Research Article

Effect of Sulphur Oxidizing Bacterial Inoculants on Microbial Population and Enzymatic Activities in Rhizosphere of *Brassica napus* (Var. GSC-7)

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Abstract

A pot experiment was conducted to evaluate the effect of sulphur oxidizing bacteria (SOB) on microbial population and enzymatic activities in rhizospheric soil of *Brassica napus* var. GSC-7. The experiment was laid down with recommended doses of inorganic fertilizers (nitrogen, phosphorus and potassium) along with two different doses of sulphur (75% and 100%). Significantly higher population of bacteria, diazotrophs and PSB was recorded in treatment having inoculation of three SOB cultures along with consortium biofertilizer (nitrogen fixers, phosphorus solubilizers and PGPRs) and 100% sulphur dose. Fungal count was observed maximum in treatment inoculated with SOB Mix (SOB10, SOB8 and SOB5) and 100% Sulphur dose. Treatment having SOB Mix + consortium biofertilizer + 75% sulphur dose showed maximum population of sulphur oxidizing bacteria, whereas highest count of actinomycetes was observed in treatment having inorganic fertilizers only. Maximum soil alkaline phosphatase, dehydrogenase and urease activity was observed in treatment having SOB Mix + consortium biofertilizer + 100% Sulphur dose. The correlation analysis revealed significant positive interaction between various soil microbial communities (except actinomycetes and fungi) and enzymatic activities at different time intervals. It was concluded that integrated application of SOB as a bio-inoculant with consortium biofertilizer was markedly effective in improving the soil health by enhancing soil microbial flora and their enzymatic activities than sole application of inorganic fertilizers hence satisfying all the criteria of a potential biofertilizer.

Keywords: Biofertilizers, Enzymatic activities, Microbial population, Sulphur, Sulphur oxidizing bacteria

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Introduction

Soil is a natural and dynamic medium, inhabited by a vast array of microbes, which include bacteria, fungi, actinomycetes and algae [1]. These soil microbial communities and their associated enzymes catalyze several biogeochemical reactions such as decomposition of organic matter and nutrient cycling (carbon, nitrogen, phosphorus and sulphur) in ecosystem. Soil nutrients such as Nitrogen (N), Phosphorus (P), Potassium (K) and Sulphur (S) play an important role in soil fertility and crop production. Sulphur is the fourth major plant nutrient after N, P & K and is one of the sixteen essential nutrient elements required for growth and development of plants. It is a crucial constituent element of various seed proteins, amino acids, enzymes, glucosinolate and plant oils. Plants take majority of sulphur in the form of sulphate (SO_4^{-2}). In soil ecosystem, sulphur comprises of an interrelated set of reactions including oxidation-reduction of inorganic and organic sulphur compounds [2]. The transformations of inorganic sulphur compounds in soil is meditated by sulphur oxidizing bacteria (SOB) that are capable of oxidizing reduced inorganic sulphur compounds into sulphate.

A sustainable agricultural management required adequate S supply along with understanding and quantification of S transformations carried out by sulphur oxidizing bacteria [3]. Deficiency of sulphur in soils due to S free fertilizers, and loss of sulphur by various processes like leaching and erosion increases the importance of sulphur (S) and sulphur oxidizing bacteria in agriculture. Keeping these points in view, study was designed to evaluate the effect of sulphur oxidizing microbial inoculants on microbial population and their function in rhizospheric soil of *Brassica napus* (GSC-7).

Materials and Methods

Experimental Design and Treatments

A pot experiment was conducted in glass house of COBS&H, Department of Microbiology, Punjab Agricultural University (PAU), Ludhiana, India. The experiment was laid down in complete randomized design (in triplicate)

having twenty treatments (**Table 1**). Two levels of sulphur fertilizer 75% and 100% (80 kg/ha) supplemented with gypsum were used with different combinations of sulphur oxidizing bacteria and consortium biofertilizer (nitrogen fixers, phosphorus solubilizers and PGPRs). Three SOB cultures SOB10, SOB38, SOB5 were used as sole and in the form of mixture as SOB Mix. Inorganic fertilizers (N, P & K) were applied to all the treatments in recommended doses as mentioned in Package & Practices for *rabi* crop, given by PAU. Nitrogen was applied @ 40 kg/ha in the form of urea, Phosphorus @ 12 kg/ha in the form of DAP and potassium @ 6 kg/ha in the form of muriate of potash. Sulphur, phosphorus and potassium fertilization was done at the time of sowing whereas nitrogen applied in 3 splits, 1/3 applied at sowing whereas other $1/3^{rd}$ applied at a gap of one month from each other (i.e. at 30 and 60 DAS). Charcoal based sulphur oxidizing bacterial culture (10^8cells/ml) and consortium biofertilizer were applied to seeds before sowing.

Table 1 Different combinations of treatments designed for pot experiments

T1: Uninoculated + 75% Sulphur	T11: 75% Sulphur + Consortium
T2: Uninoculated + 100% Sulphur	T12: 100% Sulphur + Consortium
T3: 75% Sulphur + SOB10	T13: 75% Sulphur + Consortium + SOB10
T4: 100% Sulphur + SOB10	T14: 100% Sulphur + Consortium + SOB10
T5: 75% Sulphur + SOB38	T15: 75% Sulphur + Consortium + SOB38
T6: 100% Sulphur + SOB8	T16: 100% Sulphur + Consortium + SOB38
T7: 75% Sulphur + SOB5	T17: 75% Sulphur + Consortium + SOB5
T8: 100% Sulphur + SOB5	T18: 100% Sulphur + Consortium + SOB5
T9: 75% Sulphur + SOB Mix	T19: 75% Sulphur + Consortium + SOB Mix
T10: 100% Sulphur + SOB Mix	T20: 100% Sulphur + Consortium + SOB Mix

Soil Sampling and Analysis

Soil samples were collected from each replication of the treatments at 0, 80 and 160 DAS. Sampled soil was used for enumeration of bacteria, fungi, actinomycetes, diazotrophs, PSBs and SOB on Nutrient agar [4], Glucose yeast extract medium [5], Ken knight's medium, Jensen's medium [6], Pikovskaya's medium [7] and Thiosulphate agar medium [8] respectively, using standard serial dilution spread plate technique. The colonies were counted on each respective medium and recorded as CFU/g of soil. The soil samples were grounded and sieved to analyzed enzymatic activities as: alkaline phosphatase activity [9], dehydrogenase activity [10] and urease activity [11] in all treatments at different time intervals.

Statistical Analysis

Two-way analysis of variance (ANOVA) was performed to determine the effect of different combinations of treatments, different time interval and their interaction on various soil parameters. Pearson's correlation among different biological and biochemical properties of soil to observe the synergistic and/or antagonist effect of them to each other. For statistical analysis of data, OP-stat software was used [12]. The level of significance in the results was $p< 0.05$.

Results and Discussion

The initial microbial population of soil was determined at zero day of the experiment i.e. before sowing as: bacteria (20×10^6 CFU /g soil), fungi (04×10^2 CFU /g soil), actinomycetes (18×10^6 CFU /g soil), diazotroph (10×10^4 CFU /g soil), PSB (05×10^2 CFU /g soil) and SOB (09×10^4 CFU /g soil). Soil enzyme activities were recorded as alkaline phosphatase (0.33 µg pNP/hr/g soil), dehydrogenase (04.25 µg TPF/hr/g soil) and urease (205.16 µg /hr/g soil).

Effect of different treatments on microbial population of rhizospheric soil of Brassica napus (GSC-7)
Bacterial population

Bacterial population was observed higher in rhizospheric soil of inoculated treatments than uninoculated at each time interval. Maximum bacterial population (108×10^7 CFU /g soil) was observed in treatment having SOB Mix (SOB10, SOB38, SOB5) along with consortium biofertilizer (nitrogen fixers, phosphorus solubilizers and PGPRs) and 100% sulphur at 80 DAS. In case of sole application of cultures, inoculation of SOB10 culture with 100% sulphur showed higher bacterial population ($51 \text{ CFU} \times 10^7$ /g soil) than application of SOB38 and SOB5. Combination of these cultures as SOB Mix with 100 % sulphur dose showed significantly improved bacterial population as compared to

sole application of SOB cultures (**Table 2**). Treatment (T12) having consortium biofertilizer gives higher bacterial count than sole SOB and SOB Mix. However, application of SOB10 culture with consortium biofertilizer and 100% sulphur showed a significant increase in higher bacterial population (91 x 10^7 CFU /g soil) as compared to inoculation of SOB38 and SOB5 culture with consortium. Increased in number of microbial count in inoculated treatments was due to the establishment and secretion of polysaccharides by inoculated microbial species that enhanced the multiplication of indigenous bacterial population [13]. Statistically significant impact of combined application of sulphur levels and sulphur oxidizers on soil bacterial population in addition to inorganic fertilizer was also observed by Bhagwan [14].

Table 2 Microbial population in different treatments at different time intervals in rhizospheric soil of *Brassica napus* (GSC-7)

Treatment	Bacteria (× 10^7 CFU g^{-1} soil)		Diazotroph (× 10^5 CFU g^{-1} soil)		Fungi (×10^3 CFU g^{-1} soil)		Actinomycetes (× 10^5 CFU g^{-1} soil)		PSB (×10^3 CFU g^{-1} soil)		SOB (× 10^5 CFU g^{-1} soil)	
DAS	80	160	80	160	80	160	80	160	80	160	80	160
T1	38	24	14	29	6	5	62	78	7	11	13	19
T2	42	32	18	31	9	7	56	66	11	15	9	16
T3	46	34	20	32	13	10	49	55	10	16	20	33
T4	51	40	25	35	14	11	41	52	15	20	14	29
T5	43	34	23	36	19	15	47	50	13	18	23	39
T6	48	38	28	39	21	18	39	43	16	21	17	35
T7	41	29	26	37	17	14	42	49	9	15	16	32
T8	47	37	31	43	20	16	37	41	13	18	12	29
T9	62	41	29	38	21	19	35	43	17	21	31	48
T10	71	52	37	49	25	22	29	32	19	24	29	40
T11	69	49	39	46	10	5	36	45	21	27	15	25
T12	72	58	42	51	11	5	32	37	23	29	13	21
T13	83	62	45	49	9	5	34	38	27	34	36	50
T14	91	80	49	55	10	7	29	32	31	42	29	44
T15	78	61	48	53	15	10	33	34	30	35	38	52
T16	85	67	52	59	18	15	28	30	33	41	32	47
T17	76	55	51	54	14	9	31	33	26	32	33	48
T18	84	69	55	63	18	11	26	28	30	39	28	42
T19	97	66	54	60	21	17	25	30	36	45	48	59
T20	108	78	63	76	23	19	21	26	39	48	39	53
CD @ 5%												
Treatments	1.48		5.33		3.16		3.76		3.23		4.68	
Days	0.47		1.68		1.00		1.19		1.02		1.481	
Interaction	2.10		NS		NS		5.32		NS		NS	

Diazotrophic population

Diazotrophic population improved significantly in treatments having application of SOB cultures as compared to uninoculated treatments. Significantly higher diazotrophic population was recorded at maturation stage (160 DAS) as compared to flowering stage (80 DAS) in all treatments. This might due to the application of 1/3rd dose of N-fertilizers (urea) at flowering stage that diminish the population of diazotrophs (Table 2). Maximum diazotroph population (76 x 10^5 CFU /g soil) was recorded in treatment having SOB Mix with consortium biofertilizer and 100% dose of sulphur. Minimum count of diazotrophs (14 CFU x 10^5 /g soil) was observed with uninoculated treatment (T1) at 80 DAS. Inoculation of SOB5 culture with 100% sulphur showed higher diazotrophs population as compared to SOB10 and SOB38 culture. Combined application of three cultures in treatment having SOB Mix and 100% sulphur recorded with significantly increased (49 CFU x 10^5/g soil) population of diazotrophs. Whereas, integrated application of SOB5 culture with consortium biofertilizer and 100% sulphur dose showed higher diazotrophic population as compared to treatments having SOB10 and SOB38 culture along with consortium biofertilizer. In contrary to this, the addition of sulphur and nitrogen with *Azotobacter* to soil increased the rate of nitrogen fixers by satisfying their energy requirements of nitrogen fixation in soil [15]. Combined application of SOB biofertilizers with nitrogen fixers significantly improved population of diazotrophs [16].

Fungal population

Inoculation with SOB cultures resulted in significantly increased fungal population over uninoculated treatments. Maximum fungal count (25 x 10^3 CFU /g soil) was reported in treatment (T10) having SOB Mix with 100% sulphur at 80 DAS whereas, minimum fungal population (05 CFU x 10^3 /g soil) was observed with uninoculated treatment (T1) at 160 DAS (Table 2). Application of SOB38 culture in treatment T6 resulted in higher (21 x 10^3 CFU /g soil) count of fungi as compared to SOB10 and SOB5 culture. Application of consortium biofertilizer in treatment T12 reported with lower fungal count as that of inoculation of SOB10, SOB38, SOB5 and SOB Mix. Combined application of SOB cultures with consortium biofertilizer showed decrease in fungal count as compared to sole application of SOB cultures. This might be due to application of sulphur oxidizing bacteria that increase nutrient availability in the rhizosphere attributed to positive impact on soil fungi [14]. Dual inoculation of arbuscular mycorrhizal fungi and sulphur oxidizing bacteria showed a synergetic effect on population of each other along with better plant growth [17].

Actinomycetes population

Actinomycetes act as efficient decomposers of nutrient compounds and can increase in the number under lower mineral conditions. Maximum population (78 x 10^5 CFU /g soil) of actinomycetes was found in uninoculated treatment (T1) with 75% sulphur dose. Combined application of SOB culture with consortium biofertilizer showed lowest actinomycetes population as compared to the treatments with sole application of SOB cultures (Table 2). In case of application of SOB cultures, higher actinomycetes count (49 CFU x 10^5 /g soil) was observed in treatment having SOB10 + 100% sulphur as compared to SOB38 and SOB5. Application of consortium biofertilizer in treatment T12 reported with lower actinomycetes count as that of inoculation of SOB10, SOB38 and SOB5. The actinomycetes population was observed to be higher at 160 DAS as compared to 80 DAS. This might be due to activation of other microbial groups at flowering stage of plant growth which could have increased the competition for nutrients, hence diminished the growth of slow growing actinomycetes [18].

PSB population

The population of phosphorus solubilizer improved significantly in treatments having application of SOB inoculants as compared to uninoculated treatments. Increase in dose of inorganic sulphur from 75% to 100% positively influenced the PSB population. Maximum PSB count (48 x 10^3 CFU /g soil) was recorded in treatment having SOB Mix along with consortium biofertilizer at 160 DAS whereas, minimum (07 x 10^3 CFU /g soil) in uninoculated treatment (T1) at 80 DAS. In case of sole application of SOB cultures, inoculation of SOB38 culture showed higher PSB population as compared to SOB10 and SOB5 culture. However, combined application of these cultures in form of SOB Mix with 100% sulphur showed significantly higher PSB population (24 x 10^3 CFU /g soil) than sole culture application. Integrated application of SOB38 culture with consortium biofertilizer showed higher PSB population as compared to treatments having SOB10 and SOB5 culture with consortium biofertilizer (Table 2). The results were supported by the findings that phosphorus solubilizers derive their nutrition from soil reservoir which increases in presence of other soil microbial inoculants [19]. A significant effect of application of phosphorus sources on PSB population [20] especially with application of sulphur and *Acidithiobacillus* and triple super phosphate (TSP).

SOB population

Population of sulphur oxidizing bacteria (SOB) was found significantly higher in all microbial inoculated treatments over the uninoculated control at each time interval. Sulphur oxidizing bacterial population was found higher in treatments with 75% sulphur as compared to 100% sulphur dose (Table 2). Maximum count of SOB (59 CFU x 10^5 /g soil) was observed in treatment having application of SOB Mix along with consortium biofertilizer at 160 DAS. Application of SOB38 culture significantly increased the total sulphur oxidizing bacteria count as compared to SOB10 and SOB5 culture. However, combined application of three cultures as SOB Mix with 75% sulphur significantly improves the population of sulphur oxidizing bacteria (48 CFU x 10^5/g soil). Treatment T15 and T16 inoculated with SOB38 culture along with consortium biofertilizer showed increased SOB count as compared to treatments having SOB10 and SOB5 culture with consortium biofertilizer. Results were in accordance to the findings of Namwar and Khandana [21] that application of N, P, K alongwith biofertilizer helps in increased population and activity of SOB as well as nutrient uptake of plants. Higher population of sulphur oxidizing bacteria was found at harvesting stage of crop [22].

Effect of different treatments on enzymatic activities of rhizospheric soil of Brassica napus (GSC-7)

Alkaline phosphatase activity

Alkaline phosphatase activity was found to be higher in inoculated treatments than uninoculated control. Maximum alkaline phosphatase activity (3.21 µg PNP/ hr / g soil) was observed in treatment having SOB Mix with consortium biofertilizer at 160 DAS whereas, minimum (0.39 µg PNP/ hr / g soil) in uninoculated treatment at 80 DAS (**Figure 1**). In case of sole application of cultures, inoculation of SOB38 culture showed higher (1.33 µg PNP/ hr /g soil) alkaline phosphatase activity as compared to SOB10 and SOB5. Combined application of these three cultures in treatment T10 (SOB Mix + 100% sulphur) showed increased alkaline phosphatase activity. However, integrated application of SOB38 culture with consortium biofertilizer resulted in higher enzyme activity (2.69 µg PNP/ hr /g soil) than SOB10 and SOB5 culture with consortium biofertilizer. This indicated that application of SOB microbial inoculants with consortium biofertilizer (having nitrogen fixer and phosphorus solubilizer) showed stronger control on alkaline phosphatase activity as both N and P are essential for phosphatase enzyme. Alkaline phosphatase activity increased with addition of N and P fertilizers [23].

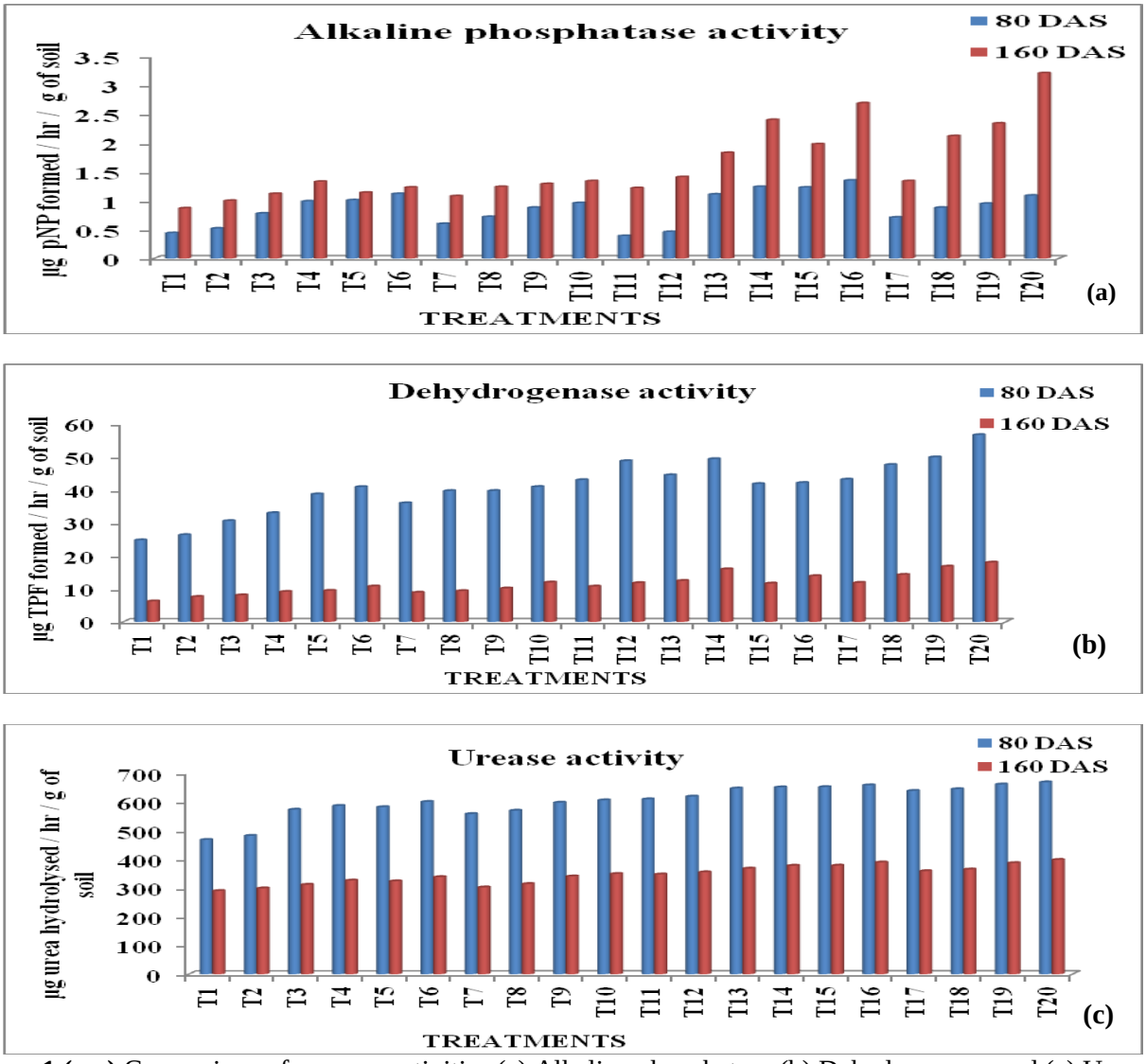

Figure 1 (a-c) Comparison of enzyme activities (a) Alkaline phosphatase (b) Dehydrogenase and (c) Urease in different treatments at different time intervals

Dehydrogenase activity

Dehydrogenase activity is a direct measure of active microbial biomass in soil. Dehydrogenase activity was found significantly higher with application of SOB cultures as compared to uninoculated treatments. Maximum dehydrogenase activity (56.62 µg TPF formed/ hr / g soil) was observed in treatment T20 having SOB Mix along with consortium biofertilizer and 100% sulphur at 80 DAS (Figure1). This might be due to increase in microbial populations as a consequence of inoculation with biofertilizer. Dehydrogenase activity was recorded higher (40.87 µg

TPF formed/ hr /g soil) in treatment inoculated with SOB10 culture as compared to SOB38 and SOB5. Combined application of these three cultures in treatment T10 (SOB Mix + 100% sulphur) resulted in increased dehydrogenase activity. However, application of SOB10 culture with consortium biofertilizer observed with higher (49.33 µg TPF formed/hr/g soil) dehydrogenase activity as compared to SOB38 and SOB5 culture with consortium biofertilizer. The results were in accordance to findings that sulphur oxidizers significantly increased the dehydrogenase activity in soil [14]. Higher dehydrogenase activity was observed with inoculation of bacterial cultures [24].

Urease activity

Urease activity was found to be higher in inoculated treatments as compared to uninoculated at each time interval. At 80 DAS, maximum urease activity (668.10 µg urea formed/ hr /g soil) was observed in treatment having SOB Mix along with consortium biofertilizer and 100% sulphur. Urease activity was recorded higher (599.98 µg urea formed/ hr/g soil) in treatment T6 having SOB38 culture with 100% sulphur as compared to SOB10 and SOB5 (Figure 1). Application of SOB Mix with 100% sulphur (T10) was recorded with higher (605.51 µg urea formed/ hr /g soil) urease activity than sole SOB cultures. However, application of SOB38 culture with consortium biofertilizer resulted in higher (657.52 µg urea formed/hr/g soil) enzymatic activity as compared to SOB10 and SOB5. Application of microbial biomass (due to inoculation) increased the rhizodeposition of soluble carbon fractions which could stimulate enzymatic activity [25]. The results were supported by the studies that treatments inoculated with biofertilizers had higher urease activity than uninoculated treatments [26].

Correlation between microbial population and enzyme activities at different time intervals

Correlation analysis revealed significant interaction between soil microbial population and enzyme activities at different time intervals. At each time interval, significant positive correlation was found between microbial population (except actinomycetes and fungi) and enzyme activities under study.

Table 3 Correlation between microbial population and enzyme activities at (a) 80 DAS (b) 160 DAS

	Bac	Diazo	Fungi	Actino	PSB	SOB	Phos	Deh	Urease
(a) 80 DAS									
Bac	1	0.957**	0.223NS	-0.891**	0.977**	0.803**	0.573**	0.891**	0.864**
Diazo		1	0.279NS	-0.884**	0.967**	0.752**	0.532*	0.847**	0.896**
Fungi			1	-0.465*	0.240NS	0.399NS	0.600**	0.295NS	0.384NS
Actino				1	-0.879**	-0.720**	-0.657**	-0.937**	-0.951**
PSB					1	0.803**	0.636**	0.879**	0.882**
SOB						1	0.727**	0.670**	0.765**
Phos							1	0.621**	0.661**
Deh								1	0.915**
Urease									1
(b)160 DAS									
Bac	1	0.927**	0.027NS	-0.835**	0.959**	0.656**	0.896**	0.948**	0.948**
Diazo		1	0.223NS	-0.818**	0.959**	0.687**	0.899**	0.918**	0.916**
Fungi			1	-0.301NS	0.109NS	0.445*	0.177NS	0.213NS	0.137NS
Actino				1	-0.826**	-0.754**	-0.701**	-0.838**	-0.884**
PSB					1	0.738**	0.919**	0.950**	0.957**
SOB						1	0.693**	0.717**	0.776**
Phos							1	0.927**	0.884**
Deh								1	0.927**
Urease									1

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at 0.05 level(2-tailed)
***Bac: Bacterial population; Actino: Actinomycetes population; Diazo: Diazotrophic population;
PSB: Phosphate Solubilizing Bacteria; SOB: Sulphur Oxidizing bacteria; Phos: Alkaline Phosphatase activity; Deh: Dehydrogenase activity

At 80 DAS, a significant positive correlation was observed between SOB population (r= 0.727) and alkaline phosphatase activity followed by bacterial population (r= 0.573), fungal population (r= 0.600) and PSB population (r= 0.636) at 0.01 level of significance (**Table 3**). Whereas, soil dehydrogenase activity had greater positive correlation with bacterial (r= 0.891) and PSB (r= 0.879) population at 0.01 level of significance than other microbial populations.

Similarly bacteria (r= 0.864), diazotrophic (r= 0.896), PSB (r= 0.882) and SOB (r= 0.765) population showed significant positive correlation (at 0.01 level of significance) with soil urease activity. Fungal population had non-significant correlation with soil dehydrogenase (r= 0.295), and urease (r= 0.384), activity (Table 3). However, actinomycetes population had significant negative correlation with soil dehydrogenase (r= -0.937), alkaline phosphatase (r = -0.657) and urease (r = -0.951) activity at 0.01 level of significance.

Similarly, after 160 DAS, soil bacterial, diazotroph, PSB and SOB population showed significant positive correlation with soil enzyme activities. PSB population had significant (@ p=0.01) positive correlation with dehydrogenase activity (r= 0.950), urease activity (r= 0.957) and alkaline phosphatase activity (r= 0.919). However, the correlation analysis revealed greater positive correlation of bacterial (r= 0.948), diazotroph (r=0.916), PSB (r= 0.957) and SOB (r= 0.776) population with soil urease activity than dehydrogenase and phosphatase activity at 0.01 level of significance (Table 3). Whereas, fungal population showed non-significant interrelationship with soil alkaline phosphatase (r= 0.177), dehydrogenase (r= 0.213) and urease (r= 0.137) activity. Actinomycetes population was found with significant negative correlation (at 0.01 level of significance) with all three enzyme activities 160 DAS. Negative correlation of actinomycetes population with soil enzymatic activities was also observed by Khipla [27].

Conclusion

It was concluded that combined application of microbial inoculants (single SOB cultures, SOB Mix, consortium biofertilizer) certainly influenced the indigenous microflora and their activities in *Brassica napus* (GSC-7). Application of SOB Mix and 100% sulphur along with consortium biofertilizer increased the population of various microbial communities such as total bacteria, diazotrophic, PSB and SOB; and all soil enzymatic activities. Different microbial population and soil enzymatic activities showed a significant positive correlation with each other except actinomycetes and fungi at all time intervals. Sulphur oxidizer's population showed significant positive correlation with other soil microbial flora and enzyme activities thus, indicating a positive impact of SOB on microbial dynamics of rhizosphere. This will lead to directly boost the soil health and hence ecosystem sustainability.

References

[1] D. V. Murphy, E. A. Stockdale, P. C. Brookes and K. T. Goulding, Impact of microorganisms on chemical transformation in soil. In: Abbott L K, Murphy D V (ed) Soil biological fertility- A key to sustainable land use in agriculture, 2007, p 37-59.

[2] D. Y. Sorokin, Oxidation of inorganic sulphur compounds by obligately organotrophic bacteria. Microbiol., 2003, 72(6), 725-39.

[3] M. A. Kertesz, E. Fellows and A. Schmalenberger, Rhizobacteria and plant sulphur supply. Advances. Appl. Microbiol., 2007 62: 235-68.

[4] H. D. Wright, The importance of adequate reduction of peptone in the preparation of media for the pneumococcus and other organisms. J. Path. Bact., 1933, 37, 257.

[5] D. A. A Mossel, A. M. C. Kleynen-Semmeling, H. M. Vincentie, H. Beerens and M. Catsaras, Oxytetracycline-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol., 1970, 33, 454–57.

[6] H. L. Jensen, Nitrogen fixation in leguminous plants II. Is symbiotic nitrogen fixation influenced by Azotobacter. Pro Line Soc NSW, 1942, 57, 205-12.

[7] R. I. Pikovskaya, Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya, 1948, 17, 362–70.

[8] M. W. Beijerinck, Arch Sci Exactes Nat Haarlem Ser 2, 1904, 9131-157.

[9] O. A. Bessey, O. H. Lowry and M. J. Bruck, A method for the rapid determination of alkaline phosphatase with fine cubic millimeters of serum. J Biol Biochem, 1969, 164, 321-29.

[10] V. W. Mersi and F. Schinner, An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. Biol. Fertil. Soils., 1991, 11, 216-22.

[11] J. W. McGarity and M. G. Myers, A survey of urease activity in soils of northern New South Wales. J. Pl. Soil., 1967, 27, 217-38.

[12] O.P. Sheoran, D.S. Tonk, L.S. Kaushik, R.C. Hasija, and R.S. Pannu, Statistical Software Package for Agricultural Research Workers. Department of Mathematics Statistics, CCS HAU, Hisar, 1998, p 139-143.

[13] G. Jilani, A. Abida, Z. H. fauzia, H. S. Imran, M. A. Raja and A. Muhammad, Prospects of supplementing chemical fertilizers with organic and bio fertilizers for crop production in rainfed conditions. Afr. Crop. Sci. Conference Proceedings, 2007, 8, 1513-16.

[14] G. H. Bhagwan, Effect of sulphur oxidizing bacteria and sulphur levels on growth, yield and nutrient uptake by pigeon pea. M.Sc. thesis, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (M.S.), India (2017).
[15] S. Varin, J. B. Cliquet, E. Personeni, J. C. Avice, S. Lemauviel Lavenant, How does sulphur availability modify N acquisition of white clover (Trifolium repens L.). J. Exp. Bot., 2010, 61(1), 225-34.
[16] R. Anandham and R. Sridar, Use of sulphur bacteria for increased yield and oil content of groundnut. In: Proceedings of the National Workshop on 'Recent Developments in Biofertilizers for Rice Base Cropping System', Coimbatore, India, 16-18 August 2001 and published in book entitled Biofertilizers technology, 2004 pp. 365-371, ISBN 81-7233-359-5, edited by Kannaiyan S. Kumar K and Govindarajan K.
[17] A. A. Mohamed, E. E. Wedad, A. M. Eweda, E. A. H. Heggo, Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur-oxidizing bacteria on onion (*Allium cepa* L.) and maize (*Zea mays* L.) grown in sandy soil under green house conditions. Annals. Agric. Sci., 2014, 59(1), 109-18.
[18] W. Barabasz, D. Albinska, M. Jaskowska, J. Lipiec, Biological effects of mineral nitrogen fertilization on soil microorganisms. Pollut. J. Environ., 2002, 11(3), 193-98.
[19] S. Chand, L. L. Somani and S. C. Bhandari, Effect of fertilizer, farmyard manure (FYM) and biofertilizer on the population of *Azotobacter* and Phosphate Solubilizing Bacteria (PSB) in the soil. J. Indian. Soc. Soil. Sci., 2010, 58(4), 460-63.
[20] N. P. Stamford, P. R. dos Santos, A. M. M. F. de Moura, C. E. R. S. Santos, A. D. S. de Freitas, Biofertilizers with natural phosphate, sulphur and *Acidithiobacillus* in a soil with low available-P. Scientia Agricola, 2003, 60(4), 767-73.
[21] A. Namvar and T. Khandana, Inoculation of Rapeseed under Different Rates of Inorganic Nitrogen and Sulfur Fertilizer: Impact on Water Relations, Cell Membrane Stability, Chlorophyll Content and Yield. Archives Agron Soil Sci, 2014, DOI: 10.1080/03650340.2014.982550.
[22] A. M. Pujar, B. N. A. Kumar and G. S. Geeta, Response of sunflower (*Helianthus annuus* l.) to graded levels of sulphur and sulphur oxidising biofertilizer (*Thiobacillus thiooxidans*). Biochem Cell Arch, 2014, 14(2), 339-42.
[23] R. M. Alison and B. Z. Houlton, Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol, 2011, 193, 696-704.
[24] G. K. Aseri, N. Jain, J. Panwar, A. V. Rao, P. R. Meghwal, Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) Indian Thar Desert. Scientia Horti, 2008, 11, 7130-35.
[25] M. C. Rivera- cruz, A. T. Narcia, G. C. Ballona, J. Kohler, F. Caravaca, A. Roldan, Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops soil biology and biochemistry. Soil Biol Biochem, 2008, 40(12), 3092-95.
[26] L. J. Vandana, P. C. Rao and G. Padmaja, Effect of herbicides and nutrient management on soil enzyme activity. J. Rice. Res., 2012, 5, 50-56.
[27] N. Khipla, Effect of biofertilizers and inorganic fertilizers on soil health and growth of Poplar under nursery conditions. M.Sc. thesis, Punjab Agricultural University, Ludhiana, Punjab, India, 2017.

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