Research Article

Response of Spring and Autumn Sunflower to Chemical and Bio-Fertilizers for Yield and Quality Traits

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Abstract | Sunflower (*Helianthus annuus* L.) is one of the major oilseed crops grown worldwide for the production of edible oils. In order to increase the oil production there is dire need to grow more sunflower by using the economical and environment friendly techniques. Present study was proposed to evaluate the response of spring and autumn sunflower to chemical and bio-fertilizers for yield and quality traits during 2014 at University of Agriculture, Faisalabad, Pakistan. The treatments comprised on the combination of chemical fertilizers (No chemical fertilizer, half recommended dose of fertilizer and full recommended dose of chemical fertilizer) and Plant Growth Promoting Rhizobacteria (PGPR) (No PGPR, PGPR-1, PGPR-2 and PGPR-1 + PGPR-2). The PGPR used in this study were pre-isolated and characterized, taken from soil microbiology and biochemistry laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad. In this experiment Randomize Complete Block Design (RCBD) with factorial arrangements having three replications was used. Significantly maximum 1000 achene weight, achene yield, biological yield, harvest index and protein content were recorded where seed inoculation with PGPR-1 + PGPR-2 and full dose of recommended chemical was applied during both the growing seasons. Overall the performance of sunflower was better in spring as compared to autumn season. However, it was also noted that the combination of PGPR-1 + PGPR-2 and half recommended doses of chemical fertilizer gave results as full recommended dose of chemical fertilizer alone. Therefore, PGPR enhanced the yield by reducing the use of chemical fertilizer, leading towards the sustainable agriculture.

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops containing high quality of edible oil worldwide. It is easy to cultivate different type of soils (Kaya and Kolsarici, 2011; Lopez-Valdez et al., 2011). In Pakistan sunflower is also oil seed crop with many qualities (Nasim et al., 2011; 2012), however, average yield of sunflower is very lesser than the genetic potential of various varieties under cultivation. Factors responsible for low yield are such as improper nutrition, less fertile soil, poor management practices, run-off and leaching of N and P fixation in calcareous soils along with high fertilizer prices.

Extensive use of chemical fertilizers is causing the environmental and health hazards. Considering these issues, regarding the usage of chemical fertilizers many attentions has been drawn towards the use of bio-fertilizers. The application of bio-ferti-

lizers is encouraged in order to establish a pollution free, environmental friendly and sustainable farming ecosystem (Galavi et al., 2011). The combination of chemical fertilizers with bio-fertilizers is one of the most important ways for the sustainable agriculture production (Ali et al., 2008).

Rhizobacteria (PGPR) are the groups of becteria that colonize in the roots and improve the growth and yield of plant by several mechanisms such as organic acid, phytohormone, siderophore and biologically active compounds production as well as phosphate solublization (Mirshekari et al., 2012) . Previous study showed that chemical fertilizers and PGPR significantly improved the height of plant, branches per plant and leaf chlorophyll (Nosheen and Bano 2014). Application of combination of PGPR and chemical fertilizer improved the grain yield, yield components and dry matter production of barley (Hassanzadeh et al., 2006). It was also reported that the application of *Azospirillum* and *Azotobecter* improved canola yield, number of branches, pod per plant and weight of 1000 grain (Yasari and Patwardhan, 2007).

Sunflower is a crop that can be grown in two seasons spring and autumn in Pakistan. Hence, from germination to maturity of sunflower crop two opposite sets of environmental condition exist. Temperature is the major environmental factor that determined the plant growth and development has been widely studied for different crops. Khalifa et al. (2000) reported that germination and vegetative growth of sunflower during autumn takes place under high temperature, takes less time and completes life cycle.very soon. It was reported that favorable growing conditions during flowering and seed setting period for the crop of spring resulted in greater achene yield (Sumangala and Giriraj, 2003).

Being grown in diverse environmental conditions all phases are affected accordingly. Thus, availability of nutrients to plants is also affected. Therefore this study is designed to evaluate the response of spring and autumn sunflower to chemical and bio-fertilizers for yield and quality traits during spring and autumn season 2014.

Materials and Methods

A field trail was conducted at the University of Agriculture Faisalabad, (semi-arid area, 73^{0}E longitude, 31^{0}N latitude and at the altitude of 184.4 m above sea level) Pakistan. A randomized complete block design with factorial arrangements having three replications was used. A net plot size 4.5 m × 6 m was kept. Hysun-33 variety of sunflower was sown as test crop. Crop was sown in spring (March 2014) and autumn season (August 2014) to compare seasonal difference. The soil sampling was done before sowing according to Chapman and Pratt (1978). Soil analysis showed that the soil was sandy loam having pH, 7.7 during both the seasons; organic matter, 0.80% and 0.83%; phosphorus, 7.9 and 8.4 ppm; potassium, 140 and 142 ppm, determined in soil of spring and autumn planted crop respectively before sowing.

Seed bed was prepared by cultivating the soil 2-3 times with tractor mounted cultivator to a depth of 10-12 cm, each followed by planking (planking is done to crush the hard clods to smoothen the soil surface and to compact the soil lightly). Crop was sown with the help of hand drill by maintaining 75 cm row to row and 22.5 cm plant to plant distance with seed rate of 6 kg ha^{-1} in both seasons. Irrigation was applied at critical growth stages of crop; first irrigation was applied twenty days after germination in spring crop while fifteen days after germination in autumn sown crop, then next at twenty days interval in spring and fifteen days interval in autumn crop, then next was applied at the time of flowering, then at grain formation and next at milking stage in both crops by keeping in mind the rainfall and temperature during the experiment. Weather data (temperature, relative humidity and rainfall) during both seasons was obtained (Figure 1).

The experiment (as given in Table 1) was comprised of 2 kinds of treatments.

Table 1: *Treatments.*

Plant Growth Promoting Rhizobacteria (PGPR)	
P_0	No PGPR
P_1	PGPR-1
P_2	PGPR-2
P_3	PGPR-1 + PGPR-2
Chemical fertilizers	
F_0	No chemical Fertilizers
F_1	Half dose of recommended chemical fertilizers (75-50-31 kg ha^{-1} NPK)
F_2	Full dose of recommended chemical fertilizers (150-100-62 NPK kg ha^{-1})

By the combination of PGPR and chemical fertilizers there were twelve plots in each replication and thirty six plots in total three replications. The PGPR used in

this study were pre-isolated and characterized, taken from soil microbiology and biochemistry laboratory, Institute of Soil and Environmental Sciences (ISES), University of Agriculture Faisalabad, Pakistan.

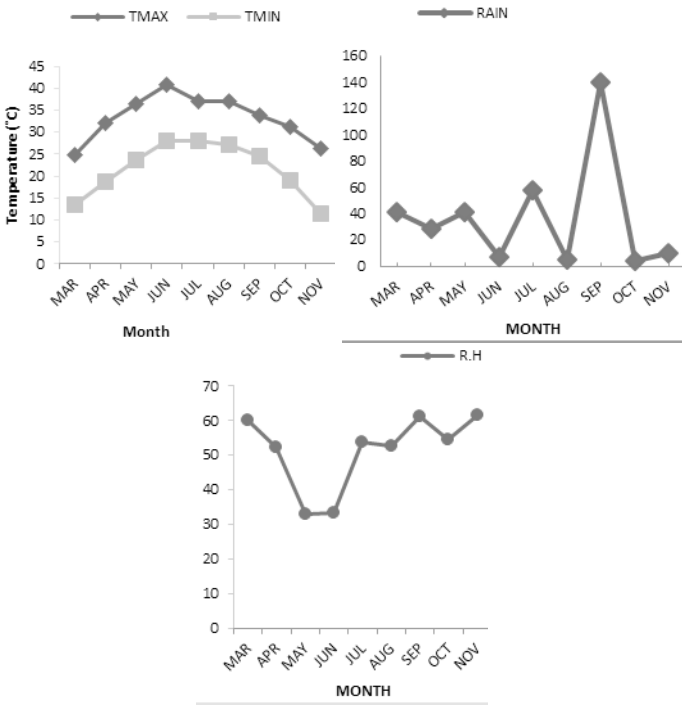

Figure 1: *Monthly maximum and minimum temperature, relative humidity and rainfall during both (spring and autumn) crop season (2014)*

Collection of samples

Soil samples were collected from rhizospheric soil of sunflower. Samples were collected in polyethylene bags and then placed in refrigerator before beginning of the process of isolation.

Isolation and screening of rhizobacteria

Bacteria were isolated from collected soil samples and these different types of isolated bacteria are termed as (PGPR-1) and (PGPR-2). These becteria were isolated from soil samples by dilution plate technique using glucose peptone agar media (GPAM) (Wollum II, 1982). Ten grams of each soil sample was put into100 mL conical flask having 95 mL sterilized water. The flask was shaken thoroughly to mix the contaminated soil with water. Further dilutions were made by taking 1 mL from 100 mL flask and transferred it to test tube that contained 9 mL of sterilized distilled water. Following the same procedure, five dilutions 10^{-1} to 10^{-5} was prepared. From each dilution 1 mL was transferred to petri plates having sterilized glucose peptone agar media (GPAM) to serve as a growth medium for bacteria. After solidification of the media these petri plates was inverted and incubated at 28±1°C for 72 hours. Proper optical density was maintained to obtain uniform population of bacteria [10^{8}-10^{9} colony forming units (cfu) mL^{-1}] in the broth at the time of inoculation in experiment.

Seed was inoculated with PGPR's before sowing. Diammonium phosphate (DAP) as a source of phosphorus, Urea as a source of nitrogen and Sulphate of potash (SOP) as a source of Potash was used in solid form. All P, K and half dose of N was applied at the time of sowing by incorporating in the soil during final seed bed preparation, while remaining N was applied in two equal splits as per plan.

Parameters such as 1000 achene weight, achene yield, biological yield, harvest index, oil content and protein content were recorded. Data were analyzed through analysis of variance technique and treatment means were further separated and compared using LSD test at 0.05 level of probability (Steel et al., 1997).

Results and Discussion

1000-achene weight has a key role in defining the yield potential of a seed crop, as it expresses the extent of seed development. Various PGPR inoculation and chemical fertilizer rates had significant effect on 1000 achene weight during both growing seasons. In both seasons the treatment F_2P_3 (full recommended dose of fertilizer and seed inoculation with PGPR-1 + PGPR-2) produced significantly the heaviest 1000-achene in weight (55.56 and 49.92 g) during spring and autumn respectively compared to all other treatments (Table 2a). Two season's data exhibited the same trend. However, in spring increased 1000 achenes weight was recorded as compared to autumn season. Present results were in line with findings of Agele, (2003) whose concluded that due to better environment driven resource capture and utilization during spring, greater hundred achene weight was recorded than in autumn. Mirshekari et al. (2012) concluded that the application of nitrogen and phosphorus fertilizers in combination with *Azospirillum* and *Azotobacter* resulted in maximum seed yield, 1000-seed weight as compared to control treatment.

Different PGPR inoculation and chemical fertilizer rates significantly affected the achene yield (Table 2b). Significantly, maximum achene yield (2960 kg ha^{-1}) was produced in plots of the treatment F_2P_3 where full dose of recommended fertilizer and seed inoculation

Table 2a: *Effect of chemical and bio-fertilizers on 1000-achene weight (g) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	30.42 ^{h}	40.20 ^{f}	46.16 ^{cd}	38.93 ^{C}	29.95 ^{g}	41.32 ^{d}	47.70 ^{b}	39.65 ^{C}
P_1	34.37 ^{g}	44.20 ^{e}	47.82 ^{bc}	42.13 ^{B}	35.85 ^{f}	43.62 ^{c}	47.85 ^{b}	42.44 ^{B}
P_2	34.45 ^{g}	44.44 ^{de}	48.43 ^{b}	42.44 ^{B}	35.76 ^{f}	44.66 ^{c}	48.14 ^{b}	42.85 ^{B}
P_3	35.69 ^{g}	45.70 ^{de}	55.56 ^{a}	45.65 ^{A}	38.19 ^{e}	48.00 ^{b}	49.92 ^{a}	45.37 ^{A}
Mean	33.73 ^{C}	43.63 ^{B}	49.49 ^{A}		34.93 ^{C}	44.40 ^{B}	48.40 ^{A}	

LSD for PGPR=1.0, for fertilizer=0.866, for interaction=1.733 LSD for PGPR=0.615, for fertilizer=0.533, for interaction=1.066
Any two mean not sharing a letter in common differ significantly at $p \leq 0.05$
P_0: *No PGPR;* **P_1:** *PGPR-1;* **P_2:** *PGPR-2;* **P_3:** *PGPR-1 + PGPR-2;* **F_0:** *No chemical fertilizers;* **F_1:** *half dose of recommended chemical fertilizers (75-50-31NPK kg ha^{-1});* **F_2:** *Full dose of recommended chemical fertilizers (150-100-62NPK kg ha^{-1}).*

Table 2b: *Effect of chemical and bio-fertilizers on achene yield (kg ha^{-1}) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	1024 ^{h}	2018 ^{e}	2489 ^{c}	1843 ^{C}	794 ^{h}	1623 ^{d}	2153 ^{c}	1523 ^{C}
P_1	1476 ^{g}	2225 ^{d}	2650 ^{b}	2117 ^{B}	1114 ^{g}	1875 ^{d}	2376 ^{b}	1788 ^{B}
P_2	1424 ^{g}	2256 ^{d}	2672 ^{b}	2117 ^{B}	1141 ^{g}	1865 ^{d}	2348 ^{b}	1785 ^{B}
P_3	1568 ^{f}	2531 ^{c}	2960 ^{a}	2353 ^{A}	1487 ^{f}	2162 ^{c}	2503 ^{a}	2050 ^{A}
Mean	1373 ^{C}	2257 ^{B}	2693 ^{A}		1134 ^{C}	1881 ^{B}	2345 ^{A}	

LSD for PGPR=39.49, for fertilizer=34.20, for interaction=68.41 LSD for PGPR=40.67, for fertilizer=35.22, for interaction=70.44
Any two mean not sharing a letter in common differ significantly at $p \leq 0.05$; For detail of treatments, see Table 2(a)

with PGPR-1 + PGPR-2 was applied. During autumn trend was same and significantly the highest achene yield found in F_2P_3 was (2503 kg ha^{1}). However, spring was recorded more productive by giving higher yield than autumn season. Lesser achene yield during the autumn season might be due to decrease in the duration of seed filling during which the assimilates partitioned to seed was decreased. Similarly less number of achenes per head with lower hundred achene weight during autumn as compared to higher values of these components during spring might have also reduced achene productivity in autumn season. In the same way, it was reported that favorable growing conditions during flowering and seed setting resulted in greater achene yield (Sumangala and Giriraj, 2003). Chandrasekar et al. (2005) reported that growth and yield of crop showed better results when applied in combination of chemical fertilizers and PGPR instead of applying any method alone.

The term biological yield refers to the total yield of plant material (above ground vegetative matter and achene yield). The outcome related to biological yield kg ha^{-1} was maximum in treatment F_2P_3 (full dose of recommended fertilizer and seed inoculation with PGPR-1 + PGPR-2) which produced significantly the highest biological yield (11664 and 11564 kg ha^{-1}) than other treatments while lowest biological yield (6898 and 6871 kg ha^{-1}) was produced by F_0P_0 treatment in spring and autumn seasons respectively (Table 3a). Similar trend was noted in two seasons, However, the treatments F_2P_2, F_2P_1 are statistically at par with F_2P_3 in case of maximum biological yield in autumn while seasonal effect on biological yield was also considerable with higher values in spring than in autumn season. The results were in conformity with (Smiderle, 2001) who evaluated the sunflower cultivation in varying seasons, and reported that spring sowing was more suitable than autumn sowing in accumulation of maximum biological yield potential. Ali et al. (2004) reported that inoculation in combination with P in chickpea increased biological yield.

The effect of the PGPR and chemical fertilizer were significant in case of harvest index (Table 3b). During both seasons significantly maximum harvest index (25.38 and 21.64%) was given by treatment F_2P_3 (full dose of recommended fertilizer and seed inoculation with PGPR-1 + PGPR-2), against the rest of inoculation source and fertilizers rates and statistically minimum harvest index was recorded (14.85 and11.56%) in treatment F_0P_0(no fertilizer and no

Table 3a: *Effect of chemical and bio-fertilizers on Biological yield (kg ha^{-1}) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	6898 ^{g}	9675 ^{d}	10528 ^{bc}	9034 ^{C}	6871 ^{g}	10119 ^{d}	11172^{b}	9387 ^{C}
P_1	8782 ^{e}	10157 ^{c}	10858 ^{b}	9932^{AB}	7860 ^{f}	10546 ^{c}	11481 ^{a}	9963 ^{B}
P_2	8249 ^{f}	10219 ^{c}	10789 ^{b}	9752 ^{B}	7914 ^{f}	10590 ^{c}	11485 ^{a}	9996 ^{B}
P_3	8464 ^{ef}	10357 ^{c}	11664 ^{a}	10162 ^{A}	9700 ^{e}	11108 ^{b}	11564 ^{a}	10791^{A}
Mean	8098 ^{C}	10102 ^{B}	10960 ^{A}		8086 ^{C}	10591 ^{B}	11426^{A}	

LSD for PGPR=231.6, for fertilizer=200.6, for interaction=401.2 LSD for PGPR=136.7, for fertilizer=118.4, for interaction=236.8
Any two mean not sharing a letter in common differ significantly at p ≤ 0.05; For detail of treatments, see Table 2(a)

Table 3b: *Effect of chemical and bio-fertilizers on Harvest index (%) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	14.85 ^{h}	20.86 ^{e}	23.64 ^{c}	19.78 ^{C}	11.56 ^{h}	16.04 ^{e}	19.28 ^{c}	15.62 ^{C}
P_1	16.83 ^{g}	21.91 ^{d}	24.41 ^{b}	21.05 ^{B}	14.17 ^{g}	17.78 ^{d}	20.44 ^{b}	17.55 ^{B}
P_2	17.29 ^{g}	22.08 ^{d}	24.77 ^{ab}	21.38 ^{B}	14.42 ^{g}	17.62 ^{d}	20.69 ^{b}	17.49 ^{B}
P_3	18.54 ^{f}	24.44 ^{b}	25.38 ^{a}	22.78 ^{A}	15.33 ^{f}	19.47 ^{c}	21.64 ^{a}	18.81 ^{A}
Mean	16.87 ^{C}	22.32 ^{B}	24.55 ^{A}		13.87 ^{C}	17.73 ^{B}	20.51 ^{A}	

LSD for PGPR=0.369, for fertilizer=0.320, for interaction=0.640 LSD for PGPR=0.403, for fertilizer=0.349, for interaction=0.699
Any two mean not sharing a letter in common differ significantly at p ≤ 0.05; For detail of treatments, see Table 2(a)

inoculation with PGPR) was applied. Similar trend was noted in two season's data. Season effect showed that greater harvest index was obtained in spring than in autumn. The results of harvest index are in conformity with those of (Baydar and Erbas, 2005) who conclude that seasons with high temperature and less rainfall humidity at pollination (autumn), affected pollination thus producing achenes of low weight, empty and sterile, causing less yield and ultimately less harvest index.

Achene oil content were also significantly affected by PGPR and chemical fertilizers in both growing seasons (Table 4a). The treatment F_0P_0 (no fertilizer and no inoculation with PGPR) produced significantly the highest achene oil content (42.06 and 40.41%) while lowest achene oil content (38.48 and 35.13%) were produced by treatment F_2P_0 (Full dose of recommended fertilizer without inoculation of PGPR) having a negative effect on oil content in spring and autumn respectively. However, treatments of fertilizers with different PGPR inoculation sources improved the achene oil content as compare to full dose of fertilizer alone, depicting a positive effect on oil content. This type of result might be due to the adverse effect of nitrogen on oil content, compensated by an increase in protein content (Munir et al., 2007). Bakht et al. (2010) reported that sunflower seed oil decreased with an increase in the rate of NP fertilizers. Seasonal effect of achene oil content showed higher oil content in spring than in autumn. Temperature is a main factor of environment that determines the rate of development as well as oil accumulation in sunflower and greater achene oil content were recorded from spring sunflower crop which matured at higher temperature (Qadir et al. 2006).

Significantly the highest achene protein content (19.91 and 21.48 %) were received from the plots treated with full dose of recommended fertilizer and seed inoculation with PGPR-1+PGPR-2, against rest of the treatments in both seasons of trialing (spring and autumn) (Table 4b). Our result are in accordance with those of (Kandil et al., 2008) who noted increased protein content in autumn sown sunflower crop as compared to spring crop, higher protein content during autumn might be due to less temperature prevailed during achene development in addition to other factors of environment. Tahir et al. (2009) concluded that *Rhizobium* inoculation with combination of NP in soybean increased oil and protein content.

It might be concluded from the present study that the sunflower crop which was planted in spring have

Table 4a: *Effect of chemical and bio-fertilizers on achene Oil content (%) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	42.06 ^{a}	39.23 ^{d}	38.48 ^{e}	39.92 ^{B}	40.41 ^{a}	38.49 ^{c}	35.13 ^{h}	38.01 ^{A}
P_1	39.30 ^{d}	41.30 ^{b}	40.32 ^{c}	40.31 ^{A}	40.03 ^{b}	37.09 ^{e}	35.47 ^{g}	37.53 ^{D}
P_2	39.31 ^{d}	41.60 ^{ab}	40.18 ^{c}	40.36 ^{A}	39.82 ^{b}	37.14 ^{e}	35.88 ^{f}	37.61^{C}
P_3	40.05 ^{c}	40.09 ^{c}	39.17 ^{d}	39.77 ^{B}	39.87 ^{b}	38.19 ^{d}	36.11 ^{f}	38.05 ^{A}
Mean	40.18 ^{B}	40.56 ^{A}	39.54 ^{C}		40.03 ^{A}	37.73 ^{B}	35.64 ^{C}	

LSD for PGPR=0.288, for fertilizer=0.249, for interaction=0.499 LSD for PGPR=0.136, for fertilizer=0.117, for interaction=0.235
Any two mean not sharing a letter in common differ significantly at $p \leq 0.05$; For detail of treatments, see Table 2(a)

Table 4b: *Effect of chemical and bio-fertilizers on achene Protein content (%) of spring and autumn sunflower*

	Spring				Autumn			
Treatment	F_0	F_1	F_2	Mean	F_0	F_1	F_2	Mean
P_0	14.06 ^{g}	16.93 ^{d}	18.33 ^{c}	16.44 ^{C}	14.30 ^{h}	16.62 ^{g}	19.52 ^{d}	16.82 ^{D}
P_1	14.82 ^{f}	17.00 ^{d}	19.36 ^{b}	17.06 ^{B}	16.21 ^{g}	18.11 ^{f}	20.09 ^{c}	18.14 ^{C}
P_2	14.83 ^{f}	17.13 ^{d}	19.56 ^{ab}	17.17 ^{B}	16.28 ^{g}	18.74 ^{e}	20.84 ^{b}	18.62 ^{B}
P_3	16.44 ^{e}	18.14 ^{c}	19.91 ^{a}	18.16 ^{A}	16.43 ^{g}	19.46 ^{d}	21.48 ^{a}	19.12 ^{A}
Mean	15.03 ^{C}	17.30 ^{B}	19.29 ^{A}		15.81 ^{C}	18.23 ^{B}	20.48 ^{A}	

LSD for PGPR=0.239, for fertilizer=0.207, for interaction=0.415 LSD for PGPR=0.238, for fertilizer=0.206, for interaction=0.413
Any two mean not sharing a letter in common differ significantly at $p \leq 0.05$; For detail of treatments, see Table 2(a)

It might be concluded from the present study that the sunflower crop which was planted in spring have the ability to fully utilize the existing environmental conditions and express the higher yield and quality attributes than the autumn crop. PGPR-1+ PGPR-2 in combination with full dose of recommended chemical fertilizers improved plant yield and quality attributes. However, it was also noted that, PGPR-1+ PGPR-2 in combination with half dose of recommended chemical fertilizers gave the yield as with full recommended dose of chemical fertilizers alone. Therefore, PGPR enhanced the yield by reducing the use and harmful effects of chemical fertilizers on soil health and environment, leading towards the sustainable agriculture.

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Authors Contribution

MT conceived the idea, did overall management of the article and supervised the trials.MS conducted field trials, data collection, data entry in SPSS and analysis, wrote the research paper.

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