INFLUENCE OF SULPHUR OXIDIZING FUNGI ON GROWTH AND YIELD OF SOYBEAN

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Abstract:
Efficacy of two sulphur oxidizing fungal cultures viz., Trichoderma harzianum and Aspergillus niger individually and in combination with sulphur was evaluated in soybean crop in field trial at research farm of Plant Pathology Section, College of Agriculture, Nagpur, during kharif, 2012. The yield parameters viz., number of pods plant-1, grain yield and 1000 grains weight increased significantly due to inoculation of cultures under study with or without sulphur fertilizer. The treatment of T. harzianum and recommended dose of fertilizer (30:75:30 NPK kg ha-1) with sulphur (15 kg ha-1) had given maximum response in respect of number of pods plant-1, grain yield and 1000 grains weight. The number of pods plant-1, grain yield and 1000 grains weight were increased from 47.30 to 68.34, 823.00 to 1590.00 kg ha-1 and 111.21 to 127.43 g by this treatment respectively over uninoculated control. The inoculation of T. harzianum + Sulphur (15 kg ha-1) + RDF could increase available sulphur content in soil from 13.36 kg ha-1 in control to a maximum of 19.60 kg ha-1 and sulphur uptake ranging from 250.00 to 322.33 mg 100g-1 plant dry weight when compared with control.

Keywords:
Sulphur oxidizing fungi

Introduction:

Soybean [Glycine max (L.) Merrill] is well known oilseed as well as pulse crop which is grown in various countries. Pulses and oilseeds are important constituents of Indian diet and supply a major part of the protein requirement. Soybean supplies 20 per cent oil and 40 per cent protein. Sulphur is one of the seventeen essential nutrients and it is a structural component of amino acids, proteins, vitamins and enzymes and is essential to produce chlorophyll. Sulfur ranked equal to nitrogen for optimizing crop yield and quality. It enhances the efficiency of nitrogen for protein manufacture (Tucker, 1999). The major reservoir of sulphur in soil is an unavailable elemental and reduced form of sulphur. Plants generally utilize the oxidised state of sulphate (SO_4^{-2}) and therefore, it must first be oxidized before it can be used by crops. Most agricultural soils contain some micro organisms that are able to oxidize sulphur. The micro organisms are mainly responsible to make available sulphate from elemental or reduced forms of sulphur through its oxidation process with the intermediate formation of thiosulphate (S2O32–) and tetrathionate (S4O62–) in soils (Anandham and Sridar, 2004). Sulphur oxidizing fungi appear particularly useful for oxidation purpose since their spores or mycelia can be produced economically in large quantities and have good survival characteristics, both in the inoculants and in soil. Reports showed that combined inoculation of Thiobacillus thioxidans and Aspergillus niger, Trichoderma harzianum, Myrothecium cinctum, Aspergillus terreus with sulphur improved soybean grain yield and protein content (Shinde et al., 2004). Therefore, the study was undertaken to determine performance of sulphur oxidizing fungi on yield parameters of soybean, available sulphur in soil and on sulphur uptake.

Material and Method:

The sulphur oxidizing fungi were isolated from soil samples collected from selected spots of different oilseed crops i.e. sesame, mustard, linseed and soybean fields of Agronomy Section, College of Agriculture, Nagpur. These isolates were further purified and screened for sulphur oxidizing ability in potato dextrose agar medium and czapek dox medium respectively. From these, two isolates i.e. Aspergillus niger and Trichoderma harzianum were selected for further experiment. A field experiment was conducted in randomized block design with three replications during kharif, 2012. There were nine treatments comprising of two fungal cultures and two sulphur levels (Table 1). The recommended dose of NPK (30:75:30 kg ha-1) was applied in the form of DAP and MOP, sulphur in the form of elemental sulphur powder. The cultures were multiplied in modified sulphur medium (Wainwright, 1978) and when they attained 10-7 cfu ml-1 strength, each one was mixed in 1:2 (v/w) carrier. Then each of the cultures @ 250 g 10 kg-1 of JS-335 seed of soybean were treated. The yield parameters of soybean viz., number of pods plant-1, grain yield and 1000 grains weight were studied. Five plants from each net plot were uprooted at harvest and all the developed pods were plucked and counted to estimate number of pods plant-1. The available sulphur content in soil and sulphur uptake by plant was analyzed by turbidimetric method (Chopra and Kanwar, 1980).

Result and Discussion:

The influence of sulphur oxiding fungal cultures with and without sulphur on the growth and yield of soybean plant was found to be significant. Particularly, inoculation of Trichoderma harzianum culture along with sulphur (15 kg ha-1) showed significantly superior performance over all other treatments under study. It is revealed from the data that number of pods plant-1 were increased due to the cultures under study with and without sulphur over control. The maximum number of pods (68.34 plant-1) were recorded in the treatment Trichoderma harzianum culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) and it was significantly superior over rest of the treatments and control. Next to this treatment application of Aspergillus niger culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) significantly better than the remaining treatments except Trichoderma harzianum + recommended dose of fertilizer + Sulphur (10 kg ha-1). The remaining less effective treatments were viz., Aspergillus niger + recommended dose of fertilizer + Sulphur (10 kg ha-1), Sulphur (20 kg ha-1) + recommended dose of fertilizer, Trichoderma harzianum + recommended dose of fertilizer, Aspergillus niger + recommended dose of fertilizer, recommended dose of fertilizer in a descending manner. The minimum number of pods (47.30 plant-1) was recorded in uninoculated control. Similar trend of results was noticed in respect of grain yield. The maximum grain yield (1590 kg ha-1) was obtained in Trichoderma harzianum culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) treatment and minimum weight of 1000 grains per treatment was also noticed to be the maximum (127.43 g) with the treatment of Trichoderma harzianum culture and recommended dose of fertilizer along with sulphur (15 kg ha-1). Shinde et al. (2004) in soybean reported that the number of pods, grain yield and 1000 grains weight were significantly increased due to combined inoculation of Thiobacillus thioxidans and Aspergillus niger, Trichoderma harzianum, Myrothecium cinctum, Aspergillus terreus with sulphur. These results were conformed during the present studies so far as T. harzianum and A. niger are concerned. Shinde et al. (2000) in cotton reported that the number of picked bolls and seed yield were significantly increased due to combined inoculation of Scolecobasidium constrictum, Myrothecium cinctum, Aspergillus terreus, Thiobacillus thioparus and T. thiooxidans. The available sulphur content in soil increased due to inoculation of sulphur oxidizing fungi in conjunction with sulphur application. The maximum available sulphur (19.60 kg ha-1) was observed in the treatment of Trichoderma harzianum culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) and it was at par with Aspergillus niger culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) treatment. Next to these treatments the treatments were applicaton of Trichoderma harzianum + recommended dose of fertilizer + Sulphur (10 kg ha-1) and Aspergillus niger + recommended dose of fertilizer + Sulphur (10 kg ha-1). The sulphur treatment alone also helped in increasing available sulphur level in soil from 13.36 kg ha-1 to 17.40 kg ha-1. The less effective treatments were viz., Trichoderma harzianum + recommended dose of fertilizer and Aspergillus niger + recommended dose of fertilizer. The minimum available sulphur (13.36 kg ha-1) was recorded in uninoculated control. Kadam et al. (2004) and Shinde et al. (2010) in soybean found increased available sulphur content in soil due to combined inoculation of Thiobacillus thioxidans and Aspergillus niger, Trichoderma harzianum, Myrothecium cinctum, Aspergillus terreus with sulphur. The data on sulphur uptake by soybean crop revealed that two fungal cultures with or without sulphur treatment increased sulphur uptake ranging from 250.00 to 322.33 mg 100-1 g dry weight of plant and maximum was recorded in Trichoderma harzianum culture and recommended dose of fertilizer along with sulphur (15 kg ha-1) and it was significantly superior over rest of the treatments. Next to this treatment the treatments were application of Aspergillus niger culture and recommended dose of fertilizer along with sulphur (15 kg ha-1), Trichoderma harzianum + recommended dose of fertilizer + Sulphur (10 kg ha-1) and Aspergillus niger + recommended dose of fertilizer + Sulphur (10 kg ha-1). The sulphur uptake also increased due to sulphur application alone from 250.00 to 262.00 mg 100-1 g dry weight of plant. The less effective treatments were viz., Trichoderma harzianum + recommended dose of fertilizer and Aspergillus niger + recommended dose of fertilizer. The minimum sulphur uptake (250.00 mg 100-1 g) was recorded in uninoculated control. Shinde et al. (2004) reported increase in sulphur uptake in soybean due to combined inoculation of Thiobacillus thioxidans and Aspergillus niger, Trichoderma harzianum, Myrothecium cinctum, Aspergillus terreus with sulphur Earlier, Shinde et al. (2000) recorded similar results in groundnut and cotton crops. The significant effect of different cultures on the performance of soybean may be attributed to the increased rate of microbial oxidation of elemental sulphur to sulphate (SO4) sulphur in soil which in turn might have increased the sulphur uptake and thereby the yield.

Table 1: Enumeration of Bacteria, Actinomycetes and Fugal population associated with different samples collected from various sites

LOCATION	SITES	SAMPLES	Microbial count (×10^4 cfu / g soil)			Actinomycetes count (×10^3cfu / g soil)	Fugal count (×10^3 cfu / g soil)
			Nutrient Agar (NA)	Pikovaskaya's agar (PVK)	Soil extract media (SEM)		
SOLAN	NAUNI	Rural compost	121.00	83.00	106.13	67.11	12.13
		Mushroom compost	103.34	72.33	104.03	54.66	10.45
		Soil	126.34	84.33	116.01	55.12	17.11
	CHAIL	Rural compost	111.31	74.00	68.02	58.66	18.01
		Mushroom compost	90.64	53.67	62.00	33.15	13.21
		Soil	113.00	77.34	84.66	37.33	20.21
	CHAMBAGHAT	Rural compost	97.30	89.00	98.33	45.62	21.28
		Mushroom compost	76.33	54.00	72.03	28.12	15.11
		Soil	80.34	61.01	82.31	31.22	19.42
	SAPROON	Rural compost	78.03	59.10	100.13	51.00	22.31
		Soil	98.89	63.11	103.88	42.12	17.26
	BAROG	Mushroom compost	121.76	78.33	111.65	34.27	11.34
	SALOGRA	Municipal solid waste	87.12	34.12	45.13	21.21	8.34
	DHARAMPUR	Rural compost	132.44	67.88	167.43	56.18	24.81
		Soil	104.54	44.32	93.24	39.23	15.23
SHIMLA	SHOGI	Rural compost	144.15	66.11	102.44	51.22	19.44
		Soil	98.66	76.65	113.77	44.31	17.63
	LAALPANI	Municipal solid waste	77.22	34.62	55.53	19.34	9.45

Table 2: Morphological and Biochemical characterization of the bacterial isolates

Isolates	Morphology			Simple staining	Grams staining
	Form	Elevation	Margin		
DB1	Irregular	Raised	Erose	Rods	+
DB2	circular	Raised	Entire	Rods	+
DB3	Irregular	Flat	Undulate	Rods	+
DB4	Irregular	Flat	Erose	Rods	+
DB5	circular	Flat	Entire	Rods	+
DB6	circular	Raised	Undulate	Rods	+
DB7	Irregular	Convex	Entire	Rods	+
DB8	circular	Convex	Entire	Rods	+
DB9	circular	Flat	Entire	Rods	+
DB10	circular	Flat	Undulate	Rods	+

DB11	Irregular	Raised	Undulate	Rods	-
DB12	circular	Convex	Entire	Rods	-
DB13	circular	Convex	Entire	Rods	-
DB14	Irregular	Flat	Erose	Rods	+
DB15	circular	Flat	Entire	Rods	+
DB16	Irregular	Raised	Entire	Rods	-
DB17	circular	Convex	Entire	Rods	+
DB18	circular	Flat	Undulate	Rods	+
DB19	circular	Flat	Undulate	Rods	-
DB20	Irregular	Flat	Erose	Rods	+
DB21	circular	Convex	Entire	Rods	+
DB22	circular	Raised	Undulate	Rods	-
DB23	circular	Convex	Entire	Rods	+
DB24	Irregular	Raised	Erose	Rods	+
DB25	Irregular	Flat	Undulate	Rods	+
DB26	Irregular	Flat	Undulate	Rods	+

Table 3: Biochemical characteristics of the bacterial isolates

Isolates	Catalase test	Oxidase test	MR test	VP test	Carbohydrate fermentation	Casein hydrolysis	Gelatin hydrolysis	Hydrogen sulphide production
DB1	+	-	-	+	+	+	+	-
DB2	+	-	-	+	+	-	+	-
DB3	+	-	-	+	+	-	+	-
DB4	+	-	-	+	+	-	+	-
DB5	+	-	-	+	+	+	+	-
DB6	+	-	-	+	+	+	-	-
DB7	+	-	-	+	+	+	+	-
DB8	+	-	-	+	+	+	+	-
DB9	+	-	-	+	+	-	+	-
DB10	+	-	-	+	+	-	+	-
DB11	-	-	-	+	+	-	-	-
DB12	-	-	-	+	+	+	+	-
DB13	+	-	-	+	+	+	+	-
DB14	+	-	-	+	+	+	-	-
DB15	+	-	-	+	+	+	+	-
DB16	+	-	-	+	+	-	+	-
DB17	+	-	-	+	-	+	-	-
DB18	+	-	-	+	+	+	+	-
DB19	+	-	-	+	+	+	+	-
DB20	+	-	-	+	+	-	+	-
DB21	+	-	-	+	+	+	+	-
DB22	+	-	-	+	-	-	-	-
DB23	+	-	-	+	+	+	-	-
DB24	+	-	-	+	+	+	+	-
DB25	+	-	-	+	+	-	+	-
DB26	+	-	-	+	+	-	+	-

Table 4: Screening of selected bacterial isolates for multifarious plant growth promoting traits

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic	Antagonism against	
						Fusarium oxysporum	*Pythium ultimum*
DB1	+++	++	+++	++	+	+	+
DB2	++	++	-	++	-	-	-
DB3	+	+	+	-	+	+	-
DB4	++	-	+++	+	-	-	-
DB5	++	+++	-	+	+	+	-
DB6	-	-	++	+++	-	+	-
DB7	+++	+	+	++	-	-	-
DB8	-	++	++	-	-	-	-
DB9	-	-	+	+	+	+	-
DB10	++	+	-	+++	-	+	-
DB11	++	+	+	-	+	-	-
DB12	-	++	++	+	-	+	-
DB13	+	+	-	+	-	+	-
DB14	+++	+++	+	+++	-	-	-
DB15	-	-	-	++	-	+	-
DB16	-	+++	-	++	+	-	+
DB17	++	-	-	+	-	-	-
DB18	++	+++	+	-	-	-	+
DB19	-	-	++	-	-	+	-
DB20	+++	-	-	++	+	-	+
DB21	-	-	+++	+++	-	+	-
DB22	++	+++	+	+	-	-	+
DB23	++	-	-	-	-	-	+
DB24	+	++	-	-	-	-	+
DB25	+++	++	-	++	-	-	-
DB26	+	++	++	+++	-	+	-

*values ranging from 55-75 % (++), ≤ 55% (+), ≥ 75 % (+++), no activity (-) ; ** values ranging from 20-25 (++), ≤ 20 (+), ≥ 25 (+++), no activity (-) ; ***values ranging from 50-80 % (++), ≤ 50% (+), ≥ 80 % (+++), (-) no activity ; # Values ranging from 20-35 (++), ≤ 20 (+), ≥ 35 (+++), no activity (-)

Table 5: Screening of selected actinomycelial isolates for multifarious plant growth promoting traits

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic
DA1	+++	-	-	++	+
DA 2	++	+++	-	+	-
DA 3	+++	-	-	-	-
DA 4	++	+	++	+	-
DA 5	++	+++	+	-	-
DA 6	+	-	++	+++	-
DA 7	+++	++	++	+++	+
DA 8	-	++	++	-	-
DA 9	+++	-	+	+	-
DA 10	++	+	-	+++	-
DA 11	+++	++	-	-	-
DA 12	-	-	+++	+++	-
DA 13	+++	++	-	+++	-
DA 14	-	-	+	++	-
DA 15	-	-	-	++	+
DA 16	-	++	-	++	-
DA 17	+++	++	+++	++	-

Table 6: Screening of selected fungal isolates for multifarious plant growth promoting traits

Isolates	P solubilisation	Growth on N free medium	HCN production	Siderophore production	Cellulolytic
DF1	++	++	+	++	-
DF2	+++	+	-	++	-
DF3	+	+	+	-	+
DF4	+++	-	+++	+	-
DF5	++	+++	+	-	-
DF6	+	-	++	+++	-
DF7	++	-	+	++	+
DF8	-	++	++	-	-
DF9	+++	-	+	+	+
DF10	++	+	-	+++	-
DF11	+++	++	-	-	-
DF12	++	++	+	++	+
DF13	-	++	++	+	+
DF14	+++	++	++	+++	+
DF15	++	-	-	++	-
DF16	-	++	-	++	+
DF17	+++	++	-	++	-
DF18	++	++	+	+	-
DF19	-	-	++	-	-
DF20	+++	-	-	++	+
DF21	-	-	+++	+++	-
DF22	++	+++	-	+	-
DF23	+++	-	-	-	-

Table 7: Dual culture compatibility assessment amongst eleven bacterial isolates of tomato seedlings

Bacterial Isolates	Synergism with actinomycetes isolates	Synergism with fungal isolates	Compatibility
DB1	DA7	DF14	+
DB1	DA17	DF12	-
DB1	**DA7**	**DF12**	-
DB1	DA17	DF14	-
DB 22	DA7	DF14	-
DB 22	DA17	DF12	-
DB 22	DA7	DF12	-
DB 22	DA17	DF14	+

Table 7: Dual culture compatibility assessment amongst eleven bacterial isolates of tomato seedlings

Conclusion:

The influence of sulphur oxiding fungal cultures with and without sulphur on the growth and yield of soybean plant was found to be significant. Particularly, inoculation of Trichoderma harzianum culture along with sulphur (15 kg ha-1) showed significantly superior performance over all other treatments under study.

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