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Research Paper

Quantifying response of *azospirillum* and phosphate solubilizing bacteria liquid inoculants seed treatment on productivity and profitability of *kharif* grain sorghum (*Sorghum bicolor*)

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ABSTRACT

A field study was carried out during 2017–19 at Sorghum Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra to assess the effect of liquid bio-fertilizers inoculation over existing powder form inoculants on *kharif* grain sorghum [*Sorghum bicolor* (L.) Moench]. The experiment comprised of 6 treatments, viz. no bio-fertilizer control i.e. with RDF alone (T_1); RDF + *Azo.* (*Azospirillum)* + PSB @50 g each/kg seed (T_2); RDF + *Azo.* + PSB @2 ml each/kg seed (T_3); RDF + *Azo.* @2 ml and PSB @4 ml/kg seed (T_4); RDF + *Azo.* @4 ml + PSB @2 ml/kg seed (T_5); and RDF + *Azo.* + PSB @4 ml each/kg seed (T_6) conducted in randomized block design (RBD) with 4 replications. Pooled data revealed that seed inoculation with *Azo.* + PSB @2 ml each/kg seed (T_3) has improved the sorghum biological, grain yield and net returns 21.13, 19.83 and 17.92% over their powder form of inoculants i.e. T_2 (14.71 t/ha, 3.56 t/ha and 73,116). These enhancements in biomass, grain yields and net income was owing to liquid bio-fertilizer seed treatment. Liquid-based inoculants were found to boost growth and yield parameters, which was ascribed to the concomitant increase in plant height and number of grains/panicle, respectively. Increase in quantity of liquid bio-fertilizers beyond 2 ml/kg seed proved not-rewarding from both productivity and profitability point of view. Sorghum seed inoculation with liquid bio-fertilizers @2 ml each/kg seed proved to be a potential substitute for existing powder form without any additional cost.

Key words: Azospirillum, Bio-fertilizer, Liquid Formulation, PSB, RDF, Sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] is the second most important coarse cereal staple crop of India after pearl millet. It is cultivated during both rainy (*kharif)* and winter (*rabi*) seasons on 4.80 million ha area producing 4.60 million tonnes of grain at an average productivity of 958 kg/ha in 2021–22 (DES, 2022). Though, substantial improvements in productivity of this crop have been made in the past two decades (764 in 2000 to 958 kg/ha in 2022) through development of high yielding varieties and hybrids, it is far below the potential yield of 3.13 t/ha (Murthy *et al.,* 2009). It's rainfed cultivation is leading to productivity variations depending on the rain fall received and thus has been at its lowest during 2015–16 (697 kg/ha). However, vast differences lie in the quantity of fertilizers used under rainfed sorghum as compared to its irrigated crop (FAO, 2005). Low use of fertilizers in rainfed sorghum is done to avoid unproductive use of moisture for biomass production without improving grain yields. In this context, use of nitrogen fixing (*Azospirillum*) and phosphorous solubilizing (especially bacteria) bio-fertilizers assume prominence as an alternative or additional source to chemical fertilizers. Worldwide studies for 20 years on *Azospirillum* inoculation indicated a 60–70% success with 3–50% improvements in grain yields of crops on account of N fixation and associated root growth stimulation (Bashan and Bashan, 2005) and phyto-hormonones synthesis (Cassan *et al.,* 2011). Phosphorus, the second most important fertilizer nutrient that upon application to soil as fertilizers gets fixed with di-calcium phosphate dehydrate and becomes unavailable to plants (Zaidi *et al.,* 2009). Application of phosphorous solubilizing microbes (PSM) especially bacteria (PSB) was found to enhance the availability of this fixed P from soil for crop uptake (Sharma *et al*., 2013) that ultimately leads to significant sorghum

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crop yield enhancements. Significant response to combined inoculation of *Azospirillum brasilense* and PSB (*Pseudomonas striata* or *Bacillus polymyxa*) along with NP fertilizers as compared to either bio-fertilizers of fertilizers alone indicated the need for integrated sorghum nutrition. *Azospirillum* alone and in combination with PSB were reported to increase yield of sorghum substantially (Patidar and Mali, 2004). The carrier-based bio-fertilizers (*Azospirillum* and PSB) were found to have low efficacy under moisture limiting environments and are subjected to contamination, have low viable counts and poor shelf-life. In this context, liquid formulation-based bio-fertilizer inoculants with long shelf-life and ability to tolerate moisture, salinity etc. stresses have been evolved (Pindi and Satyanarayana, 2012). Studies have indicated the superiority of liquid based *Azospirillum* + *Bacillus* + *Pseudomonas* cultures for maize crop (Geeta *et al*., 2017). However, their impact assessment studies on *kharif* sorghum are meagre. Hence, a study was framed under All India Coordinated Sorghum Improvement Project (Agronomy) to ascertain the impact of liquid based *Azospirillum (Azo.)* + Phosphorus Solubilizing Bacteria (PSB) inoculant on *kharif* grain sorghum as compared to its traditional carrier-based inoculants.

MATERIALS AND METHODS

Field studies were carried out during 2017–19 at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (23°43' N and 77°64' E with an altitude of 281 m amsl), Maharashtra. The experiment consisted of 6 treatments, viz. no biofertilizer control i.e. with RDF alone (T_1); RDF + *Azo.* (*Azospirillum*) + PSB @50 g each/kg seed (T_2); RDF + *Azo.* + PSB @2 ml each/kg seed (T_3); RDF + *Azo.* @2 ml and PSB @4 ml/kg seed (T_4); RDF + *Azo.* @4 ml + PSB @2 ml/kg seed (T_5); and RDF + *Azo.* + PSB @4 ml each/kg seed (T_6) conducted in randomized block design (RBD) with 4 replications. The liquid bio-fertilizers were sourced from the production laboratory at University of Agricultural Sciences, Dharwad, Karnataka. Sorghum variety CSH 16 was sown on 1st July, 3rd July and 30th June during 2017, 2018 and 2019, respectively. The experimental clayey soil was slightly alkaline in reaction (*p*H 8.1), low in organic carbon content (0.48%) and available nitrogen (174 kg/ha), medium in available phosphorus (19.4 kg/ha) and high in available potassium (320 kg/ha). Sowing was done in rows at 45 cm apart with a plant to plant spacing of 15 cm. Sorghum crop was raised under rainfed conditions with all recommended package of practices. Data on growth, yield attributes and yield were collected as per standard and uniform procedures and economics were worked out based on output of crops and input costs. For carrier based biofertilizer seed treatments, inoculants of nitrogen fixing bacteria (*Azospirillum brasilense*) and PSB were mixed with the seeds and kept in shade for 20 min and sown immediately. The quantity of biofertilizer culture was used @50 g/kg seed. To apply liquid biofertilizer, seed treatment is effective and economic way. The plastic bag of 21 cm × 10 cm size was used for the seed treatment of approximately 10 kg seed and applied @2 ml and 4 ml/kg liquid biofertilizer for *Azospirillum* and PSB as per the treatment. The bag was squeezed for 2 to 4 min or more until the seeds were become uniformly wetted. Then bag was opened, inflated again and shaked well. Stopped the bag shaking after each sorghum seed gets a uniform layer of *Azospirillum* and PSB culture coating. Finally opened the bag and seeds were dried under shade for 20 min. The sorghum crop fertilized with a RDF of 80:40:40 kg/ha N-P_2O_5-K_2O through urea, single super phosphate (SSP) and muriate of potash (MOP), respectively. Entire P and K along with 50% N were applied as basal and remaining N was top dressed at 30 days after sowing (DAS). Biofertilizers treated seeds were sown as per the treatments. A rainfall of 518.1, 830.6 and 930.2 mm was received in 43, 40 and 55 rainy days during 2017, 2018 and 2019 and crop did not face any moisture stress. However, the rainfall received was lesser during 2017 and higher during 2018 and 2019 than the mean rainfall (734 mm). As similar trend of results was observed during 2017 and 2019 for all the characters, hence pooled analysis was done. Year and treatment impacts were significant while their interaction were not significant; hence, mean data for years were also presented. All the parameters were subjected for statistical analysis and interpretation as per Gomez and Gomez (1987).

RESULTS AND DISCUSSION

Growth and grain yield attributes

Pooled data pertaining to growth and yield attributes of sorghum (Table 1) showed that, plant height (cm), grain weight/panicle (g) and number of grains/panicles were significantly affected by seed treatment with biofertilizers in general and in their liquid form in particular. Seed treatment with powder form of *Azospirillum* + PSB @50 g each/kg seed did not improve the plant height, grain weight/panicle and number of grains/panicles of sorghum significantly over no biofertilizer (RDF alone) treatment. However, in their liquid form, *Azospirillum* + PSB seed treatment @2 ml each/kg seed has brought significant gains in plant height and grain yield over their powder form treatment and un-inoculated control. Plant height of *Azospirillum* + PSB (2 ml each/kg seed) treated seeds of sorghum crop (184 cm) was 5.75 and 6.36% higher than powder form of biofertilizers and no biofertilizer control treatments. Similarly, number of grains/panicle and grain

Table 1. Growth and yield attributes of sorghum as influenced by seed inoculation with biofertilizers (pooled data of 3 years)

Treatment	Plant height (cm)	Days to 50% flowering	Test weight (g)	Grain weight/ panicle (g)	Grains/ panicle	Panicle weight (g)	Panicle length (cm)
No biofertilizer control (RDF alone)	173	64.6	20.72	40.61	1,996	191	26.57
RDF + *Azo.* + PSB @50 g each/kg seed	174	63.9	20.37	42.62	2,160	208	29.29
RDF + *Azo.* @2 ml + PSB @2 ml/kg seed	184	64.6	20.34	48.83	2,495	267	31.58
RDF + *Azo.* @2 ml + PSB @4 ml/kg seed	180	64.8	19.04	45.34	2,408	221	28.41
RDF + *Azo.* @4 ml + PSB @2 ml/kg seed	180	64.7	19.42	46.96	2,414	244	31.25
RDF + *Azo.* @4 ml + PSB @4 ml/kg seed	181	65.1	19.93	46.34	2,353	237	30.99
SEm±	1.7	0.49	0.28	0.86	34.1	7.2	0.67
CD (P=0.05%)	5.1	NS	0.88	2.59	102.7	21.6	2.00
2017	135	63.7	26.40	43.37	2,413	237	30.44
2018	187	64.2	26.60	43.02	2,526	241	31.07
2019	194	65.5	24.88	49.04	1,977	206	27.54
CD (P=0.05%)	S	NS	S	S	S	S	S

weight/panicle of sorghum in liquid *Azospirillum* + PSB (2 ml each/kg seed) treatment were 15.51 and 14.57% higher than its powder form inoculants (2, 160 counts and 42.62 g). The improvements in grains/panicle and grain weight/ panicle of sorghum have noted still higher (25.0 and 19.36%) due to liquid biofertilizers seed treatment when compared to no biofertilizers. The significant increase in panicle weight and panicle length support the increases in grain weight/panicle and grain number/panicle, respectively. Days to 50% flowering and test weight of sorghum were not significantly altered by the biofertilizer seed inoculation treatments. This was ascribed to increased microbial activity in the rhizosphere resulted in solubilization of bound form of soil minerals and enhanced availability of nutrients in the soil for plant growth and development by the liquid biofertlizer leading to improved seedling germination, vigour, emergence and productivity (Puenete *et al.,* 2009 and Yadav *et al.,* 2011).

Yield

The biological, grain and stover yield and harvest index data obtained in response to biofertilizer seed treatment on pooled basis is given in Table 2. The result shows significant responses of sorghum to biofertilizer seed treatment. Seed treatment with *Azospirillum* + PSB in powder form could bring significant improvements in biological and stover yields of sorghum (10.4 and 9.0%) over no biofertilzer treated one (13.49 and 11.16 t/ha). However, the improvements in grain yield (5.33%) due to powder form of biofertilziers over no biofertilizer (3.38 t/ha) were not significant. Liquid form of *Azospirillum* + PSB @2 ml each/ kg seed has resulted in 11.08, 13.77 and 10.01% increase in biological, grain and stover yield over the powder form of inoculants. The magnitude of increase was still higher (21.13, 19.82 and 21.56% for biological, grain and stover yield) when compared with un-inoculated control. The impact of biofertilizer treatments on harvest index was

Table 2. Yield and economics of sorghum as influenced by seed inoculation with biofertilizers (pooled data of 3 years)

Treatment	Biological yield (t/ha)	Grain yield (t/ha)	Harvest index (%)	GMR (₹/ha)	NMR (₹/ha)	Benefit cost ratio
No biofertlizer control (RDF alone)	13.49	3.38	25.09	81,084	56,864	3.35
RDF + *Azo.* + PSB @50 g each/kg seed	14.71	3.56	24.16	86,306	62,006	3.55
RDF + *Azo.* @2 ml + PSB @2 ml/kg seed	16.34	4.05	24.79	97,416	73,116	4.01
RDF + *Azo.* @2 ml + PSB @4 ml/kg seed	15.57	3.92	23.67	90,080	65,740	3.70
RDF + *Azo.* @4 ml + PSB @2 ml/kg seed	16.04	3.98	24.82	95,776	71,436	3.93
RDF + *Azo.* @4 ml + PSB @4 ml/kg seed	16.03	3.91	24.42	94,602	70,222	3.88
SEm ±	0.211	0.073	0.56	1,343	1,343	0.06
CD (P=0.05)	0.634	0.218	NS	4,045	4,045	0.17
2017	15.30	3.81	24.97	93,950	69,642	3.83
2018	15.47	3.88	25.09	95,010	70,713	3.90
2019	15.32	3.58	23.44	85,401	61,122	3.47
CD (P=0.05)	NS	S	S	S	S	S

non-significant. The improvement in stover and grain yields of sorghum due to liquid form of bio-fertilizers over its powder form or no bio-fertilizers could be ascribed to the increases in plant height and grain weight/panicle, respectively and the improved grain and stover yields together have boosted sorghum biological yields finally. *Azospirillum* and PSB biofertilizers seed inoculation in general has increased the microbial load and their activity in the rhizosphere leading to nitrogen fixation and phosphorous solubilization for possible plant uptake. Enhanced vigour of seedlings due to biofertilizer seed treatment might have promoted growth, yield attributes and yield formation. Biofertilizers impact could also be owing to production of amino acids, vitamins and growth promoting substances like indole acetic acid and gibberellic acid resulted in enhanced nutrient uptake, translocation and synthesis of photosynthate assimilates which ultimately resulted in increased plant growth characters and obtaining economically profitable yield (Singh *et al*., 2006; Suke *et al*., 2011). Liquid biofertilizers are more superior than solid inoculants because they have a long shelf-life of 1.5–2 years, have no contamination, do not need sticky materials, can be used with modern machinery, can withstand with high temperatures (up to 45°C), are easy for adding ingredients that enhance the growth of microbial strains and are easy to apply on both seeds and soil (Mahanty *et al*., 2017). Furthermore, Sivasakthivelan and Stella (2012) observed better growth and yield of sunflower seeds treated with liquid formulation of *Azospirillum lipoferum* (AUAz1) + *Bacillus megaterium* (AU Ba-1) + *Pseudomonas fluorescens* (AU Ps-1) than its carrier based and alginate based formulations. Geeta *et al.,* (2017) also reported superiority of liquid based formulations in maize.

Nutrient uptake and contribution by biofertilizers

Nitrogen (N) and phosphorous (N) uptake (grain + stover, kg/ha) of grain sorghum crop were significantly influenced by biofertilizer seed treatment (Table 3). Enhancement in the nutrient uptake was observed by colonizing the rhizosphere and making the nutrients easily accessible to plant root hairs. The data reveals that N and P uptake of no biofertilizer sorghum crop (RDF) was improved by 10.2 and 26.9% due to powder form of biofertilizers seed treatment. Further, use of liquid biofertilizers (*Azo.* + PSB treatment @2 ml each/kg seed) for seed treatment has further boosted N uptake significantly (by 15.93%) over its powder form of biofertilizers. However, a significant improvement in P uptake due to liquid biofertilizer was seen with *Azo.* @4 ml/kg + PSB treatment @2 ml/kg seed.

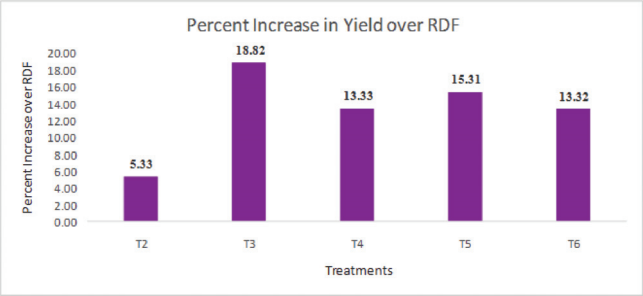
Nitrogen (N) fixation by *Azospirillum* and phosphorous solubilization by PSB in their powder form seed inoculation accounted for 7.5 and 3.85 kg/ha contribution of N and P, respectively. With the use of liquid biofertilizers (*Azo.* + PSB treatment @2 ml each/kg seed) for seed treatment, the N fixation and P solubilization was improved by 2.72 and 1.95 times over its powder form. However, highest N and P uptake due to liquid biofertilizer were obtained with treatment *Azo.* + PSB treatment @4 ml/kg seed. Liquid biofertilizers consist of living microorganisms that enhance soil properties and increase plant growth and yield. Liquid biofertilizers have been applied to different crops and yielded the best results when compared to other types of chemical (Allouzi *et al*., 2022).

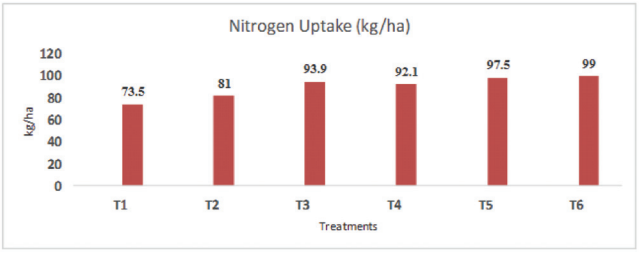
Economics

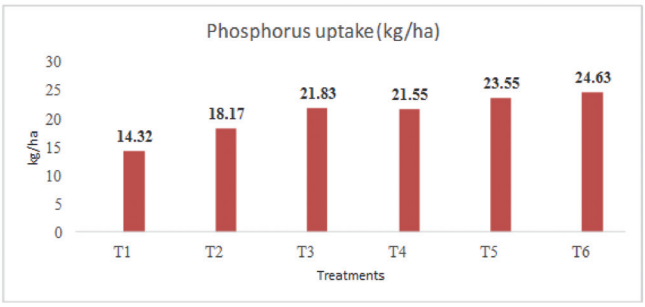
The economics of grain sorghum cultivation were significantly influenced by biofertilizer seed treatment (Table 2). The result reveals that gross and net returns obtained with the liquid biofertilizers (*Azo.* + PSB treatment @2 ml each/kg seed) treatment were 12.87 and 20.14 higher over its powder form of biofertilizers (₹86,306 and ₹62,006) that were still higher i.e. 17.92 and 28.58% when

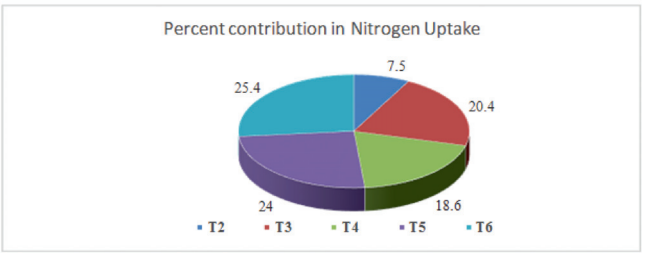
Table 3. Nutrient uptake of sorghum and contribution as influenced by seed inoculation with biofertilizers (pooled data of 3 years)

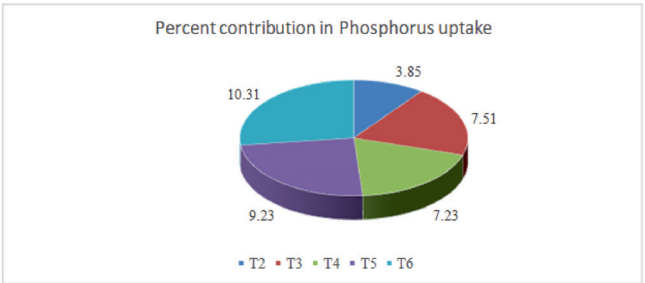
Treatment	Nitrogen uptake (kg/ha)	P uptake (kg/ha)	Contribution by N fixation (kg/ha)	Contribution by P solubilization (kg/ha)
No biofertilizer control (RDF alone)	73.5	14.32	-	-
RDF + *Azo.* + PSB @50 g each/kg seed	81.0	18.17	7.5	3.85
RDF + *Azo.* @2 ml + PSB @2 ml/kg seed	93.9	21.83	20.4	7.51
RDF + *Azo.* @2 ml + PSB @4 ml/kg seed	92.1	21.55	18.6	7.23
RDF + *Azo.* @4 ml + PSB @2 ml/kg seed	97.5	23.55	24.0	9.23
RDF + *Azo.* @4 ml + PSB @4 ml/kg seed	99.0	24.63	25.4	10.31
SEm ±	2.89	1.67		
CD (P=0.05)	9.08	5.27		
2017	86.4	20.52		
2018	92.5	22.21		
2019	84.9	18.90		
CD (P=0.05)	S	NS		











compared with no biofertilizer treatment. Gross income variations of *kharif* sorghum among biofertilizer treatments could be ascribed to the variations in grain and stover yields. Net income trend remained same as that of gross income as the additional cost of imposing biofertilizers treatments (both powder @50 g each and liquid form @2 ml each of *Azo*. + PSB) over control (RDF) was meagre i.e. ₹ 80 only. Higher gross income coupled with almost similar cost of cultivation has resulted in improvement in benefit cost (B: C) ratio due to *Azo*. + PSB @2 ml each/kg seed treatment (4.01) as compared to its powder form of biofertilizers (3.55) and no biofertilizer (3.35) control. Jat *et al.,* (2013) reported that higher profitability was insured by the application of biofertlizer in powder form compared to no biofertilizer application in *kharif* sorghum. According to Rani *et al.,* (2019) for obtaining the higher profitability, seed treatment with liquid biofertilizers is more remunerative over its powder form in *rabi* sorghum.

Based on three years it is concluded that the existing practice of *kharif* sorghum seed treatment with *Azospirillum* + PSB (@50g/kg seed each) was fruitful from biological, stover yield and economics point of view and could be made more profitable with the use of liquid form of *Azospirillum* + PSB (@2 ml/kg seed each) over and above the recommended dose of fertilizers i.e. 80-40-40 kg/ha N-P_2O_5-K_2O.

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