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Growth enrichment of *Carthamus tinctorius* (L) and reduction in dosage of chemical fertilizers with application of plant growth promoting rhizobacteria

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Abstract

Experiencing the adverse effects of synthetic chemical fertilizers in agriculture production the concept of biofertilizers has gained interest currently. The aim of the current study was to evaluate the integrated role of plant growth promoting rhizobacteria (PGPR) and chemical fertilizers on growth and yield attributes of safflower during growing season of 2009-10 and 2010-11. The chemical fertilizers were applied at full (Urea 60 Kg ha^{-1} and DAP @ 30 Kg ha^{-1}), half (Urea 30 Kg ha^{-1} and DAP 15 Kg ha^{-1}) and quarter doses (Urea 15 Kg ha^{-1} and DAP 7.5 Kg ha^{-1}). The PGPR (*Azospirillum brasilense* and *Azotobacter vinelandii*) were applied as seed inoculation @10^6cells/ml. Maximum plant height, number of branches/plant, leaf chlorophyll and leaf area was recorded in *Azospirillum* and *Azotobacter* with full dose of chemical fertilizers and with full dose of chemical fertilizers treatments alone while maximum number of capitulum/plant, capitulum weight and seed yield was shown by *Azospirillum brasilense* supplemented with half dose of chemical fertilizers. Maximum achene oil contents were recorded in *Azospirillum* treatment supplemented with half dose of chemical fertilizers and *Azotobacter*+full dose of chemical fertilizers. The PGPR improved plant growth and yield by reducing the use of chemical fertilizer (50 %), hence leading towards sustainable agriculture production.

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Introduction

Soil ecology has been subverts due to incessant use of chemical fertilizers which causes many problems such as soil pollution, soil toxicity, mortification of soil fertility status, contamination of ground water, disturbance of environment which leads to environments toxicity and consequently injurious effects on human health (Joshi *et al.,* 2006).

The integration of bio-fertilizers and chemical is one of the important ways for the sustainable agriculture production (Ali *et al.,* 2008). The irregular and excessive use of chemical fertilizers, inapt methods of energy production and consumption cost, all these factors have destructive effect on the biological cycles which directly affect the sustainable farming stability systems. In view of the current problem the application of biofertilizers is encouraged in order to tackle these problems and to establish a pollution free, environmental friendly and sustainable farming ecosystem (Galavi *et al.,* 2011).

The biofertilizers can be used in combination with mineral fertilizers to reduce the use of chemical fertilizers and increase the soil health by maintaining the nutrient status of the soil. The plant growth promoting rhizobacteria (PGPR) are the groups of bacteria which colonize the plants roots and augment plant growth and yield (Mirshekari *et al.,* 2012).

Plant Growth Promoting Rhizobacteria improves plant growth and yield by several mechanisms such as organic acid, phytohormone and siderophore and biologically active compounds production as well as phosphate solublization, nutrients uptake and antibiotic production that stifle the effect of deleterious bacteria. Plant growth promoting rhizobacteria by blending with mineral fertilizers improve plant growth by direct or indirect mechanisms. Previous study showed that mineral fertilizers and PGPR significantly improved the plant height, leaf area and stem diameter (Onasanya *et al.,* 2009). Application of combination of PGPR and chemical fertilizer improved the grain yield, yield components and dry matter production of barley (Hassanzadeh *et al.,* 2006). Yasari and Patwardhan (2007) reported that application of *Azospirillum* and *Azotobacter* augmented canola yield, number of branches, pod per plant, and weight of 1000 grain.

Carthamus tinctorius L. is the world's oldest crop originated in the Middle East and is an important oil seed crop adapted to grow in drought and salinity stress conditions. Its oil is used as edible oil for cooking, salad oil and margarine. Principally it is cultivated for the oil used both for food and industrial implications. The aim of the current research was to improve the growth, yield and oil contents of safflower by the application of PGPR either alone or in combination with mineral fertilizers.

Materials and method

Field experiment

A field experiment was conducted during 2009-2010 and 2010-2011 at the field of Department of Plant Sciences, Quaid-i-Azam University. A split plot design with three replications was used having a net plot size of 3m x 3m. Soil samples were taken from a 0-12 cm depth prior to sowing and were air dried, sieved and analyzed for various physiochemical properties. The analysis of the soil sample revealed a pH of 7.4, EC 1.5 dS m^{-1}, cation exchange capacity (CEC) 6.5 cmol (+) kg^{-1}, organic matter 2.48% and phosphorus 2.48 %.

Certified seeds of safflower cv. Saif-32 and cv.Thori obtained from National Agricultural Research Centre (NARC) were surface sterilized with 10% chlorox for three min and successively washed 3x with sterile water.

Seed inoculation

The broth culture of *Azospirillum brasilense* and *Azotobacter vinelandii* Khsr1 were grown at 28°C in Luria-Bertani (LB) medium as a rich bacterial growth medium. The PGPR were applied as seed inoculation @10^6cells/ml and the number of bacterial cells/seed were measured as 5x10^5.

Application of chemical fertilizers

Chemical fertilizers were applied into three doses i.e. full dose of Urea and Diammonium phosphate (Urea

60 Kg ha^{-1} and DAP @ 30 Kg ha^{-1}), half (Urea 30 Kg ha^{-1} and DAP 15 Kg ha^{-1}) and quarter doses (Urea 15 Kg ha^{-1} and DAP 7.5 Kg ha^{-1}). The entire amount of DAP was applied at the time of sowing while Urea was applied at three different stages at an interval of 40d, however, first dose was applied at the time of sowing as recommended. The recommended irrigations were applied.

Following treatments were made.

Treatments	Abbreviation
Control (Without inoculation and without chemical fertilizers)	C
Chemical fertilizers full dose (Urea 60 Kg ha^{-1} and DAP 30 Kg ha^{-1})	CFF
Chemical fertilizers half dose (Urea 30 Kg ha^{-1} and DAP 15 Kg ha^{-1})	CFH
Chemical fertilizers quarter dose (Urea 15 Kg ha^{-1} and DAP 7.5 Kg ha^{-1})	CFQ
Seed soaking with *Azospirillum brasilense* prior to sowing	SP
A. brasilense+full dose of chemical fertilizers	SPF
A. brasilense+half dose of chemical fertilizers	SPH
A. brasilense+quarter dose of chemical fertilizers	SPQ
Seed soaking with *Azotobacter vinelandii* prior to sowing	BT
A. vinelandii+full dose of chemical fertilizers	BTF
A. vinelandii+half dose of chemical fertilizers	BTH
A. vinelandii+quarter dose of chemical fertilizers	BTQ

The chlorophyll and carotenoids estimation of leaves were made following the method of Arnon (1949). Leaf area was measured according to Ahmed and Morsy (1999). Growth and yield parameters i.e., plant height at maturity, number of branches/plant, number of capitulum/plant, number of seed/capitulum, capitulum weight, 1000-grain weight and seed yield were recorded. Seed oil content was estimated by NMR test according to Robertson and Morrison (1979).

Statistical analysis

Data was analyzed by Statistix software version 8.1 using factorial design of Analysis of Variance (ANOVA) and mean values were compared by least significant difference (LSD) at $p < 0.05$.

Results

All the treatments exhibited significant effect on leaf chlorophyll contents; the average data of treatments showed that maximum increase (56%) was found in *Azotobacter* in the presence of full dose of chemical fertilizers (BTF) treatment followed by (50%) *Azospirillum* in combination with full dose of chemical fertilizers (SPF) (Table 1). In second year *Azotobacter* in the presence of half dose of chemical fertilizers (BTH) had pronounced effect and showed maximum increase of 53% as compared to that of control. The effect of *Azospirillum* supplemented with quarter dose of chemical fertilizers (SPQ) was at par with full dose of chemical fertilizers alone in increasing leaf chlorophyll contents.

Data regarding carotene during 2009-10 showed that all treatments increased leaf carotenoid contents in both the varieties. Maximum increase (29%) was recorded in *Azotobacter* in the presence of full dose of chemical fertilizers (BTF) in both varieties (Table 2). In the second year BTH showed highest (37%) carotenoids contents followed by SPH and BTQ respectively.

Data regarding leaf area showed the same pattern of increase in both years (Table 3). In 2009-10, the maximum increase (56%) was found in CFF followed by SPF. During second year (2010-11) CFF showed maximum significant increase (65%) as compared to the control.

Application of PGPRs and different levels of chemical fertilizers brought about significant increases in plant height in both the varieties (Table 4). The increase due to different treatments ranges 5-14%. Average data for 2009-10 indicated that maximum (14%) plant height was recorded in BTF treatments followed by SPF and CFF (12%) as compared to untreated

control. In 2010-11, all the treatments the same pattern was repeated in plant height.

During, 2009-10, maximum increase (48%) in number of branches per plant was recorded in SPF and it statistically similar with BTQ (Table 5). In year 2010-2011, all the treatments significantly increased the number of branches/plant as compared to control; maximum increase (68%) was recorded in CFF followed by SPF.

Table 1. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Leaf Chlorophyll (mg/g) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	0.07 j	0.07 j	0.07 f	0.05 i	0.08 h	0.06 f
CFF	0.14 bcd	0.14 bcd	0.14 ab	0.10 cdef	0.10 bcde	0.10 cd
CFH	0.13 bcdef	0.13 bcdef	0.13 bc	0.10 defg	0.11 abcd	0.11 bc
CFQ	0.10 ghi	0.12 cdefgh	0.11 d	0.08 fgh	0.09 efgh	0.09 e
SP	0.11 fghi	0.12 defgh	0.11 d	0.08 gh	0.10 bcde	0.09 de
SPF	0.14 bcde	0.14 abc	0.14 ab	0.09 efgh	0.13 a	0.11 bc
SPH	0.11 fghi	0.13 bcdef	0.12 cd	0.10 cdef	0.12 ab	0.11 abc
SPQ	0.13 bcdef	0.11 efghi	0.12 bcd	0.10 defg	0.10 cdef	0.10 cde
BT	0.10 hi	0.09 ij	0.09 e	0.10 defg	0.13 a	0.11 bc
BTF	0.15 ab	0.17 a	0.16 a	0.10 efgh	0.12 abc	0.11 bc
BTH	0.13 bcdef	0.12 defgh	0.12 bcd	0.13 a	0.13 a	0.13 a
BTQ	0.12 bcdefgh	0.13 bcdefg	0.12 bcd	0.12 abc	0.12 abc	0.12 ab
LSD value	0.0251 (Interaction TxV), 0.0178 (treatment Means)			0.0178 (Interaction TxV), 0.0140 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05
C: Control, CFF: Chemical fertilizers full dose, CFH: Chemical fertilizers half dose, CFQ: Chemical fertilizers quarter dose, SP: *A. brasilense*, SPF: *A. brasilense*+full dose of chemical fertilizers, SPH: *A. brasilense*+half dose of chemical fertilizers, SPQ: *A. brasilense*+quarter dose of chemical fertilizers, BT: *A. vinelandii*, BTF: *A. vinelandii*+full dose of chemical fertilizers, BTH *A. vinelandii*+half dose of chemical fertilizers, BTQ: *A. vinelandii*+quarter dose of chemical fertilizers.
LSD: Least Significant Difference
Alpha value 0.05

Table 2. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Leaf Carotenoids (ug/ml) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	4.72 i	5.86 efgh	5.29 f	2.46 k	4.80 efgh	3.63 d
CFF	5.20 efghijk	6.83 bcde	6.01 cde	8.33 efgh	21.00 a	14.66 a
CFH	3.96 jk	5.35 ghijk	4.65 fg	7.00 ghi	12.66 bc	9.83 cd
CFQ	5.30 ghi	6.58 bcdef	5.94 de	3.51 j	4.42 ghi	3.96 d
SP	5.99 defgh	5.87 efgh	5.93 de	3.60 j	5.76 abc	4.68 c
SPF	6.90 abc	6.10 cdefg	6.50 cd	3.84 ij	5.67 cd	4.76 c
SPH	5.71 fgh	7.10 ab	6.40 cd	4.29 ghij	6.48 ab	5.38 ab
SPQ	6.86 bcd	5.95 efgh	6.40 cd	4.06 hij	5.36 cdef	4.71 c
BT	5.75 fgh	5.12 hi	5.44 ef	4.02 hij	5.70 bcd	4.86 bc
BTF	7.76 a	7.25 ab	7.50 a	3.64 ij	6.10 abc	4.87 bc
BTH	6.45 bcdef	6.57 bcdef	6.51 cd	4.94 defg	6.54 a	5.74 a
BTQ	6.88 bc	6.50 bcdef	6.69 bc	4.66 fgh	6.12 abc	5.39 ab
LSD value	0.87 (Interaction TxV), 0.62 (treatment Means)			0.78 (interaction TxV), 0.5558 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05
Detail of treatment is given in Table 1
LSD: Least Significant Difference
Alpha value 0.05

Table 3. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Leaf Area (cm^2) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009-2010			2010-2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	6.67 n	8.72 lm	7.70 j	10.69 k	13.62 jk	12.15 h
CFF	16.32 b	19.03 a	17.67 a	32.98 bc	37.70 a	35.34 a
CFH	11.00 ijk	16.05 bc	13.53 de	25.14 fg	32.96 bc	29.05 cd
CFQ	11.27 ij	13.37 efg	12.32 g	21.80 h	21.69 h	21.74 f
SP	10.88 jk	14.29 de	12.58 fg	22.18 gh	22.96 fgh	22.57 f
SPF	12.91 fg	20.02 a	16.47 b	31.79 bc	32.04 bc	31.92 b
SPH	12.17 ghi	16.21 bc	14.19 cd	25.61 ef	27.98 de	26.80 e
SPQ	9.88 kl	12.76 fgh	11.32 h	22.11 h	21.95 h	22.03 f
BT	7.98 m	11.06 ijk	9.52 i	15.79 ij	18.37 i	17.08 g
BTF	11.55 hij	15.06 cd	13.31 ef	25.72 ef	30.10 cd	27.91 de
BTH	13.87 def	15.63 bc	14.75 c	26.46 e	33.63 b	30.05 bc
BTQ	9.99 k	16.24 bc	13.12 fg	26.55 e	37.48 a	32.02 b
LSD value	1.2204 (interaction TxV), 0.8629 (treatment Means)			2.9584 (interaction TxV), 2.0919 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at $P<0.05$

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 4. Effect of plant growth promoting rhizobacteria and chemical fertilizers on plant height (cm) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	112.61 g	125.31 ef	118.96 f	104.00 l	103.67 l	103.83 h
CFF	135.47 bc	137.16 ab	136.31 ab	139.00 de	144.33 cd	141.67 cd
CFH	123.61 ef	124.46 ef	124.04 e	136.00 efg	141.00 de	138.50 def
CFQ	121.07 f	137.16 ab	129.12 d	124.33 ijk	122.33 jk	123.33 g
SP	128.69 de	134.62 bc	131.66 cd	121.00 jk	130.00 ghi	125.50 g
SPF	138.85 ab	139.70 ab	139.28 a	154.67 a	144.33 cd	149.50 a
SPH	135.47 bc	134.62 bc	135.04 bc	132.33 fgh	135.67 efg	134.00 f
SPQ	127.00 de	131.23 cd	129.12 d	127.33 hij	141.33 de	134.33 ef
BT	121.07 f	134.62 bc	127.85 de	119.33 k	125.33 ijk	122.33 g
BTF	138.01 ab	141.39 a	139.70 a	144.67 cd	148.00 bc	146.33 ab
BTH	135.47 bc	131.23 cd	133.35 bc	137.33 ef	151.67 ab	144.50 bc
BTQ	127.00 de	128.69 de	127.85 de	138.33 def	139.00 de	138.67 de
LSD value	5.4244 (Interaction TxV), 3.8356 (treatment Means)			6.5095 (Interaction TxV), 4.6029 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at $P<0.05$

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 5. Effect of plant growth promoting rhizobacteria and chemical fertilizers on number of branches/plant of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	4.33 ijk	3.75 k	4.04 g	4.66 i	4.66 i	4.66 f
CFF	5.20 efghijk	6.83 bcde	6.01 cde	8.33 efgh	21.00 a	14.66 a
CFH	3.96 jk	5.35 fghijk	4.65 fg	7.00 ghi	12.66 bc	9.83 cd
CFQ	4.08 ijk	6.10 defgh	5.09 efg	7.00 ghi	7.66 fgh	7.33 e
SP	5.27 efghijk	6.70 bcde	5.98 cde	6.00 hi	8.00 efgh	7.00 e
SPF	5.50 efghij	10.25 a	7.87 a	11.66 bcd	14.0 b	12.83 ab
SPH	5.55 efghij	7.60 bcd	6.57 bcd	8.33 efgh	8.33 efgh	8.33 de
SPQ	4.60 hijk	5.76 efghi	5.18 efg	6.00 hi	10.0 cdef	8.00 de
BT	4.50 hijk	4.78 ghijk	4.64 fg	6.00 hi	8.00 efgh	7.00 e
BTF	5.61 efghij	8.13 bc	6.87 abc	10.66 cde	9.33 defg	10.00 cd
BTH	4.96 fghijk	6.35 defg	5.65 def	7.00 ghi	12.00 bcd	9.50 cd
BTQ	8.38 b	6.53 cdef	7.45 ab	8.00 efgh	14.00 b	11.00 bc
LSD value	1.7003 (Interaction TxV), 1.2023 (treatment Means)			2.889 (Interaction TxV), 2.0430 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at $P<0.05$

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

In terms of capitulum/plant, there were different responses to inoculated PGPR and chemical fertilizers application in the two years (Table 6). In 2009-10, the effect of treatment BTQ, BTH and SP was greater than that of CFF alone. Maximum increase (56%) was shown by BTQ treatment. Treatments SPH and BTF also showed 50% increase over control. In second year maximum increase 67% was recorded in CFF. Treatment SPF and CFH showed 61% and 53% increase as compared to the control.

The data during 2009-10 indicated that all the treatments significantly increased the number of seed capitulum^{-1} (Table 7). Maximum increase (46%) was recorded in CFF followed by SPQ (42%). In year 2010-11, BTQ showed maximum (40%) increase in number of seed capitulum^{-1} as compared with untreated control. Treatment SPH was at par with BTQ. Single inoculation of SP showed 36% increase as compared to control. Results (2009-10) showed that SPH exhibited maximum increase (53%) in capitulum weight followed by BTH and BTQ treatments over the control (Table 8). In 2010-11 treatments BTH, BTQ showed maximum (44%) increase followed by CFH at $P<0.05$.

Results for 1000 seed weight indicated that during both years all treatments significantly increased 1000 seed weight and maximum increase (13%) was recorded in BT and BTQ treatment as compared to that of the control (Table 9).

Data for seed yield indicated that in 2009-2010 all the treatments significantly increased the seed yield as compared to control; maximum significant increase (61%) was found in SPH treatment as compared to the control (Table 10). Similarly, the data for second year showed that maximum increase in seed yield was recorded in SPF and SPH (56% and 55%) as compared to the control.

Results showed that seed oil contents were significantly enhanced by all the treatments by both the varieties cvv. Thori and Saif-32 during 2009-2010 (Table 11). Maximum increase (18%) was recorded in

SPF and BTH treatments in variety Saif-32. In cv. Thori maximum increase 17% was recorded in SPF as compared to untreated control. In year 2010-2011, maximum increase 20% was recorded in BTH treatment in cv. Saif-32. In cv. Thori SPF and BTF showed maximum increase of 20% over control while SPH showed 19% increase.

Table 6. Effect of plant growth promoting rhizobacteria and chemical fertilizers on number of capitulum/plant of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	4.66 hi	4.33 hi	4.50 f	5.00 j	5.33 ij	5.16 h
CFF	5.66 ghi	11.00 bcd	8.33 cd	8.33 efghi	23.33 a	15.83 a
CFH	5.33 ghi	5.66 ghi	5.50 ef	7.00 fghij	15.00 b	11.00 c
CFQ	4.00 i	5.66 ghi	4.83 f	6.33 fghij	8.66 defgh	7.50 efg
SP	9.33 de	10.00 cde	9.66 ab	5.66 hij	6.00 ghij	5.83 gh
SPF	6.00 gh	10.00 cde	8.00 d	11.66 cd	15.00 b	13.33 b
SPH	6.00 gh	12.00 ab	9.00 bcd	7.66 fghij	9.33 def	8.50 def
SPQ	5.66 ghi	7.00 fg	6.33 e	6.00 ghij	11.33 cde	8.66 de
BT	6.00 gh	5.33 ghi	5.66 ef	6.00 ghij	6.66 fghij	6.33 fgh
BTF	6.66 fg	11.33 bc	9.00 bcd	7.33 fghij	9.00 defg	8.16 ef
BTH	8.33 ef	10.66 bcd	9.50 abc	6.33 fghij	11.33 cde	8.83 cde
BTQ	13.66 a	7.00 fg	10.33 a	7.33 fghij	14.00 bc	10.66 cd
LSD value	1.724 (Interaction TxV), 1.2193 (treatment Means)			3.266 (Interaction TxV), 2.309 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 7. Effect of plant growth promoting rhizobacteria and chemical fertilizers on number of seed/capitulum of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatment	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	20.00 no	21.00 mno	20.50 g	28.00 i	20.00 k	24.00 g
CFF	36.00 bc	40.333 a	38.16 a	20.00 k	39.66 b	29.83 f
CFH	25.00 kl	30.33 efg	27.66 e	36.66 cd	32.00 fgh	34.33 c
CFQ	22.00 mn	29.66 fg	25.83 ef	25.00 j	34.00 def	29.50 f
SP	25.66 ijkl	25.33 jkl	25.50 f	25.00 j	50.00 a	37.50 b
SPF	29.00 gh	34.33 cd	31.66 d	29.33 hi	37.66 bc	33.50 cd
SPH	33.33 cd	32.33 def	32.83 cd	47.66 a	30.33 hi	39.00 ab
SPQ	34.66 cd	38.00 ab	36.33 ab	30.66 ghi	33.33 efg	32.00 de
BT	23.66 klm	19.00 o	21.33 g	21.00 k	40.33 b	30.66 ef
BTF	36.00 bc	33.00 de	34.50 bc	24.66 j	35.00 cde	29.83 f
BTH	26.33 hijk	28.00 ghij	27.16 ef	33.66 ef	36.00 cde	34.83 c
BTQ	23.00 lm	28.33 ghi	25.66 ef	40.01 b	40.00 b	40.00 a
LSD value	2.8635 (Interaction TxV), 2.0248 (treatment Means)			2.876 (Interaction TxV), 2.0338 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 8. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Capitulum Weight (g) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	1.53 hij	1.43 ij	1.48 h	1.56 hijk	1.20 k	1.38 g
CFQ	1.69 ghij	1.80 fghi	1.74 fgh	1.69 ghij	2.13 def	1.91 de
CFF	1.59 hij	1.61 hij	1.60 gh	1.54 ijk	1.62 ghij	1.58 fg
CFH	2.70 bc	1.52 hij	2.11 de	2.97 a	1.82 fghi	2.39 ab
SP	1.38 j	2.12 def	1.75 fgh	1.38 jk	1.38 jk	1.38 g
SPF	2.11 def	1.89 efgh	2.00 def	2.15 def	2.41 cd	2.28 bc
SPH	2.99 ab	3.39 a	3.19 a	2.70 abc	1.33 jk	2.01 d
SPQ	2.04 defg	2.41 cd	2.22 cd	1.91 efgh	2.37 cd	2.14 bcd
BT	1.64 ghij	2.22 de	1.93 ef	1.52 ijk	1.97 efg	1.74 ef
BTF	1.67 ghij	1.89 efgh	1.78 fg	1.64 ghij	2.60 bc	2.12 cd
BTH	2.87 b	2.67 bc	2.77 b	2.19 de	2.78 ab	2.48 a
BTQ	1.92 efgh	2.96 b	2.44 c	1.92 efg	2.96 ab	2.44 a
LSD value	0.4091 (Interaction TxV), 0.2893 (treatment Means)			0.361 (Interaction TxV), 0.2553 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 9. Effect of plant growth promoting rhizobacteria and chemical fertilizers on 1000 Seed Weight (g) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatments	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	37.80 mno	38.00 lmn	37.90 f	39.70 h	38.80 i	39.25 de
CFF	40.46 i	38.52 klm	39.49 e	40.60 f	34.20 m	37.40 g
CFH	42.03 fg	37.10 o	39.56 e	42.05 e	37.00 l	39.52 de
CFQ	37.48 no	38.70 kl	38.09 f	37.55 kl	38.70 ij	38.12 f
SP	39.70 ij	42.48 f	41.09 d	38.10 jk	42.95 d	40.52 c
SPF	45.41 b	39.72 ij	42.57 b	45.10 b	39.75 gh	42.42 b
SPH	43.65 de	40.58 hi	42.11 bc	39.00 i	40.35 fgh	39.67 d
SPQ	39.41 jk	43.91 d	41.66 cd	43.75 c	43.95 c	43.85 a
BT	42.76 ef	44.45 cd	43.60 a	43.00 d	44.65 b	43.82 a
BTF	32.70 p	45.20 bc	38.95 e	33.00 n	45.10 b	39.05 e
BTH	41.36 gh	44.00 d	42.68 b	41.50 e	40.40 fg	40.95 c
BTQ	40.47 i	46.69 a	43.58 a	41.50 e	46.35 a	43.92 a
LSD value	0.886 (interaction TxV), 0.6265 (treatment Means)			0.6819 (interaction TxV), 0.4822 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 10. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Seed Yield (Kg/ha) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatment	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	641.1 o	1057.7 i	849.4 i	474.3 x	1169.9 m	822.1 l
CFF	740.8 mn	2432.3 b	1044.7 h	688.0 v	1774.0 g	1682.6 b
CFH	959.5 j	1129.9 h	1586.6 d	916.1 q	2449.0 b	1231.0 i
CFQ	682.0 no	1877.3 e	1279.7 e	639.1 w	1612.5 i	1125.8 j
SP	851.6 l	1571.2 f	1211.4 f	848.3 r	2069.4 f	1458.8 e
SPF	1147.5 h	2173.1 d	1660.3 c	1044.2 o	3272.0 a	2158.1 a
SPH	1183.4 gh	3201.0 a	2192.2 a	925.2 p	2149.9 e	1537.5 c
SPQ	958.5 j	2207.6 d	1583.1 d	1150.1 n	1645.0 h	1397.5 h
BT	763.3 m	1529.7 f	1146.5 g	750.0 u	2161.6 d	1455.8 f
BTF	923.3 jk	2223.3 cd	1573.3 d	820.0 t	2237.1 c	1528.6 d
BTH	1220.1 g	2277.0 c	1402.9 b	829.0 s	1285.6 k	1057.3 k
BTQ	882.3 kl	1517.5 f	1199.9 f	1220.1 l	1586.4 j	1403.2 g
LSD value	64.893 (interaction TxV), 45.886 (treatment Means)			1.415 (interaction TxV), 1.0008 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Table 11. Effect of plant growth promoting rhizobacteria and chemical fertilizers on Oil content (%) of safflower. PGPR were applied at @ 10^6 cells/ml as seed soaking prior to sowing. The data represent mean of three replicates.

Treatment	2009–2010			2010–2011		
	Thori	Saif-32	Treatment Means	Thori	Saif-32	Treatment Means
C	24.88 h	25.63 gh	25.50 g	25.36 n	25.63 n	25.50 g
CFF	27.53 f	29.10 de	28.31 d	28.63 ghi	30.36 de	29.50 c
CFH	25.69 gh	29.19 cd	27.44 e	27.60 ijkl	29.53 efg	28.56 d
CFQ	25.37 gh	26.11 g	25.74 g	25.56 n	26.00 mn	25.78 g
SP	27.53 f	26.137 g	26.58 f	27.96 ijk	27.10 kl	27.53 ef
SPF	29.40 bcd	29.38 bcd	29.39 bc	30.03 def	30.83 cd	30.43 b
SPH	30.14 bc	31.68 a	30.91a	30.90 bcd	31.90 ab	31.40 a
SPQ	25.89 gh	27.47 f	26.68 f	27.43 jkl	29.10 fgh	28.26 de
BT	28.17 ef	26.26 g	27.21 ef	28.00 ijk	26.90 lm	27.45 f
BTF	29.23 cd	30.37 b	29.80 b	30.90 bcd	31.80 abc	31.35 a
BTH	26.10 g	31.59 a	28.85 cd	28.20 hij	31.96 a	30.08 bc
BTQ	27.75 f	30.34 b	29.05 c	28.33 hij	30.46 de	29.40 c
LSD value	1.0198 (interaction TxV 0.7211 (treatment Means)			1.0662 (interaction TxV), 0.7539 (treatment Means)		

All such means which share a common English letter are similar; otherwise differ significantly at P<0.05

Detail of treatment is given in Table 1

LSD: Least Significant Difference

Alpha value 0.05

Discussion

The PGPR enhance productivity by increasing the local availability of nutrients, synthesizing phytohormones and facilitating the uptake of nutrients by the plants (Burd *et al.,* 2000). Similarly, Gholami *et al.* (2009) reported that inoculation with the bacterial strains significantly increased plant height, leaf area, number of seed per ear and 100 seed weight of maize. The content of photosynthetic pigments was higher following PGPR inoculation and supplemented with mineral fertilizers. These results are in agreement with previous findings of Yasari and Patwardhan (2007) who have reported that the chemical fertilizers were highly effective in increasing leaf chlorophyll content, number of branches per plant, number of siliqua per branch, number of seeds per siliqua, yield and yield attributes in canola. Generally, Plant Growth Promoting Rhizobacteria improve plant growth by improving the germination rate, root growth, leaf area, protein contents, chlorophyll contents, mineral uptake, increasing yield and yield attributes, enhancing tolerance against stress, increasing root and shoot weight as well as acting as biocontrol agent against pathogenic bacteria and delaying leaf senescence.

The application of PGPR in combination with full and half doses of chemical fertilizers gave the best results in terms of plant height, number of branches per plant, leaf area and yield attributes. Akhtar *et al.* (2009) reported that maximum increase in plant height, number of tillers and number of spikelets/ spike, grain, straw yield, grain yield and 1000 grain weight were recorded with the use of PGPR inoculated seeds in combination with compost and recommended dose of chemical fertilizers in wheat. Similarly, Adesemoye *et al.* (2009) reported that supplementing the recommended fertilizer rate (75%) with inoculants produced higher plant growth, yield, and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer dose without inoculants. The 1000 seed weight and seed yield were higher in treatments having inoculation with PGPR and supplemented with mineral fertilizer at full and half doses. This might be because that PGPR improved the dry matter accumulation resulting in heavier seeds. The higher yield of SPF over SP alone was because of larger leaf area, greater chlorophyll contents and number of branches plant^{-1}. Previous studies have shown that the application of nitrogen and phosphorus fertilizers in combination with *Azospirillum* and *Azotobacter* resulted in maximum grain yield, 1000 grain weight as compared to control treatment in barley (Mirshekari *et al.,* 2012).

The seed oil contents were significantly improved by PGPR in combination with full, half and quarter doses of chemical fertilizers, similar results were reported by Asghar *et al.* (2004) that inoculation with selected PGPR increased plant height, 1000-grain weight, grain yield, and oil content over a range of 7–57% above the uninoculated control.

Conclusion

The integration of bioinoculants with chemical fertilizers augmented plant growth and yield attributes. Agronomic traits were improved by combination of PGPR with full dose of chemical fertilizers while yield and yield components showed maximum increase in PGPR supplemented with quarter dose of chemical fertilizers. Application of PGPR reduces the extent of chemical fertilizers to obtain optimum growth and yield of safflower and reduce the deleterious effects of chemical fertilizers on soil health and ecosystem.

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