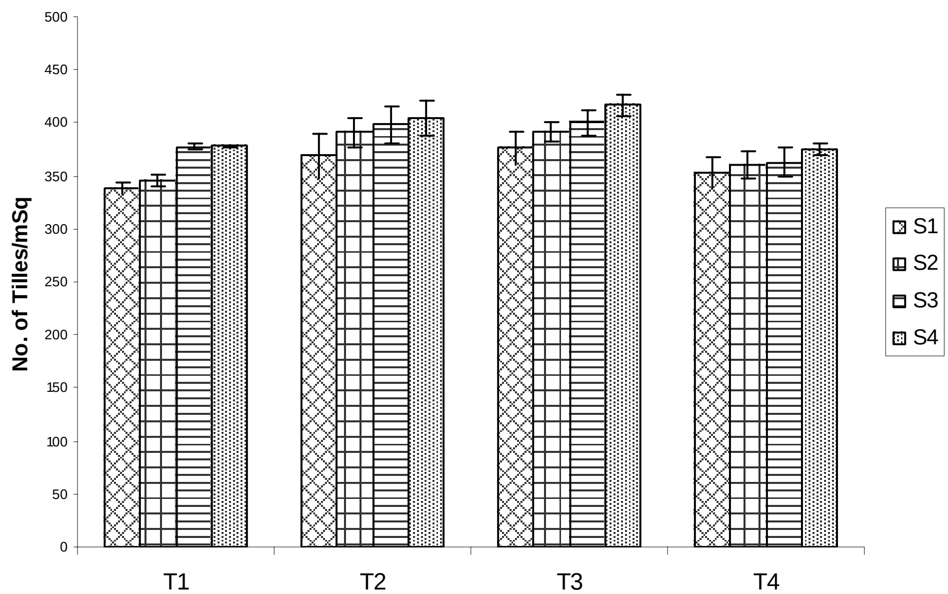

Fig. 2. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on tillering of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

The data regarding tillering revealed that maximum number of tillers m^{-2} (396), was observed with treatment T_3 resulting in 10.00 % increase in tillering over control (Fig. 2). Regarding plant spacing, in all the treatments, S_4 showed maximum response with maximum number in T_2 and T_3. Maximum number of spikelets $spike^{-1}$ (19) was also observed in T_4, 5.88% higher than control. Regarding plant spacing S_4 proved best in all the treatments in increasing the number of spikelets $spike^{-1}$ (Fig. 3).

The application of recommended N fertilizer alongwith seed inoculation (T_4) proved most effective with a 13.84% increase in the 1000-grain weight over control. Regarding plant spacing, in T_1, T_2 and T_3, S_4 (25 cm plant spacing) showed maximum response whereas in T_4 S_1 (10 cm plant spacing and S_4 (25 cm plant spacing) exhibited statistically similar and highest effect (Fig. 4).

Maximum straw yield (8760 kg ha^{-1}) was also observed in T_3 which showed 12.75% increase over control (Fig. 5). Regarding plant spacing, in T_1, T4 S_4 (25 cm plant spacing) showed maximal response. In T_2, S_1 (10 cm plant spacing) showed maximum response, while in T_3, S_2 (15 cm plant spacing) showed maximal response.

All the treatments had a statistically significant effect on grain yield but maximum response was seen in T_4 where recommended N fertilizer was applied alongwith seed inoculation and it resulted in 6.86% increase in grain yield over control (Fig. 6). Regarding plant spacing, in T_1, S_4 (25 cm plant spacing); T_2, S_3 (20 cm plant spacing) and S_4 (25 cm plant spacing); and in T_3, S_4 (25 cm plant spacing) showed maximum response whereas in $T_{4,}$ S_2 (15 cm plant spacing), S_3 (20 cm plant spacing) and S_4 (25 cm plant spacing) statistically had a similar effect on grain yield.

Chemical analysis of the wheat straw and grains for N content revealed that all the treatments significantly influenced the nitrogen content (Figs. 7 & 8). Maximum N contents 0.66% and 1.74% in straw and grains respectively were recorded with seed inoculation with PGPR alongwith the application of compost and recommended chemical N fertilizer (T_3) that caused an increase of 144.44% and 34.88% increase over control respectively. Regarding plant spacing, S_4 (25 cm plant spacing) showed maximum response in all the treatments.

Discussion

This study demonstrated the positive effect of the integrated use of seed inoculation with plant growth promoting rhizobacteria, compost and chemical fertilizer on growth and yield of wheat sown at different plant spacing in a field experiment. In the case of plant height, number of tillers m^{-2}, number of spikelets $spike^{-1}$ and straw yield, best results were recorded in T_3 where seed inoculation with plant growth promoting rhizobacteria (PGPR) in combination with compost and chemical fertilizer was applied which caused 6.67% increase in plant height, 10% increase in number of tillers m^{-2}, 5.88% increase in number of spikelets/spike and 12.66% increase in straw yield over control. These findings are supported by Marcos *et al*., (1995) and Naveed *et al*., (2008). The increase in plant height recorded was significantly higher with increasing plant to plant distance. Highest plant height and number of tillers was recorded in S_4 (250 cm plant to plant spacing). This might be due to the reason that the greater plant spaces provided more space to plants to receive light resulting in more photosynthesis and thus more growth. Furthermore the plant roots had more chance to spread and take nutrients from greater surface area.

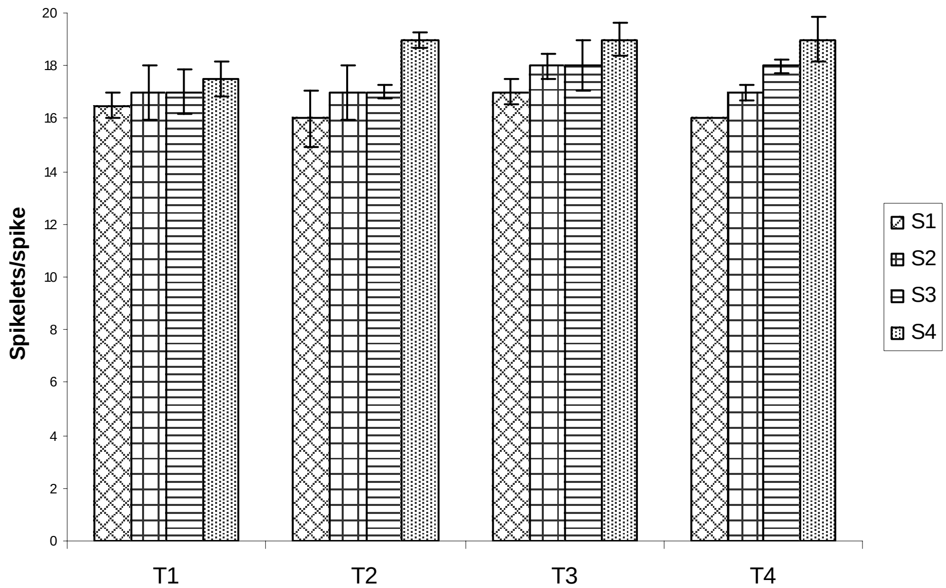

Fig. 3. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on number of spiklets of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

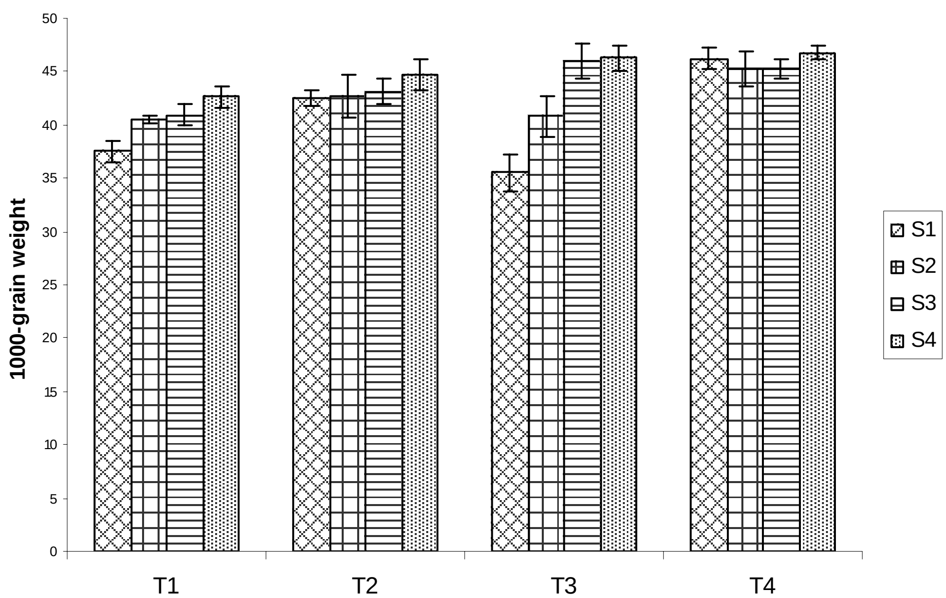

Fig. 4. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on 1000 grain weight of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

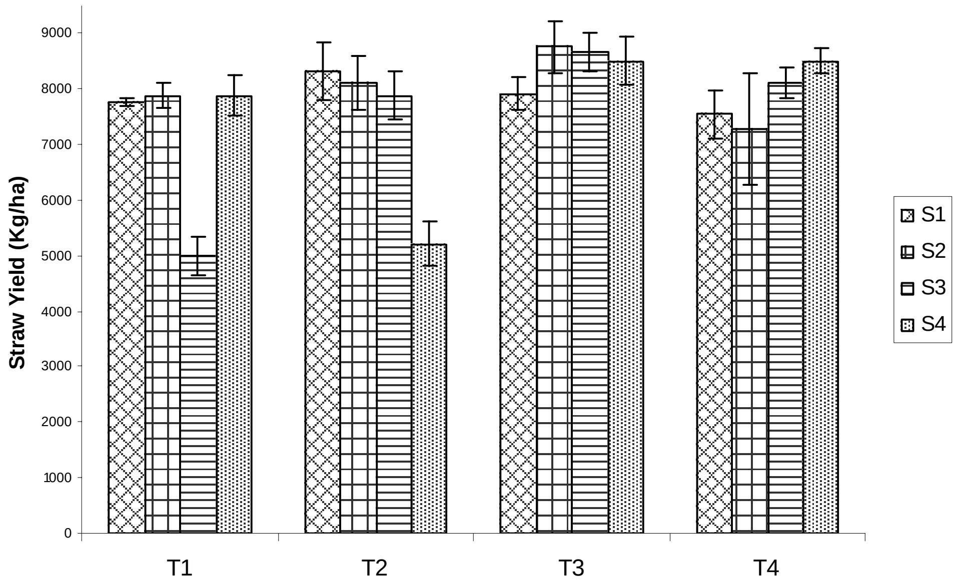

Fig. 5. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on straw yield of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

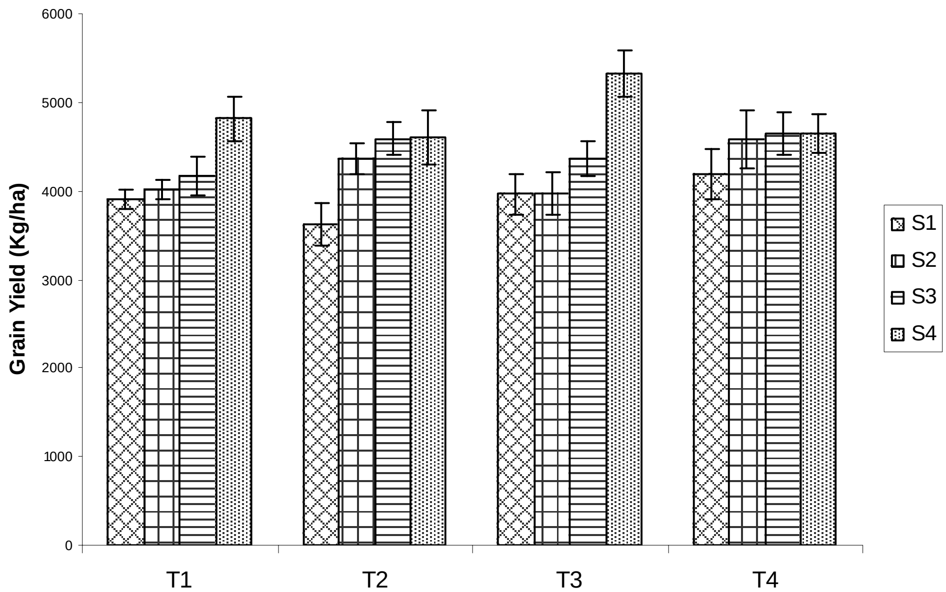

Fig. 6. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on grain yield of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

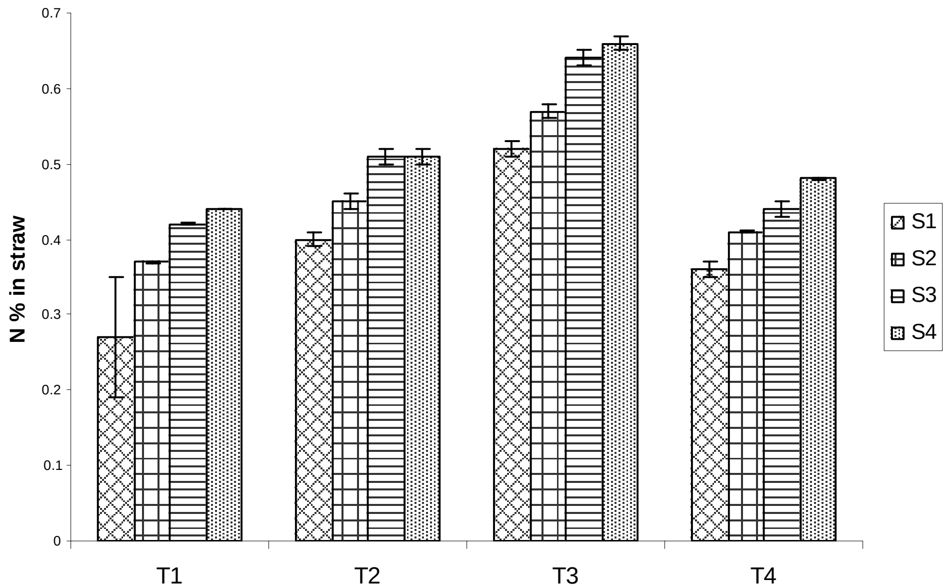

Fig. 7. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on N content straw of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

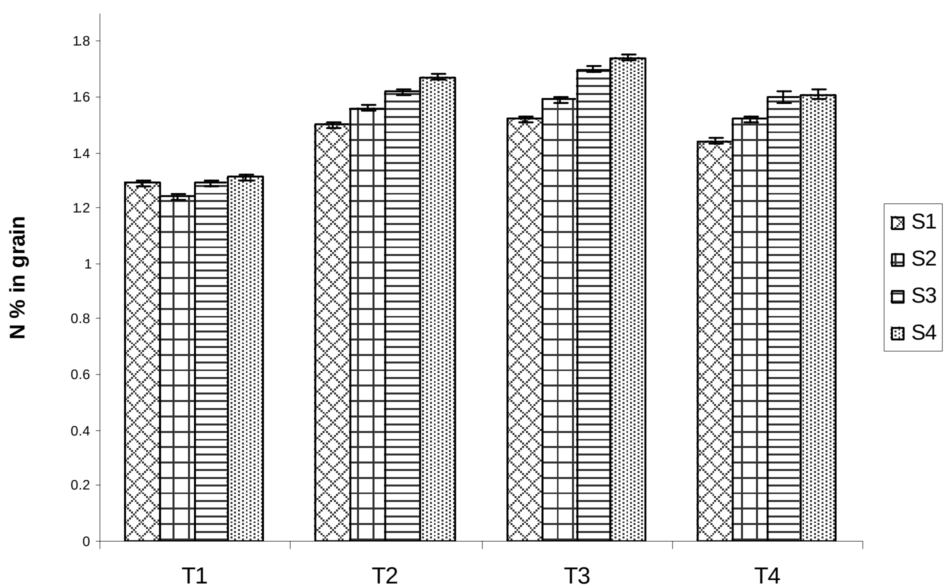

Fig. 8. Effect of compost, plant growth promoting rhizobacteria and chemical fertilizers on N content of grains of wheat sown at different plant spacing (Average of four replicates). S_1, S_2, S_3 and S_4 are 10, 15, 20 and 25 cm plant to plant distance respectively.

In the case of grain yield and 1000 grain weight, T_4 where inoculation of seeds with PGPR was used in combination with recommended chemical fertilizers showed best results which caused 6.86% and 13.84% increase over control respectively. These results are in agreement with the findings of many other workers (Mei, 1989; Vacura, 1989; Xiia *et al*., 1990; Chen *et al.*, 1994; Javed, 1995; & Zahir *et al*., 1996). Similarly, our findings in case of 1000- grain weight were also in accordance with the findings of Hussain *et al*., (1985) and Zahir *et al*., (1996).

Higher N content were recorded with the application of seed inoculation with PGPR alongwith compost and recommended chemical fertilizers. These findings are in agreement with the findings of Marcos *et al*., (1995), Das *et al*., (2004) and Zahir *et al*., (2007). This may be due to a possible reduction of N losses due to leaching, denitrification or volatilization. This demonstrated that the inoculation of wheat seeds with PGPR and addition of compost resulted in improved N use efficiency. Furthermore, in compost applied plots nutrients remained available over a long period of time due to slow and long-term release of nutrients from the compost.

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