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Research Article

EFFECT OF ZINC SOLUBILIZING MICROORGANISMS IN ENHANCING ENZYME ACTIVITY AND NUTRIENT AVAILABILITY IN GROUNDNUT GROWN ON VERTISOL

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Abstract- The experiment was conducted on zinc deficient soil at farmer's field in Kehal village, Taluka Jintoor, Dist. Parbhani, Maharashtra during summer season of 2014 to assess the ability of zinc solubilizing microorganisms to enhance the zinc availability and enzyme activity in soil. Bioinoculants used such as *Burkholderia cepacia, Burkholderia cenocepacia, Pseudomonas fluorescens, Pseudomonas striata, Trichoderma viride, Trichoderma harzianum* and *Bacillus megaterium*. Tenth day after sowing 24 hrs old fresh culture of microbial isolates was inoculated at the rate 10 ml. Activity of alkaline phosphatase and acid phosphatase was noted significantly highest in treatment RDF + *Rhizobium* + *Bacillus megaterium* and respectively. However, dehydrogense activity and nutrient availability was found significantly highest in treatment whereas, significantly highest periodical Zn content in soil was found with *Pseudomonas striata* followed by *Pseudomonas fluorescens* and *Trichoderma viride*, respectively.

Keywords- Enzyme activity, Groundnut, Nutrient availability, Zinc solubilizing microorganism

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop and food grain legume. Groundnut cultivation occurs in 108 countries around the world, which is grown in all tropical and subtropical countries. It is a valuable cash crop planted by millions of small farmers because of its economic and nutritional value. Oil seed crops have been the backbone of agricultural economy of India and the world. It is originated from Brazil in South America. The word *Arachis* is a Latin word meaning 'legume' and *hypogaea* meaning 'below ground' which refers to the geocarpic nature of pod formation. It is a predominant oilseed crop, of which approximately 53 per cent of total global production is crushed for high quality edible oil, 32 per cent for confectionary consumption, and the remaining 15 per cent is used for food and seed production. Groundnut is good source of dietary proteins, minerals and vitamins. It is also known as poor man's almond. Its calorific value is 349 per hundred grams of seed. The residual oil cake contains 7 to 8 per cent of N, 1.5 per cent of P_2O_5 and 1.20 per cent of K_2O and hence it is used as a fertilizer. The cake is also used for making of reconstituted food because of its high protein content. Being a legume crop it can fix atmospheric nitrogen and thereby improves soil fertility [13]. India ranks first in respect of area and second in respect of production after China, contributing about 40 per cent of world's production. According to 4th advanced estimates in India area, production and yield of groundnut during 2013-14 was 4.19 million hectares, 5.62 million tones and 1341 kg ha^{-1} and in Maharashtra 0.29 million hectares, 0.36 million tones and 1341 kg ha^{-1} respectively. In Maharashtra, total yield of oilseeds during the year 2012-13 was 1394 kg ha^{-1} and during 2013-14 reduced to 1228 kg ha^{-1} and yield of *kharif* and *rabi* oilseeds during 2013-14 was 1271 kg ha^{-1} and 720 kg ha^{-1} [2]. Zinc is one of such elements, which plays significant role in various enzymatic and physiological activities in the plant system. It is well reflected with increased area being deficient in nutrients like sulphur and zinc or showing hidden hunger of these. At several places normal yield of crops could not be achieved despite judicious use of NPK fertilizers due to deficiency of micronutrients in soil, in general, that of Zn in particular. Numerous microorganisms especially those associated with roots, have the ability to increase plant growth and productivity. In soil, both macro and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by the soil pH and microflora and that ultimately affects their accessibility to plant roots for absorption [1]. Zinc is an essential micronutrient required for growth and metabolism of microorganisms and plants. Zinc is present in the enzyme system as co-factor and metal activator of many enzymes. The role of zinc in the nutrition and physiology of both eukaryotic and prokaryotic organisms, especially its importance for activity of many enzymes is widely studied. Many bacterial enzymes contain zinc in the active centre or in a structurally important site. Bacteria can contribute to metal immobilization by several processes such as precipitation and adsorption [3]. Zinc occurs in soil as sphalerite, olivine, hornblende, augite and biotite. However, availability of Zn from these sources is guided by many factors among which biochemical actions of rhizo micro-organisms play an important role in converting such unavailable sources into available ones. Exogenous application of soluble zinc sources similar to fertilizer application has been advocated to various crops. This causes transformation of about 96-99 per cent of applied available zinc to various unavailable forms. The zinc thus made unavailable can be reverted back to available form by inoculating a bacterial strain capable of solubilizing it [14]. A number of microorganisms are considered as challenging agents for agriculture to promote better nutrient uptake and availability for plant use particularly zinc mobilizing and acidifying cultures may help to increase the zinc and other nutrient availability for the crops like Groundnut. Since the information on groundnut to inoculation with different zinc solubilizing (ZnS) microbial inoculants is meagre. Keeping these points in consideration, field trial was conducted to enhance the enzyme properties and nutrient availability in soil using ZnS microbial cultures in groundnut grown on Vertisol.

Materials and Methods

The experiment was conducted at farmer's field in Kehal village, Tq. Jintoor, Dist. Parbhani on Vertisol during summer season of 2014-15 on groundnut. The soil was clayey in texture, moderately alkaline in reaction, medium in available nitrogen, phosphorus and sufficient in available potassium, iron, sulphur and low in zinc. The initial soil pH was 8.0, EC- 0.28 dSm^{-1}, organic carbon- 4.86 g kg^{-1}, $CaCO_3$- 37.0 g kg^{-1} available N – 133.26 kg ha^{-1}, P_2O_5 – 14.0 kg ha^{-1}, K_2O - 530 kg ha^{-1} and S – 13.90 mg kg^{-1}. The initial micron utrient status were DTPA Cu 2.33 mg kg^{-1}, Mn 6.30 mg kg^{-1}, zinc 0.53 mg kg^{-1}, iron 5.18 mg kg^{-1}. The soil was clayey in texture, low in organic carbon, available nitrogen, medium in phosphorus, sufficient in potassium and low in sulphur. As among the micronutrient status Cu, Fe and Mn were above the critical limits but Zn content in experimental soil was found to be deficient. The treatments comprising inoculation with liquid inoculants of *Bradyrhizobium* and *Bacillus megaterium* (PSB) for Groundnut seed in alone and in combinations. Total eight treatments of bioinoculants were replicated three times in RBD. The experiment consists of 8 treatments of laboratory tested P and Zn solubilizers T_1 RDF+ *Rhizobium*; T_2 RDF + *Rhizobium* + *Bacillus megaterium*; T_3 RDF + *Rhizobium* + *Burkholderia cepacia*; T_4 RDF + *Rhizobium* + *Burkholderia cenocepacia*; T_5 RDF + *Rhizobium* +*Pseudomonas fluorescens;* T_6 RDF + *Rhizobium*+ *Pseudomonas striata;* T_7 RDF + *Rhizobium* + *Trichoderma viride;* T_8 RDF + *Rhizobium* + *Trichoderma* harzianum. Seed treatment was done before sowing with liquid bioinoculants each @ 50 ml 10 kg^{-1} seed. The crop was raised following recommended agronomic practices. The recommended dose of chemical fertilizers were applied @ 30:60:30 NPK kg ha^{-1} at the time of sowing. Intercultural operations like thinning, weeding, spraying of insecticides, fertilizer application and schedule of irrigation for groundnut crop was carefully followed. The crop variety used was TAG 37A. The soil samples were collected after harvest of groundnut for analysis of enzyme activity, chemical properties and available nutrient status as per standard procedures. The data obtained was statistically analyzed and appropriately interpreted as per the methods described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme, (1985) [11].

Results and Discussion

The data noted in [Table-1] indicates that the enzymatic activity in soil after harvest of groundnut crop was influenced by different ZnS microbial inoculants. Activity of alkaline phosphatase was noted significantly highest in treatment T2 RDF + Rhizobium + Bacillus megaterium (76.38 µg g-1) and T6 was noted at par with T2 treatment. Enzymatic activity of acid phosphatase was also noted significantly highest in treatment T2 (43.07 µg g-1) and at par with T6 (42.87µg g-1). However, dehydrogenase activity was found significantly highest in T6 treatment (57.75µg g-1). The treatment T4 (RDF + Rhizobium + Burkholderia cenocepacia) and T5 (RDF + Rhizobium + Pseudomonas fluorescens) was noted at par with treatment T6 (RDF + Rhizobium + Pseudomonas striata). Lowest enzymatic activity was observed in treatment T1 (RDF + Rhizobium). These results are corroborate with the findings of Nihorimbere et al. (2011) [9] who reported more microbial activities increased the dehydrogenase activity in rhizosphere due to more availability of food material for its growth. Whereas, Activities of both acid and alkaline phosphatases were significantly improved over control levels in the rhizosphere upon inoculation. This might be due to increased microbial and root activities as previously reported by Kaleeswari, (2007) [8]. Increase in dehydrogenase and phosphates activity in soil due to inoculation were also noticed by Panwar et al., (2003) [12] which might be due to increased microbial population in soil because of the presence of greater organic substances as a result of rhizo-deposition and leaf fall. The results presented in [Table-2] regarding changes in soil pH, EC and $CaCO_3$ after harvest of groundnut indicates non-significant results. However, organic carbon was recorded significantly highest in treatment T6 (5.78 g kg-1) with inoculation of RDF+ Rhizobium + Pseudomonas striata and treatment T2 (RDF+ Rhizobium+ Bacillus megaterium), T5 (RDF+ Rhizobium+ Pseudomonas fluorescens) and T8 (RDF+ Rhizobium+ Trichoderma harzianum) was noted at par with treatment T6 and lowest organic carbon was noted in treatment T1.

Table-1 *Effect of zinc solubilizing microorganisms on enzyme activities in soil after harvest of summer groundnut*

Tr. no.	Treatment	Enzyme activities in soil after harvest of groundnut (µg g^{-1})		
		Alkaline Phosphatase	Acid Phosphatase	Dehydrogenase
T_1	RDF+*Rhizobium*	56.03	35.42	49.81
T_2	T1+*Bacillus megaterium*	76.38	43.07	54.96
T_3	T1+*Burkholderia cepacia*	65.33	38.85	53.03
T_4	T1+*Burkholderia cenocepacia*	69.25	38.66	53.75
T_5	T1+*Pseudomonas fluorescens*	72.76	39.67	57.43
T_6	T1+*Pseudomonas striata*	75.58	42.87	57.75
T_7	T1+*Trichoderma viride*	75.18	39.50	53.38
T_8	T1+*Trichoderma harzianum*	66.65	39.36	54.52
	S.E.±	0.16	0.19	0.10
	C.D. at 5 %	0.51	0.58	0.32
	C.V. %	4.1	8.3	3.4
	Initial	60.60	33.95	38.00

Table-2 *Effect of zinc solubilizing microorganisms on chemical properties of soil after harvest of summer groundnut*

Sr. No.	Treatment	pH(1:2.5)	EC (dSm^{-1})	$CaCO_3$(%)	Organic carbon (g kg^{-1})
T_1	RDF+*Rhizobium*	7.98	0.28	35.00	5.01
T_2	T1+*Bacillus megaterium*	7.97	0.25	36.10	5.69
T_3	T1+*Burkholderia cepacia*	7.61	0.26	36.20	5.37
T_4	T1+*Burkholderia cenocepacia*	7.66	0.26	36.00	5.33
T_5	T1+*Pseudomonas fluorescens*	7.57	0.27	36.10	5.75
T_6	T1+*Pseudomonas striata*	7.60	0.25	35.50	5.78
T_7	T1+*Trichoderma viride*	7.68	0.25	36.10	5.57
T_8	T1+*Trichoderma harzianum*	7.69	0.23	36.40	5.60
	S.E.±	0.20	0.02	0.43	0.12
	C.D. at 5 %	NS	NS	NS	0.36
	C.V. %	4.69	14.1	2.10	3.81
	Initial	8.00	0.28	37.00	4.86

The data presented in [Table-3] indicates that significant increase in nutrient availability in soil after harvest of groundnut crop. Significantly highest values of soil available N, K2O and S after harvest of groundnut were recorded in treatment T6 (171.3, 628.4 kg ha-1 and 16.27 mg kg-1) respectively receiving RDF + Rhizobium + Pseudomonas striata and treatment T5 (RDF + Rhizobium +Pseudomonas fluorescens) and T8 (RDF + Rhizobium + Trichoderma harzianum) was found at par with T6 treatment. However coming to value of P, it was found maximum in T2 treatment (18.22 kg ha-1) i.e with the inoculation of Rhizobium + Bacillus megaterium and treatment T3 (17.32 kg ha -1), T5 (17.09 kg ha -1) and T8 (16.58 kg ha-1) were noted at par with T2 treatment. These results are in accordance with the reports of Singh and Yadav (2008)[16] who noticed that co-inoculation of Rhizobium + PSB significantly increased nitrogen fixation and increased soil available nitrogen as compared to initial value. Further, Bodkhe et al. (2014) [4] also found that more availability of major nutrients in dual inoculated plots as compare to inoculation of Rhizobium or PSB alone. Similar findings were also reported by Dubey et al. (1997) [6]. Whereas, Nirmal et al., (2006) [10] reported that the dual inoculation of Rhizobium and PSB resulted more availability of soil N and P because of their associative effect plus solubilization from non exchangeable to labile form. Similarly, Trivedi et al. (1997) [17, 18] found that application of PSB there was greater mobilization of insoluble phosphorus along with enhanced transport of soil nutrients within the plant system. The results presented in [Table-4] revealed that the available micronutrients in soil after harvest of groundnut crop were also influenced significantly with the microbial treatments. DTPA Zn and Fe were noted maximum in T6 treatment (0.68 and 5.72 mg kg-1) receiving inoculation of Rhizobium + Pseudomonas striata and treatment T3 (RDF + Rhizobium + Burkholderia cepacia); T4 (RDF + Rhizobium + Burkholderia cenocepacia), T5 (RDF + Rhizobium + Pseudomonas fluorescens) and T7 (RDF + Rhizobium + Trichoderma viride) were found at par with the T6 treatment. Regarding, DTPA Mn and Cu, these were also noted maximum in T6 (6.71 and 2.75 mg kg-1) receiving inoculation of Rhizobium + Pseudomonas striata along with RDF and in case of Mn, T5 (RDF + Rhizobium + Pseudomonas fluorescens) and T7 (RDF + Rhizobium + Trichoderma viride) was found at par with T6. In Cu, treatment T4 (RDF + Rhizobium + Burkholderia cenocepacia) and T5 (RDF + Rhizobium + Pseudomonas fluorescens) was found at par with T6 and the lowest was noted in treatment T1. Our results are also concur with the reports of Saravanan et al., (2007) [15], they also studied the solubilization of insoluble zinc compound by G. diazotrophicus and observed that the zinc solubilization by plate assays revealed effective solubilization of insoluble zinc compounds by G. diazotrophicus strains. These might to be due to the production of protons and organic acids, which are considered as the significant mechanism affecting the solubilization of metal compounds through excretion of other metabolites, siderophore and the like also contribute to the solubilizing process. Further, Di Simine et al.,(1998) [5] considered that 5-ketogluconic acid was the major organic acid produced in the intermediary of solubilization while in Pseudomonas it was 2-ketogluconic acid that mediated solubilization process. Furthermore, Fasim et al. (2002) [7] studied the solubilization of zinc salts by a bacterium isolated from the air environment of tannery and results revealed that the strain of Pseudomonas aeruginosa solubilized both ZnO and $Zn_3(PO4)_2$ when incorporated in solid and liquid growth medium.

Table-3 *Effect of zinc solubilizing microorganisms on major nutrient availability in soil after harvest of groundnut*

Tr.no.	Treatment	Available N (kg ha^{-1})	Available P_2O_5 (kg ha $^{-1}$)	Available K_2O (kg ha $^{-1}$)	Available S (mg kg $^{-1}$)
T_1	RDF+*Rhizobium*	145.37	14.61	532.57	14.45
T_2	T1+*Bacillus megaterium*	147.04	18.22	538.67	14.61
T_3	T1+*Burkholderia cepacia*	151.71	17.32	555.79	14.39
T_4	T1+*Burkholderia cenocepacia*	146.32	15.80	573.33	15.21
T_5	T1+*Pseudomonas fluorescens*	156.44	17.09	589.33	16.15
T_6	T1+*Pseudomonas striata*	171.53	16.07	628.40	16.27
T_7	T1+*Trichoderma viride*	124.58	16.03	489.59	12.48
T_8	T1+*Trichoderma harzianum*	153.87	16.58	588.85	14.84
	S.E.±	7.54	0.64	21.41	0.58
	C.D. at 5 %	22.88	1.97	64.95	1.77
	C.V. %	8.73	6.82	6.60	6.84
	Initial	133.26	14.00	530.00	13.90

Table-4 *Effect of zinc solubilizing microorganisms on DTPA extractable micronutrient availability in soil after harvest of groundnut*

Tr.no.	Treatment	Zn (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
T_1	RDF+*Rhizobium*	0.60	5.20	6.37	2.47
T_2	T1+*Bacillus megaterium*	0.57	5.25	6.34	2.54
T_3	T1+*Burkholderia cepacia*	0.62	5.42	5.61	2.55
T_4	T1+*Burkholderia cenocepacia*	0.62	5.22	6.26	2.73
T_5	T1+*Pseudomonas fluorescens*	0.66	5.59	6.47	2.74
T_6	T1+*Pseudomonas striata*	0.68	5.72	6.71	2.75
T_7	T1+*Trichoderma viride*	0.64	4.43	6.44	2.60
T_8	T1+*Trichoderma harzianum*	0.56	4.45	6.26	2.51
	S.E.±	0.02	0.14	0.16	0.06
	C.D. at 5 %	0.06	0.43	0.49	0.21
	C.V. %	5.68	4.81	4.44	4.64
	Initial	0.53	5.18	6.30	2.33

Conclusion

The enzyme activities in soil after harvest of groundnut crop were improved with the inoculation of zinc solubilizers. highest alkaline and acid enzyme activity and P availability were noticed with RDF + Rhizobium and Bacillus megaterium. However, dehydrogenase activity nutrient availability and was found maximum in RDF + Rhizobium + Pseudomonas striata.

Conflict of Interest: None declared

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