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Response of sulphur oxidizing bacterial inoculation on growth and yield parameters of mustard (*Brassica juncea* L.)

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Abstract

The present study was carried out to examine the effect of two sulphur oxidizing bacteria; SSA21 and SSS6 on the growth of mustard. A total of 15 treatments comprising three controls and six treatments for each bacterium were formulated. Different growth parameters like plant length, dry weight, chlorophyll content in leaves, seed oil and protein content, number of siliquae, seeds per siliqua and 100 seed weight were observed. The total plant length of mustard varied from 96.0 to 156.2 and from 97.1 to 146.8 cm during the year 2016-17 and 2017-18 respectively at maturity. The total plant weight was found in between 8.227 g/pot to 18.853 during both years. Similarly, number of siliquae (20.0-54.6), seeds per siliquae (8.0-13.3) and 100 seed weight (0.660-1.333 g), oil (28.4-33.3%), protein (18.64-24.56%), chlorophyll content (1.287-3.644mg/gFW) was recorded. A positive improvement in mustard crop growth with respect to different parameters was recorded due to inoculation of SOB.

Keywords: sulphur oxidizing bacteria, mustard yield, *Brassica juncea*, plant growth

Introduction

Rapeseed-mustard is the third most important oilseed crop comes after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.) oil in the world. It belongs to the family Brassicaceae and order Brassicales. The mustard group roughly includes Indian mustard, brown sarson, yellow sarson, raya and toria crops. Indian mustard (*Brassica juncea* (L.) is principally cultivated in Rajasthan, Uttar Pradesh, Southern Haryana, Madhya Pradesh and Gujarat. India is placed fourth in terms of oilseed production and holds a premier position in rapeseed-mustard economy of the world with 2nd and 3rd rank in area and production, respectively (Anonymous, 2016) [1]. The productivity of only five states viz. Haryana, Gujarat, Rajasthan, Uttar Pradesh and Madhya Pradesh was found above 1000 kg/ha (Rathore *et al*, 2018) [2].

Oilseed crops like mustard require more sulphur (S) than cereals as these need as much sulphur as phosphorous. The areas of sulphur scarcity are becoming more prominent around the world due to intensive agriculture, low sulphur returns with farmyard manure (FYM), high yielding varieties and use of chemical fertilizers having very less amount of S (Jamal *et al*, 2010) [3]. The deficiencies of sulphur in soils of tropical and subtropical regions have been documented for many years (Pasricha and Fox, 1993) [4] and reported from approximately 70 countries, including India. At least 57 million hectares (~41%) of total arable land of India (142 million hectare), is affected from various degrees of S deficiency (Singh, 2001) [5]. With constant use of S-free fertilizers and less amount of organic manures, the sulphur deficiency has also appeared in many parts of Haryana. The maximum sulphur deficiency has been observed in Rewari and minimum in Sirsa district (Anonymous, 2016) [1]. It is prevalent in coarse textured alluvial, red and lateritic, leached acidic and hill soils and black clayey soils. The deficiency of sulphur is emerging fast in areas where, continuously sulphur free fertilizers like DAP and urea are being used.

Many microbes and plants take up sulphur in the form of sulphate, which undergoes a series of transformations before its incorporation in the main compounds (Katyal *et al*, 1997) [6]. The transformations of sulphur compounds in nature have been circulated in the so-called sulphur cycle. The soil microbial biomass is the key driving force behind all these transformations and acts as both a source and sink for inorganic sulphates (Vidhyasri and Sridar, 2011) [7]. So keeping in view, the importance of sulphur for various soil types, for oilseed crops and role of microorganisms in making them available to plants, the present investigation has been planned

with the objective to test the efficacy of selected sulphur oxidizing bacteria for growth of mustard (*Brassica juncea*).

Material and Methods

Soil and Seed Collection

The soil for pot house experiment was collected from fields of Soil Science Department and mustard seeds (RH-30) were taken from Ram Dhan Singh seed farm, CCS HAU Hisar.

Biofertilizer, Pyrite and Sulphur

Phosphoteeka (*Pseudomonas* P36) and Azoteeka (*Azotobacter chroococcum*) were taken from Bio fertilizer Unit, Department of Microbiology, COBS&H, CCSHAU, Hisar. Agricultural grade Amjhore pyrite containing 22% S was brought from M/S pyrite, Phosphates and Chemical Ltd., Dehradun (PPCL) and elemental sulphur(S⁰) from Kinjal Chemicals Bombay.

Chemicals

All chemicals used were of analytical grade (AR), obtained from Hi-media Laboratories (P) Limited, Sarabhai M. Chemicals, India, Mumbai, M/S BDH, E-Merck or Qualigens, SRL and Bioscience (P) Limited, New Delhi.

Instruments

The instruments used to carry out experiments were Remi rotary shaker, Calton B.O.D. incubator, Systronics 331 digital pH meter, UV-visible spectrophotometer, Magnus microscope with Camera (Canon 14.1 megapixels), Remi centrifuge, Shaking incubator, Gerhardt Kjeldatherm, Gerhardt vapodest 20 and Microcentrifuge (Eppendorf, Germany) etc.

Assessment of sulphur oxidizing bacteria for growth promotion of mustard

Two sulphur oxidizing bacteria SSA21 and SSS6 (Chaudhary *et al.*, 2017) ^[8] were assessed for two consecutive years for their effect on growth of mustard under pot house conditions. SOB were inoculated with mustard seeds individually and also in combination with standard biofertilizer Azoteeka and Phosphoteeka.

Analysis of soil used for pot house

The soil used was analysed for available nutrients like Organic C, N, P, K, total S and C: N ratio. The organic carbon was estimated by method of Kalembassa and Jenkinson (1973) ^[9], while total N content of soil by Kjeldhal's methods (Bremner, 1965) ^[10]. The total phosphorus of soil was evaluated by using method given by John (1970) ^[11] and potassium by method given by Jackson, 1973 ^[12] using flame photometer by direct feeding. Total sulphur content of soil was calculated by turbidometric method by Chesnut and Yien (1950) ^[13].

Treatments details

Three controls were taken; C1 = without RDF (Recommended dose of fertilizer), C2 = with 75% RDF and C3= with 100% RDF. Two bacteria isolated earlier SSA21 and SSS6 were selected and six treatments were taken for each bacterial Isolate; 1) 100% RDF + Isolate, 2) 75% RDF + Isolate, 3) 75% RDF + Azoteeka + Phosphoteeka, 4) 75% RDF + Isolate + Azoteeka, 5) 75% RDF + Isolate + Phosphoteeka and 6) 75% RDF + Isolate + Azoteeka + Phosphoteeka. So a total of 15 treatments were taken as following:

Table 1: Details of different treatments taken for experiment

S. No.	Treatments
1.	Control 1, without RDF (T1)
2.	Control 2, with 75% RDF (T2)
3.	Control 3, with 100% RDF (T3)
4.	Isolate SSA21 + 100% RDF (T4)
5.	Isolate SSA21 + 75% RDF (T5)
6.	75% RDF + Azoteeka + Phosphoteeka (T6)
7.	Isolate SSA21 + 75% RDF + Azoteeka (T7)
8.	Isolate SSA21 + 75% RDF + Phosphoteeka (T8)
9.	Isolate SSA21 + 75% RDF + Azoteeka + Phosphoteeka (T9)
10.	Isolate SSS6 + 100% RDF (T10)
11.	Isolate SSS6 + 75% RDF (T11)
12.	75% RDF + Azoteeka + Phosphoteeka (T12)
13.	Isolate SSS6 + 75% RDF + Azoteeka (T13)
14.	Isolate SSS6 + 75% RDF + Phosphoteeka (T14)
15.	Isolate SSS6 + 75% RDF + Azoteeka + Phosphoteeka (T15)

Mustard sowing (cv. RH-30) crop

Earthen pots of 30 cm inner diameter and 28 cm height were used for experiment. Air-dried soil (~7 kg) was filled in each pot and 60% moisture was maintained. Each treatment was performed in triplicates. Recommended dose of fertilizers (RDF) i.e. Urea, SSP and Zn was mixed in the upper 15 cm layer soil @ 52, 50 and 10 kg/acre respectively. Seeds were surface sterilized by using 0.1% HgCl₂ for 4-5 minutes trailed by 70% alcohol for 30 sec and wash away with double distilled water. All seeds were inoculated with one ml inoculum (10⁸cells/ml) of bacteria and sown at depth of 4-5 cm. Three controls, all in triplicates, were also kept along with one absolute control without RDF and inoculation. Pyrite was also added @ 20 kg/ha in all pots except control without RDF. Pots were irrigated as and when required.

Five seeds were sown in each pot and after appearance of seedlings; thinning was done to final three plants per pot. The crop was harvested ~135 days of sowing during both years. Different parameters were observed time to time and the harvested plants were placed into paper bags, air dried and then oven dried at 65±2oC upto constant weight for other observations to be taken. Following parameters were taken:

Determination of various growth parameters

Plant length (cm)

The shoot, root and plant length of mature plants was recorded at harvesting with centimetre scale.

Plant weight (g)

The shoot, root and plant weight of oven dried plants was recorded after getting dry with electronic weighing balance in grams.

Number of siliquae per plant

The number of siliquae per plant was counted at the time of harvesting.

Number of seeds per siliqua

Total number of seeds in one siliqua of each plant was counted manually during the threshing.

Seed weight (g)

Matured seeds (100 seeds) of plants were count up and weighed with weighing balance in grams.

Oil content (%)

Oil content of mustard seeds was determined by the standard method given in A.O.A.C. (1995) ^[14].

Seed protein content (%)

Protein content of dried mustard seeds was assessed using micro- Kjeldal method given by Markham, 1942 ^[15].

Leaves chlorophyll content

The photosynthetic pigment, total chlorophyll content of leaves of each plant taken were calculated at 30, 60 and 90 day as the method explained by Hiscox and Israelstam (1979) ^[16].

Statistical analysis

The data was analysed with the application of one or two factor complete randomized design (CRD) using OPSTAT software available on CCS HAU homepage given by Sheoran *et al*, 1998 ^[17].

Results and Discussion

Initial soil analysis

The soil used for pot house experiment was sandy loam in with the 7.33 pH. The organic C and total N were 0.38 and 0.05% respectively. Total P, K and S were found 0.028, 0.20 and 0.186 % respectively.

Plant length

The shoot, root and plant length of mustard crop at maturity of two consecutive years is presented in Table 2. It is evident from the table that the plant length increased significantly with inoculation of sulphur oxidizing bacteria over the control. Approximately 60% in treatment T9 of SSA21 (with biofertilizers; Azoteeka and Phosphoteeka with application of 75% RDF and pyrite @ 20kg/ha) and 62% increase in treatment T15 of SSS6 (with biofertilizers (Azoteeka and Phosphoteeka) with application of 75% RDF and pyrite @ 20kg/ha) was recorded for total plant length in comparison to treatment T1 (control). The total plant height of mustard during the year 2016-17, ranged from 96.0 to 156.2 and from 97.1 to 146.8 cm during 2017-18 at maturity.

Table 2: Effect of inoculation of SOB on length of mustard crop (Var. RH-30)

Treatments	Year (2016-17)			Year (2017-18)		
	Length (cm)					
	Shoot Length	Root Length	Total Plant Length	Shoot Length	Root Length	Total Plant Length
T1.	77.7	12.9	96.0	82.8	14.3	97.1
T2.	109.1	18.7	132.2	108.0	17.5	125.5
T3.	116.5	21.7	143.0	113.4	22.7	136.1
T4.	109.2	24.130	140.9	109.6	19.4	129.1
T5.	122.5	23.203	152.5	120.6	23.4	144.0
T6.	127.0	20.487	152.2	123.3	20.3	143.7
T7.	116.9	16.6	137.3	115.0	17.5	132.6
T8.	117.9	16.8	140.9	120.8	18.9	139.8
T9.	127.5	20.8	154.4	123.6	20.4	144.1
T10.	120.4	15.1	140.6	121.2	17.5	138.7
T11.	109.2	14.0	128.3	109.4	16.5	125.9
T12.	122.0	16.6	143.9	122.4	16.4	138.9
T13.	115.5	14.4	134.6	115.4	18.2	133.7
T14.	115.7	15.4	136.4	117.6	19.2	136.9
T15.	128.5	21.2	156.2	128.3	18.5	146.8
C. D. at 5% level of significance	14.2	1.5	19.3	2.4	11.8	3.2

Plant weight

The total plant weight of mustard during two consecutive years 2016-17 and 2017-18 is presented in Table 3. There was a significant increase in plant weight from 8.227 to 18.650 and from 8.373 to 18.473 g during 2016-17 and 2017-18 respectively in T15 with isolate SSS6 in comparison to control. However, no significant difference was observed among treatments inoculated with isolate SSS6 (T10-15). More than 2 fold increase was observed in the root, shoot and total plant weight of T15 (Bacterial isolate SSS6+ Phosphoteeka+Azoteeka+75% RDF + pyrite@ 20kg/ha) over the control (T1).

Table 3: Effect of inoculation of SOB on dry matter yield of mustard crop (Var. RH-30)

Treatments	Year (2016-17)			Year (2017-18)		
	Weight (g/pot)					
	Shoot Weight	Root Weight	Total Plant Weight	Shoot Weight	Root Weight	Total Plant Weight
T1.	6.350	1.877	8.227	6.653	1.720	8.373
T2.	10.593	2.803	13.397	10.967	2.950	13.917
T3.	11.743	3.870	15.613	12.150	3.907	16.057
T4.	14.833	2.717	17.550	14.650	2.803	17.453
T5.	13.340	2.900	16.240	13.713	2.717	16.430
T6.	13.830	3.110	16.940	13.970	3.183	17.153
T7.	13.693	2.763	16.457	13.470	2.880	16.350
T8.	14.347	3.220	17.567	14.420	3.357	17.777
T9.	15.277	3.577	18.853	14.603	3.567	18.170
T10.	14.013	2.490	16.503	14.690	2.600	17.290
T11.	13.033	2.320	15.353	13.623	1.547	15.170
T12.	14.077	3.100	17.177	14.517	2.503	17.020
T13.	13.760	2.797	16.557	14.040	1.913	15.953
T14.	15.057	2.600	17.657	15.030	2.643	17.673
T15.	15.203	3.447	18.650	15.077	3.397	18.473
C. D. at 5% level of significance	1.134	0.747	1.095	0.907	0.579	0.755

Microbial inoculation is very important for plant growth. For enhancement of plant growth microbes generally work by one or in combination, out of three ways either by producing some compounds for the plants or by helping the uptake of nutrients from soil through solubilization or oxidation and by defending in stress conditions or from plant diseases. The plant growth promotion can be improved by both means directly and

indirectly. Cheema *et al*, (2001) ^[18] assessed the effect of various levels of S (0, 20, 40, 60 kg/ha) fertilizer on canola (*Brassica napus* L.) and the highest plant height (165.9 cm) was reported with 60 kg S/ha. Nath *et al*, (2018) ^[19] also reported increase in height from 146.2 to 158.9 with increasing doses of sulphur. Negi *et al*, (2017), ^[20] reported increase in plant dry matter of mustard crop due to application

@ 60 kg S/ha over the control. The dry matter of mustard ranged between from 41.4 to 72.9 g and 98.8 to 128.8 g at 60th and 90th days respectively.

Number of Siliquae

It is evident from table (table 4) that during the year 2016-17, the number of siliquae per plant increased from control 1 (23.3) having only soil (T1) to control 2 (34.0) with 75% RDF and pyrite @ 20 kg/ha (T2). It further increased from T2 to 40.0 in control 3 (T3) with 100% RDF and pyrite @ 20 kg/ha. It also increased from 43.3 siliquae per plant in the treatment T4 to 47.0 and 54.6 siliquae per plant in T9 and T15 (having bacterial isolates SSA21 and SSS6 respectively with Azoteeka, Phosphoteeka, 75% RDF and pyrite @ 20 kg/ha) during 2016-17. The similar pattern was followed during the year 2017-18.

Number of seeds

The data presented in the table (table 4) revealed that there was no significant difference in number of seeds per siliqua among the controls 1, 2 and 3 during both the years. However, a significant difference was recorded between T1 (control1) with 9.3 siliquae/ plant and T15 (12.0 siliquae/ plant) having

(bacterial isolate SSS6, Azoteeka, Phosphoteeka with 75% RDF and pyrite @ 20 kg/ha).

Seed Weight

The inoculation of SOB isolate with mustard seed significantly affected the seed weight over the control (table 4). The weight of hundred seeds of mustard in the different treatments varied from (0.660) in (T1 having only soil) to (1.213 g) in T9 (having bacterial isolate SSA21, Azoteeka, Phosphoteeka with 75% RDF and pyrite @ 20 kg/ha) respectively during the year 2016-17, while during 2017-18 the maximum and minimum seed weight was 0.661 and 1.333 g respectively.

The increase in number of seeds, siliquae and seed weight with increasing level of sulphur due to activities of SOB may be credited to the role of sulphur in the growth and development of oilseed crops (Khalid *et al*, 2009)^[21]. Singh *et al*, (2015)^[22] also described that on application of 40 kg S/ha, test weight of seed and siliquae/plant increased. Siliqua/plant in mustard crop increased by 54 and 62 % on application of 40 and 60 kg S/ha respectively by gypsum (Negi *et al*, 2017)^[20].

Table 4: Effect of inoculation of bacterial isolates on yield of Mustard crop (Var. RH-30)

Treatments	Year (2016-17)			Year (2017-18)		
	No. of siliquae per plant	No. of seeds per siliqua	100 seeds weight (g)	No. of siliquae per plant	No. of seeds per siliqua	100 seeds weight (g)
T1.	23.3	9.3	0.660	20.0	8.0	0.661
T2.	34.0	8.6	0.779	32.0	8.6	0.771
T3.	40.0	10.6	0.957	37.6	10.6	0.943
T4.	43.3	9.3	1.193	44.0	10.6	1.190
T5.	40.0	8.6	1.173	41.0	9.3	1.173
T6.	45.6	10.0	1.177	45.6	9.3	1.053
T7.	44.0	9.3	1.157	44.0	10.6	1.160
T8.	43.3	10.0	1.183	45.0	10.6	1.163
T9.	47.0	10.6	1.213	46.3	11.3	1.210
T10.	47.6	10.6	1.180	47.6	11.3	1.200
T11.	41.6	10.0	1.180	41.3	9.3	1.107
T12.	45.6	10.0	1.177	45.6	9.3	1.053
T13.	48.3	10.6	1.220	47.0	10.6	1.237
T14.	50.0	10.0	1.280	49.0	12.6	1.267
T15.	54.6	12.0	1.307	53.0	13.3	1.333
C. D. at 5% level of significance	2.4	1.9	0.073	2.2	1.9	0.078

Oil content

It was found that there was a significant difference in the oil content of mustard with the inoculation of sulphur oxidizing bacterial isolates over the control (T1) during the both years (table 5). The maximum oil content was in the crop inoculated in SSA21 followed by SSS6, T3, T2 and T1 producing 33.1, 32.8, 30.3, 29.4 and 28.4% respectively among different treatments.

Inoculation of sulphur oxidizing bacteria with mustard seeds resulted into manufacture of more sulphate and thus increased the oil formation as sulphur is main nutrient for formation of seed oil due to involvement in the formation of oil composites. Mani *et al*, (2006)^[23] also reported improvement of oil formation in mustard after application of sulphur. This increase in oil formation with inoculation of SOB was mainly due to rise in glucosinolates and glucoside formation (allylthiocyanate).

Seed protein content

A significant difference was recorded in seed protein content after inoculation of sulphur oxidizing bacteria (table 5). Approximately 20% of increase in protein content of seeds of plants of treatment T9 was recorded over the control (T1) after inoculation of bacterial isolate SSA21 along with Azoteeka, Phosphoteeka and application of pyrite @ 20 kg/ha. The protein content of mustard during the present investigation varied from 19.51 to 23.54 and 18.64 to 24.56% during the years 2016-17 and 2017-18 respectively.

Mohiuddin *et al*, (2011)^[24] reported rise in protein content of mustard from an initial concentration of 19.38 to 21.81% due to increasing the S fertilization rate from 0 to 16 kg/ha. However, Ceh *et al*, 2008^[25], didn't observe any significant difference in the protein content among different treatments with application of sulphur fertilizers.

Table 5: Effect of SOB on oil and protein content of mustard seed (Var. RH-30)

Treatments	Year (2016-17)		Year (2017-18)	
	Oil Content (%)	Protein content (%)	Oil Content (%)	Protein content (%)
T1.	28.4	19.51	28.5	18.64
T2.	29.4	20.01	29.2	19.56
T3.	30.3	21.73	30.2	20.56
T4.	32.6	23.05	32.2	23.85
T5.	31.9	22.04	31.4	22.89
T6.	32.6	22.57	32.8	23.45
T7.	32.2	22.16	32.3	21.57
T8.	32.4	22.75	31.7	23.39
T9.	33.3	23.54	33.1	24.56
T10.	31.3	22.78	31.4	23.45
T11.	30.3	21.95	30.2	20.47
T12.	32.6	22.57	32.8	23.45
T13.	31.7	21.36	31.1	21.86
T14.	31.8	21.54	31.7	21.85
T15.	32.8	23.04	32.1	23.86
C. D. at 5% level of significance	0.6	0.71	0.5	0.59

Leaves chlorophyll content

The amount of total chlorophyll first increased from 30th to 60th day of sowing and after that it decreased on 90th day of sowing (table 6). At 30th day of sowing, the total chlorophyll value ranged between 1.287 in T1 to 3.008 in T9 during 2016-17 and from 1.307 to 3.102 mg/gFW during 2017-18. It increased significantly from T1 (control 1) to T2 (control 2) to T3 (control3) with addition of RDF and further increases on inoculation of sulphur oxidizing bacterial isolates. The overall amount of total chlorophyll increases from 30 to 60 days of sowing.

The increment in chlorophyll content of mustard due to inoculation of sulphur oxidizing bacterial isolates may be explained due to increase in availability of sulphur. According to study of Mishra *et al*, (2010) [26], though S is not a constituent of chlorophyll, but required for its synthesis. Jat *et al*, 2012 [27] also explained a synergistic effect of S fertilizers on the formation of chlorophyll in mustard leaves at 60th day of sowing.

Table 6: Effect of SOB on total chlorophyll content of mustard leaves at different days of sowing

Treatments	Total Chl content (mg/gFW)					
	Year (2016-17)			Year (2017-18)		
	No. of days after sowing of crop					
	30	60	90	30	60	90
T1.	1.287	1.686	1.319	1.307	1.713	1.154
T2.	1.928	2.286	1.884	1.751	2.288	1.797
T3.	2.599	2.89	3.857	2.486	2.959	2.449
T4.	2.337	2.362	2.302	1.538	2.029	1.805
T5.	2.008	3.094	2.123	1.581	2.413	2.104
T6.	2.576	3.352	2.929	2.706	3.582	3.323
T7.	2.343	2.821	2.34	2.141	2.878	2.636
T8.	2.539	2.917	2.575	2.976	3.518	3.186
T9.	3.008	3.644	3.156	3.102	3.559	3.446
T10.	2.399	2.598	3.137	2.32	2.505	2.209
T11.	2.393	2.958	2.365	2.288	2.885	2.556
T12.	2.576	3.452	2.529	2.606	2.882	2.623
T13.	2.476	2.894	2.741	2.634	2.688	2.014
T14.	1.686	2.604	2.07	2.559	2.999	2.595
T15.	2.765	3.515	3.262	2.837	3.256	2.797

C.D. For Treatments (A) = 0.227

C.D. For Days (B) = 0.059

C.D. For Factors (A X B) = 0.394

Conclusion

Inoculations of sulphur oxidizing bacteria with mustard seeds

oxidized the reduced sulphur compounds and make them available to plants in sulphate form, which further result in improvement in plant growth parameters such as length, weight, no. of siliquae, seed weight, oil content and chlorophyll.

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