

Original Research Article

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Effect of Sulphur Oxidizing Bacterial Inoculants on Soil Nutrient Availability their Uptake and Growth of *Brassica napus* (Var. GSC-7)

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ABSTRACT

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A pot experiment was conducted to evaluate the effect of sulphur oxidizing bacteria (SOB) on soil nutrient availability, nutrient uptake and other growth parameters of *Brassica napus* Var. GSC-7. The experiment was laid down in different combination of recommended inorganic fertilizers, two doses of sulphur (75 & 100%), sulphur oxidizing bacterial cultures and consortium biofertilizer (CB). Results revealed that consortium biofertilizer work in cooperative nature with sulphur oxidizing bacteria in soil. Application of single SOB38 inoculant alongwith CB significantly enhanced the nitrogen and sulphur uptake of plant. However, SOB10 with CB and 100% sulphur resulted in enhanced uptake of phosphorus and potassium nutrients. Application of SOBC + CB with 100% sulphur dose significantly increased available nitrogen (243.02 kg/ha), phosphorus (15.77 kg/ha), potassium (121.88 kg/ha) and sulphur (14.773 ppm) content of soil. Significantly higher nutrient uptake of nitrogen, phosphorus, potassium and sulphur was also recorded in same treatment. Plant growth, root shoot biomass was significantly increased with application of SOBC + CB + sulphur @ 100% dose. Maximum plant height (86.04 cm), dry root weight (0.683 g) and shoot weight (5.435g) was recorded in treatment having SOBC + CB+ 100% sulphur. So, the integrated application of SOBC with CB was markedly effective in improving the soil nutrient status, plant nutrient uptake and growth of *Brassica napus* Var. GSC-7.

Introduction

Sulphur is fourth major plant nutrient after nitrogen, phosphorus and potassium for crop production. It is a constituent of the essential amino acids, proteins, vitamins, oils and activates enzyme system in plant. It is also involved in formation of chlorophyll, glucosinolates (mustard oils) and activation of sulphhydryl (SH-) linkages that act as a source

of pungency in oilseeds of various crops (Chattopadhyay and Ghosh 2012). In Indian soil, deficiency of sulphur is widespread and scattered due to due to extensive use of sulphur free fertilizers, low organic matter and intensification of agriculture with high yielding varieties. Soils, which are deficient in sulphur, cannot supply the adequate sulphur for crop nutrient requirement So to meet demand of crop, use of sulphur with

sulphur oxidizing bacteria may improve plant growth and nutrition.

The sulphur oxidizing bacteria comprise a heterogeneous group of organisms that have the ability to oxidize inorganic sulphur compounds through oxidation process. This process results in the formation of plant available sulphate which improves soil fertility while the acidity produced during oxidation helps in solubilizing plant nutrients and improves alkali soils (Hitsuda *et al.*, 2005). *Brassica napus* (GSC-7) commonly known as Gobhi sarson is a oilseed crop and most popular amongst the farmers of Punjab and other parts of northern India. Along with critical nutrient for better productivity of crop as well as quality of oilseed adequate supply of sulphur and role of SOB in carried out sulphur transformation is very important these days (Kertesz *et al.*, 2007). Keeping these points in view, the present investigation was carried out to study the effect of sulphur oxidizing microbial on soil available nutrients, plant nutrient uptake and plant growth of *Brassica napus* (GSC-7).

Materials and Methods

Experimental Design and Treatments

A pot experiment was conducted to access the effect of SOB inoculant on soil nutrient status their uptake by plants and growth of *brassica napus* (var. GSC-7) during 2017-18 in glass house of Department of Microbiology, Punjab Agricultural University (PAU), Ludhiana, India. The experiment was designed in complete random block design in triplicate (Table 1) with recommended dose of inorganic fertilizers (Nitrogen @ 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) as per the Package and Practice of PAU, Ludhiana for *Brassica sp.* Sulphur was supplement through

gypsum at two different rate i.e. 75% and 100% of 80 kg/ ha. 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 2/3rd at 30 DAS and at 60 DAS. Experiment was comprises of two types of microbial inoculants i.e. sulphur oxidizing bacterial inoculants and consortium biofertilizer (CB). The sulphur oxidizing bacterial inoculants included SOB10, SOB38, SOB5 and mixture of all three SOB cultures as consortium. However consortium biofertilizer was combination of nitrogen fixer, phosphorus solubilizer and PGPR bacteria. Charcoal based sulphur oxidizing bacterial culture (10^8 cells/ml) and consortium biofertilizer were applied to seeds before sowing.

Analysis of soil samples for biological and chemical properties

Soil samples were collected at harvest (160 DAS) were used for enumeration of bacteria, fungi, actinomycetes, diazotrophs, PSBs and SOB population on Nutrient agar (Wright *et al* 1933), Glucose yeast extract medium (Mossel *et al* 1970), Kenknight's medium, Jensen's medium (Jensen *et al.*, 1952), Pikovskaya medium (Pikovskaya 1948) and Thiosulphate agar medium (Beijerinck 1904) respectively, using standard serial dilution spread plate technique. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 24-72 hr; and colonies were counted to record CFU/g soil. These soil samples were dried in shade, grounded and seived to access enzymatic activities viz. alkaline phosphatase, dehydrogenase and urease by method given by Bessey *et al* (1967), Mersi *et al.*, (1991) & McGarity and Myers (1967) respectively. Soil samples were analyzed for chemical nutrients available such as nitrogen, phosphorus, potassium and also sulphur content by method prescribed by Subbiah & Asija (1956), Olsen *et al.*, (1954), Merwin & Peech (1950) and Chesnin & Yien (1950) respectively.

Analysis of plant nutrient content and crop growth parameter

Plant growth parameters such as plant height was recorded at 80 and 160 DAS whereas number of primary/secondary branches, root-shoot dry weight at harvesting stage. Plant samples were collected at harvest of crop. Samples were air dried till the constant weight then milled in to determine nutrient content. Plant samples were digested in di-acid mixture of HNO₃ & H₂SO₄ then analyzed for nitrogen (Kjeldhal's method), phosphorus (Jackson (1967) Vanado Molybdo phosphoric yellow colour method), potassium (Jackson method) and sulphur uptake (Chesnin and Yien (1950)).

Statistical Analysis

Two-way analysis of variance (ANOVA) was performed (at 0.05 level of significance) to determine the effect of different combinations of treatments, time interval and their interaction on various soil and plant parameters using OP-STAT software (Sheoran *et al.*, 1998).

Results and Discussion

Effect of different sulphur doses, sulphur oxidizing bacterial inoculants and consortium biofertilizer on nutrient availability in soil

The available nutrients nitrogen, phosphorus, potassium and sulphur in soil at maturity of crop (160 DAS) was significantly influenced by different sulphur levels (75% and 100%). The maximum available nitrogen (243.02 kg/ha) and sulphur (14.773 ppm) was recorded in treatment (T20) having SOBC+ CB + 100% sulphur while the minimum was recorded in uninoculated control (237.01 kg N/ha) and (11.649 ppm S), respectively. In case of single cultures application, inoculation

of SOB38 culture in treatment (T6) with 100% sulphur showed higher available nitrogen (241.99 kg/ha) and sulphur (12.791 ppm) as compared to SOB10 and SOB5 cultures. However, application of SOBC with 100% sulphur increased the available nitrogen (242.16 kg/ha) and sulphur (13.046 ppm) in soil as that of inoculation of single SOB cultures (Fig. 1). Application of SOB with consortium biofertilizer further enhanced the nitrogen and sulphur content in the soil as compared to sole application of these cultures. Treatment T16 having SOB38 + CB + 100% S resulted in higher available nitrogen (242.89 kg/ha) and sulphur (13.832 ppm) as compared to SOB10 and SOB5 with consortium biofertilizer. These results were in closed conformity with the findings of Yadav *et al.*, (2010) and Solanki *et al.*, (2015) that each successive dose of phosphorus and sulphur resulted in significantly increase in available nitrogen. Chattopaddhyay and Ghosh (2012) reported that available S in the soil was significantly affected due to different sulphur levels after harvest of the crop. Kumar and Trivedi (2014) reported higher availability of N, P, K and S of mustard in soil with application of ammonium sulphate followed by gypsum as potent sulphur source.

The available phosphorus and potassium in soil after harvest of crop showed that their maximum value (15.77 kg/ha) and (121.88 kg/ha) was observed in treatment comprising combined application of SOBC + CB with 100% sulphur. Minimum available phosphorus (12.76 kg/ha) and potassium (110.84 kg/ha) in soil was recorded in uninoculated treatment T1 (Fig. 1). In case of single application of SOB cultures, inoculation of SOB10 culture with 100% sulphur showed higher available phosphorus (14.76 kg/ha) and potassium (114.30 kg/ha) followed by SOB38 and SOB5. However, inoculation of SOBC with 100% sulphur showed significantly higher available

phosphorus (14.83 kg/ha) and potassium (113.41 kg/ha) as that of single inoculation of SOB cultures.

Results showed that combined application of SOB cultures with CB significantly improved available phosphorus and potassium status of soil as compared to single SOB cultures. These results were in accordance to the findings of Salimpour *et al.*, (2010) and Yadav *et al.*, (2010) that the availability of potassium and phosphorus in soil increased progressively with increased levels of sulphur fertilizer application at crop harvest.

Effect of different sulphur doses, sulphur oxidizing bacterial inoculants and consortium biofertilizer on nutrients uptake of plants

Significantly maximum nitrogen (1.68 mg/plant), phosphorus (0.98 mg/plant) and potassium uptake (1.57 mg/plant) was observed in treatment having SOBC + CB + 100% sulphur whereas minimum has recorded in uninoculated control treatment. In case of application of single application of SOB cultures higher nitrogen uptake (1.46 mg/plant) was recorded in treatments having SOB38 with 100% sulphur, whereas inoculation of SOB10 culture with 100% sulphur in treatment T4 increased the total phosphorus (0.72 mg/plant) and potassium uptake (1.35 mg/plant).

Application of SOBC (having SOB10, SOB38 and SOB5) + 100% sulphur resulted in higher nitrogen, phosphorus and potassium uptake as compared to single inoculation of SOB cultures (Table 2). The integrated use of SOB cultures with CB gives better results as compared to single use of SOB cultures. So, combined application of SOB38 culture with CB and 100% sulphur showed higher nitrogen uptake (1.60 mg/plant) whereas SOB10 + CB + 100% sulphur showed with higher

phosphorus (0.93 mg/plant) and potassium uptake (1.52 mg/plant). Bhagwan (2017) reported that the nitrogen, phosphorus and potassium uptake of pigeon pea was significantly influenced with the application of graded levels of sulphur and increased levels of elemental sulphur and sulphur oxidizing microbial cultures. The inoculation of sulphur oxidizers with recommended doses of N, P, K and S resulted in nutrient uptake of various crops along with growth parameters (Mohamed *et al* 2014).

Application of 100% sulphur combined with SOBC + CB resulted in significantly maximum 1.98 mg/plant sulphur uptake whereas minimum (1.30 mg/plant) was recorded in uninoculated control treatment (T1). Application of SOB38 culture with 100% sulphur (T6) showed higher sulphur uptake (1.70 mg/plant) as compared to inoculation of cultures SOB10 (1.64 mg/plant) and SOB5 (1.61 mg/plant). However application of these three SOB cultures as SOBC + 100% sulphur showed significantly higher sulphur uptake (1.77 mg/plant) as compared to single inoculation of SOB cultures. Application of SOB cultures with CB gives better results in terms of sulphur uptake as treatment T16 having SOB38 culture + CB + 100% sulphur resulted in higher sulphur uptake 1.91 mg/plant as compared to single application of SOB cultures. The results are alliance with the findings of Balpande *et al.*, (2016) and Mohamed *et al.*, (2014) that sulphur application had positive impact on sulphur uptake by crop.

Plant growth parameters

Plant height

Plant height is an important physiological parameter of crop related to their growth and development. Maximum plant height of 59.04

cm was recorded at 80 DAS whereas 86.04 cm at 160 DAS with treatment comprising SOBC + CB + 100% sulphur. Minimum plant height was observed i.e. 55.01 cm and 80.09 cm at 80 and 160 DAS respectively. Treatment having inoculation of SOB10 culture with 100% sulphur resulted in higher plant height as 56.45 cm and 84.98 cm as compared to SOB38 and SOB5 at 160 DAS interval.

However, combined application of these three cultures as SOBC + 100% sulphur resulted in higher plant height as compared to single inoculation of SOB cultures (Fig. 2). When, SOB mix was applied in combination to CB and 100% sulphur dose significant improvement was recorded in plant height as compared to sole application of SOB cultures. better results were observed in plant height. Treatment having SOB10 + CB + 100% sulphur showed higher plant height as compared to single application of SOB cultures i.e. 58.83 cm (80 DAS) and 85.78 (160 DAS) respectively. Goud *et al.*, (2012) and Awad *et al.*, (2011) reported that optimum sulphur fertilization had positive effect on plant height due to better nutritional environment for plant growth.

Root – shoot dry weight

Combined application of SOB inoculants with CB significantly benefitted the root biomass of *Brassica napus* (GSC-7) as compared to inorganic fertilizers. Maximum dry root weight (0.683 g) and shoot weight (5.435 g) was recorded in treatment T20 having SOBC + CB + 100% sulphur followed by treatment T19 with 75% sulphur.

Minimum root-shoot dry weight was observed in uninoculated treatment T1 (Fig. 3). Treatment comprising application of SOB38 culture with 100% sulphur showed higher root dry weight (0.465 g) and shoot dry weight (4.104 g) as compared to SOB10 and SOB5.

However, combined application of SOB38 culture with CB and 100% sulphur in treatment T16 found with higher root dry weight (0.622 g) and shoot weight (5.021 g) than single application of SOB cultures. Renneberg and Lomoureaux (1990) and Lakshman *et al.*, (2017) reported that sulphur fertilization significantly increased the dry matter production per plant.

Table.1 Different combinations of treatments designed for pot experiments

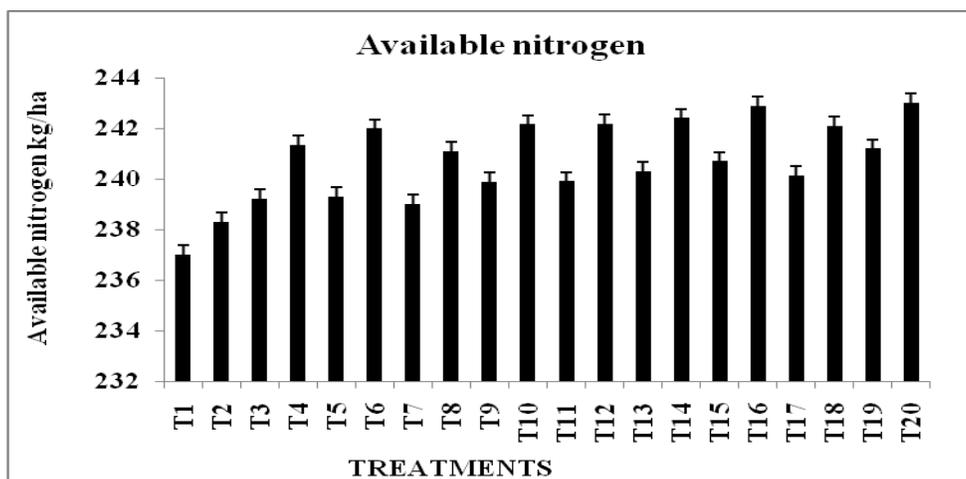
T1: Uninoculated + 75% Sulphur	T11: 75% Sulphur + CB
T2: Uninoculated + 100% Sulphur	T12: 100% Sulphur + CB
T3: 75% Sulphur + SOB10	T13: 75% Sulphur + CB + SOB10
T4: 100% Sulphur + SOB10	T14: 100% Sulphur + CB + SOB10
T5: 75% Sulphur + SOB38	T15: 75% Sulphur + CB + SOB38
T6: 100% Sulphur + SOB8	T16: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

SOB- Sulphur oxidizing bacteria; SOBC- Sulphur oxidizing bacteria consortium (SOB38+ SOB5+SOB10); CB- Consortium biofertilizer

Table.2 Effect of different sulphur doses, sulphur oxidizing bacterial inoculants and consortium biofertilizer on plant nutrient uptake of *Brassica napus* (GSC-7)

Treatments	Nitrogen uptake (mg/plant)	Phosphorus uptake (mg/plant)	Potassium uptake (mg/plant)	Sulphur uptake (mg/plant)
T1	1.20	0.49	1.10	1.30
T2	1.29	0.53	1.15	1.39
T3	1.37	0.69	1.30	1.57
T4	1.43	0.72	1.35	1.64
T5	1.40	0.65	1.27	1.63
T6	1.46	0.70	1.31	1.70
T7	1.34	0.62	1.20	1.54
T8	1.40	0.68	1.25	1.61
T9	1.44	0.73	1.35	1.71
T10	1.51	0.77	1.40	1.77
T11	1.46	0.76	1.37	1.41
T12	1.52	0.80	1.43	1.49
T13	1.53	0.87	1.47	1.78
T14	1.58	0.93	1.52	1.86
T15	1.57	0.84	1.45	1.84
T16	1.60	0.90	1.50	1.91
T17	1.50	0.81	1.41	1.72
T18	1.57	0.86	1.48	1.80
T19	1.62	0.92	1.51	1.90
T20	1.68	0.98	1.57	1.98
CD @ 5%	0.058	0.069	0.067	0.067

Fig.1 Effect of sulphur oxidizing bacteria on (A) soil available nitrogen (B) available sulphur (C) available phosphorus and (D) available potassium in different treatments at 160 DAS



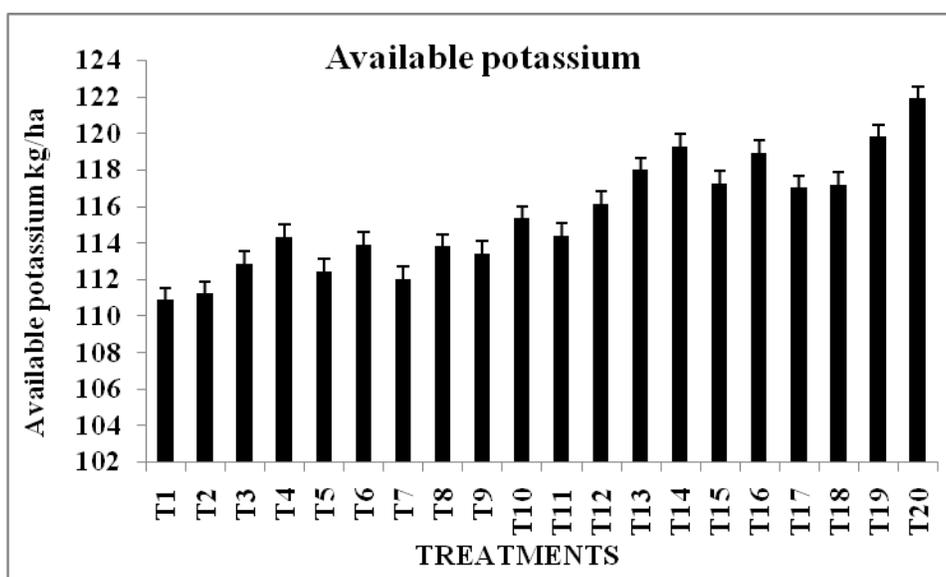
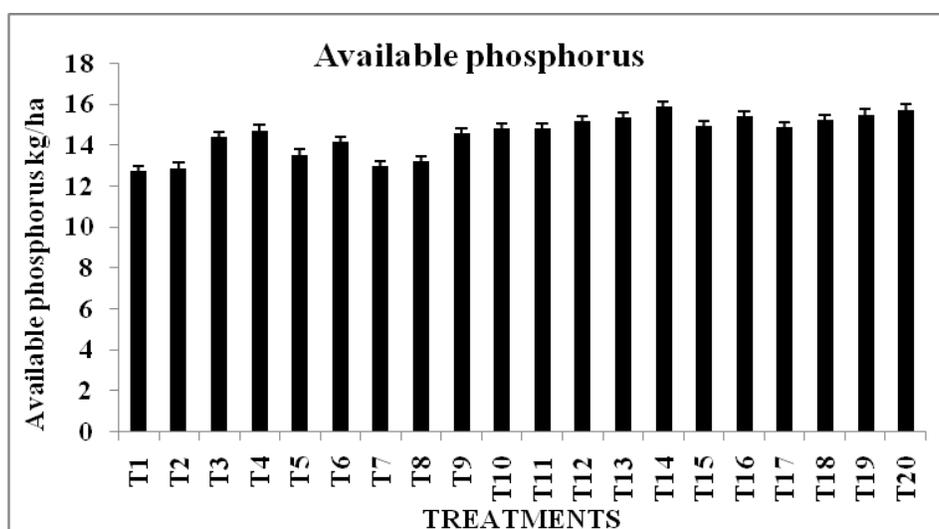
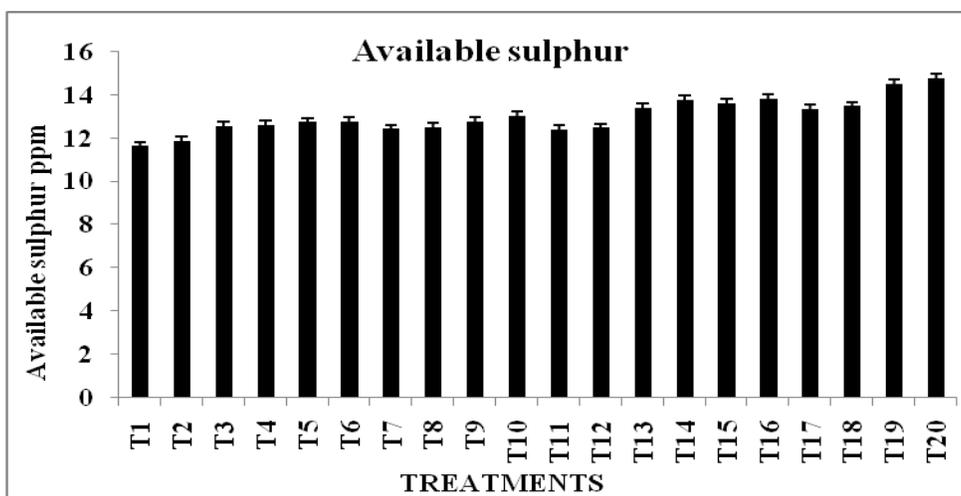


Fig.2 Effect of sulphur oxidizing bacteria on plant height in different treatments at 80 and 160 DAS

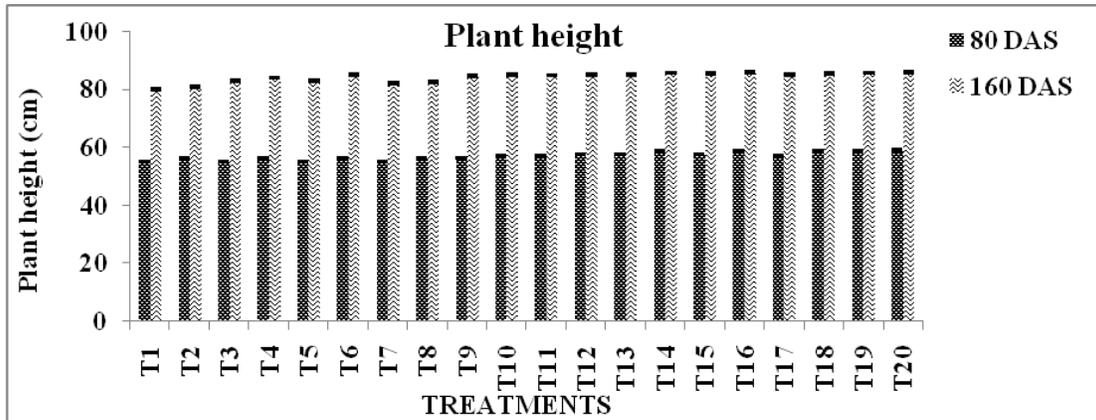


Fig.3 Effect of different sulphur doses, sulphur oxidizing bacterial inoculants and consortium biofertilizer on root and shoot biomass in different treatments at crop harvest

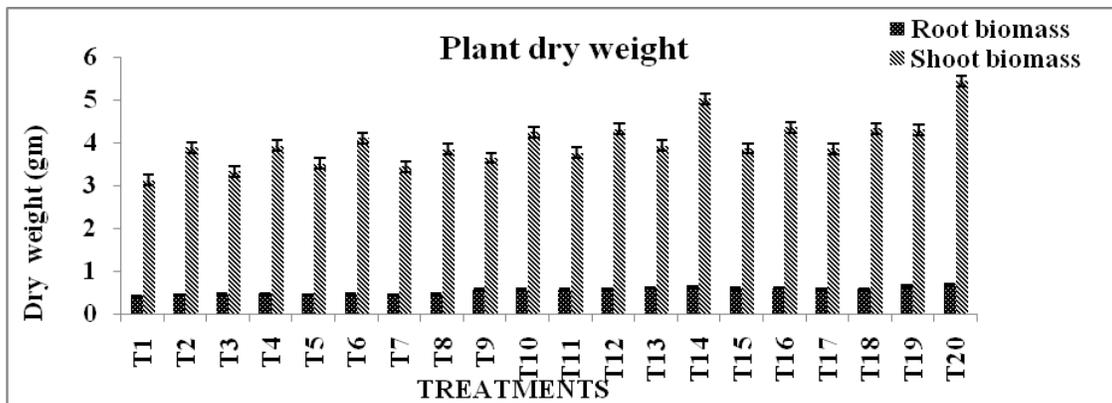
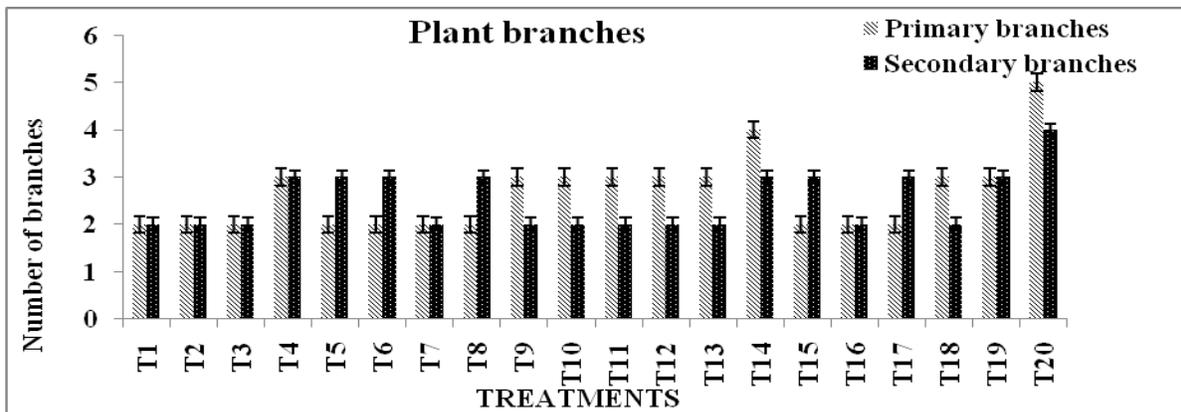


Fig.4 Effect of sulphur oxidizing bacteria on number of branches/ plant in different treatments at 160 DAS



Number of primary and secondary branches/ plant

In *Brassica napus* (GSC-7) number of branches/plant were influenced by different levels of applied sulphur and sulphur oxidizers as biofertilizers (Fig. 4). Increasing levels of sulphur from 75% to 100% have a slight effect on number of primary and secondary branches /plant. At harvesting stage, maximum number of primary branches/plant (5/plant) were observed with treatment T20 having SOB Mix + consortium biofertilizer + 100% sulphur. Similarly, maximum number of seconedry branches/plant (4/plant) was showed by treatment T20 whereas minimum in control treatment T1 (2/plant). Increased in number of primary branches with application of sulphur as compared to the untreated control was reported by Tripathi *et al.*, (2011) and Dhruw *et al*(2017).

In conclusion from the results of the experiment, it was found out that *Brassica napus* and other oil seed crops required appropriate dose of sulphur fertilizer along with microbial inoculants for proper nutritional status of soil and plants which had further significant effects on plant height, soil nutrient availability and better soil microbial growth. Most of the plant growth parameters were increased with the increasing levels of sulphur fertilizer up to 100 %. It was concluded that application of microbial inoculants significantly improved the soil biological (in term microbial population and their activities) and soil chemical (available nutrients) properties of soil. Application of SOBC significantly increase the soil available nutrients their uptake and growth of plant. However, combined application of SOBC with CB further enhanced the positive effect of these microbial formulation on soil nutritional status. So, treatment T20 having 100% S + SOBC + CB was considered to be

best treatment for soil nutrient availability and growth of *Brassica napus*.

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