Effect of biofertilizer application on rainfed sorghum in calcareous soil

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ABSTRACT

The influence of biofertilizers like *Azospirillum*, phosphobacteria, azophos (50:50 combinations of *Azospirillum* and phosphobacteria) and mycorrhiza, individually and in combination was investigated in pre-monsoon sowing with sorghum APK 1 variety in randomized replicated field trial at the Regional Research Station, Aruppukottai, Virudhunagar, Tamil Nadu. The adherence of the inoculated bacteria was recorded under all treatments. The results showed the feasibility of biofertilizer treatment to seeds in pre-monsoon sowing as the bacterial inoculants survived on seeds up to 21 days even though they were sown in dry soil and colonized until the receipt of showers. Treatment azophos + mycorrhiza with 75 per cent recommended N and P treatment was found most effective in enhancing fresh stover weight (70 tonnes/ha) and grain yield (3,536 kg/ha). This treatment also resulted in higher earhead yield (13.00 kg/plot) alongwith phosphobacteria + mycorrhiza treatment (12.80 kg/plot).

Keywords: *Azospirillum*; phosphobacteria; VAM; sorghum; black soil

INTRODUCTION

Sorghum popularly known as Jowar is the most important food and fodder crop of dryland agriculture. The sorghum grain is used primarily as human food in various forms such as Roti/Bhakri (unleavened bread) or is cooked like rice or made into porridge with pulverized sorghum grown in southern India. The sorghum grain and fodder are used for the production of ethanol, starch and paper (Doifode 2021). Indian farmers are highly dependent on rainfall and all activities of agriculture starting from sowing to postharvest are affected either directly or indirectly by weather especially by rainfall.

Sorghum is a hardy crop that can withstand moisture stress and its potentiality can be augmented by biofertilizer application. Biofertilizers improve photosynthesis performance to confer plant tolerance to stress (Chi et al 2010). Microbial inoculants are one of the components in organic farming system which help to produce the plant growth hormones, vitamins and amino acids, fixation of atmospheric nitrogen, solubilization and mobilization of P and translocation of minor elements like zinc and copper. They are cheap, ecologically safe, non-toxic and easy to apply (Bhoraniya et al 2019).

The black soil of Aruppukottai, Virudhanagar district of Tamil Nadu is characterized by higher calcium content because of the Kankar nodules as indurate layers. In certain fields, one may be able to see numerous small bits of limestone on the surface soil itself. Such a high content of Ca will exert a reversible effect even if phosphobacteria solubilize the phosphate. Calcite or calcium carbonate ($CaCO_3$) is soluble only under an acidic environment. Rainwater is slightly acidic because of the carbon dioxide present in the environment that can be dissolved in it. Phosphorus is one of most essential nutrients for plant growth and development which contributes 0.2 to 0.8 per cent of plant dry matter yield (Sharma et al 2013). The availability of P is very low due to the calcium occurrence in neutral to alkaline condition and alminium (Al) and iron (Fe) in acidic environment ultimately that can lead to fixation of P. Therefore, it is worthwhile to use phosphobacteria and mycorrhiza together in calcareous or black cotton soil (Schutz et al 2018, Vidhya 2022, Johan et al 2021)

Hence, the individual bacterial biofertilizer and combination of phosphobacterial and mycorrhizal biofertilizers have been used in this experiment which can absorb, store and mobilize phosphorus to the crops.

MATERIAL and METHODS

The effect of biofertilizers on sorghum was investigated in pre-monsoon sowing at Regional Research Station at Aruppukottai, Virudhunagar, Tamil Nadu. A randomized replicated field trial was laid out in black cotton soil. Sorghum seeds of APK 1 variety were sown in plots of 5.2 m x 3.8 m size with a spacing of 45 cm x 15 cm. Three replications were maintained. The treatments used are given in Table 1.

Seed treatment

Seed treatment was done employing rice gruel as adhesive. For each treatment 300 g seeds were taken and 12 g of bacterial inoculant was mixed with 12 ml of rice gruel and prepared as slurry so that the biofertilizers are well applied on the seeds. The seeds were shade-dried for 30 min and sown. Mycorrhizal inoculum was applied @ 2 per cent on seeds before sowing in plots. Uniform quantity of seeds was sown in each plot to maintain uniform number.

Survival of biofertilizers on sorghum seeds

The pre-monsoon sowing practiced in certain tracts of southern districts was simulated in pots with soil brought from RRS, Aruppukottai, Tamil Nadu. In pre-monsoon sowing, seeds were sown in soil anticipating rain in 2-3 weeks; the seeds were subjected to stress there in hot sun. Seeds were sown in earthen pots of 27 cm in diameter and left buried in the soil for 3 weeks. The soil was kept dry without irrigation and the soil temperature was recorded. The survival of inoculants on the seeds after sowing and prior to germination was investigated.

Assessment of VAM infection and enumeration of VAM spores in soil

The rhizosphere soil was examined for the presence of VAM spores by the wet sieving and decanting method of Gerdemann and Nicolson (1963).

The roots of sorghum plants were analyzed for VAM infection by clearing and staining method of Phillips and Hayman (1970). The mycorrhizal infection was calculated by using the following formula:

$$\text{Root infection (\%)} = \frac{\text{Number of root segments infected}}{\text{Number of root segments examined}} \times 100$$

Determination of calcium carbonate content of soil

Calcium carbonate content was determined by wet oxidation method (Jackson 1973). The soil sample was first passed through 0.2 mm sieve. A sample containing considerably less than 0.625 g of $CaCO_3$ equivalent (10 g of soil containing 5% $CaCO_3$ or less or 0.25 g of limestone was taken) was weighed and transferred to the sample flask. The sample flask was attached to the apparatus. Twenty five ml of 0.5 N NaOH was pipetted into the O_2 absorption flask followed by 50 ml of CO_2 free water. The bead tower was lowered to dip into the NaOH until the solution reached the beads and then the bead tower was raised so that no more NaOH solution entered. Fifty ml of 1N HCl (with 5% $SnCl_2$) was added through the separate funnel slowly at first if the sample was high in carbonate. A small flame with a windshield was placed under the sample flask and the suspension was slowly brought to boiling. The boiling continued for 5 min. The CO_2 evolved was collected in the absorption flask and determined by back titration with standard HCl after addition of $BaCl_2$:

$$\text{Meq of } CO_2 = (S - T) \times N$$

where S = Standardization blank titration value, T = Back titration value, N = Normality of the standard HCl

$$\% \; CaCO_3 = \text{Meq of } CO_2 \times \frac{5}{S}$$

where S = Sample weight in g

Factor 5 derived from the meq weight of $CaCO_3 \frac{100}{200} \times 100$

RESULTS

Survival of biofertilizers on sorghum sown in soil (pot culture)

The results on the survival of bacterial inoculant on sorghum seeds in pre-monsoon sowing and left in the soil up to 3 weeks (Table 2) at varied soil temperature conditions prevailed during the crop growth

Table 1. Details of treatments used in the study

Treatment	Composition
T_1	*Azospirillum* 3 packets/ha seed treatment, 10 packets/ha field application
T_2	Phosphobacteria 3 packets/ha seed treatment, 10 packets/ha field application
T_3	Azophos 3 packets/ha seed treatment, 10 packets/ha field application
T_4	Mycorrhiza 2% on seed rate (seed inoculation only)
T_5	Azophos 3 packets/ha seed treatment, 10 packets/ha field application + Mycorrhiza 2% on seed rate (seed inoculation only)
T_6	Phosphobacteria 3 packets/ha seed treatment, 10 packets/ha field application + Mycorrhiza 2% on seed rate (seed inoculation only)
T_7	Control (no biofertilizers)
T_8	Recommended dose of NPK fertilizer (NPK 40:20:0 kg/ha)

Each packet contained 200 g biofertilizer; Treatments T_1-T_6 received only 75% of N and P

Table 2. Survival of biofertilizers on sorghum sown in soil (pot culture)

Treatment	*Azospirillum* (x 10^4) at DAS				Phosphobacteria (x10^3) at DAS			
	0	7	14	21	0	7	14	21
Azospirillum 3 packets/ha seed rate	0.47	0.17	0.13	0.20	-	-	-	-
Phosphobacteria	-	-	-	-	12	6	3	3
Azophos	0.45	0.44	0.36	0.13	41	29	26	14
Azophos + Mycorrhiza	0.47	0.41	0.38	0.33	20	12	8	9
Phosphobacteria + Mycorrhiza	-	-	-	-	20	9	8	12
Control (uninoculated)	0.17	0.17	0.13	0.13	12	3	2	3
Azospirillum culture	0.14*	-	-	-	-	-	-	-
Azophos culture	0.38*	-	-	-	5*	-	-	-
Phosphobacteria culture	-	-	-	-	4*	-	-	-

DAS = Days after sowing; Moisture content of soil at 0 DAS = 8.4, at 7 DAS = 5.1; *cfu 10^7 on wet weight basis

period were recorded. Both *Azospirillum* and phosphobacteria survived on seeds when the seeds were sown in a dry soil and left for 21 days without water simulating the condition in pre-monsoon sowing. Not only they survived but also exhibited an increase at certain sampling periods. The *Azospirillum* survived on seeds up to 21 days. The azophos + mycorrhiza-treated seeds showed a higher population on 7, 14 and 21 DAS as compared to other treatments. Control seeds also recorded a population of 0.13 to 0.17 x 10^4 cfu/g population which was negligible. The results indicated the survival of *Azospirillum* on seeds when they were sown and left in the field under dry condition.

Azophos-treated seeds showed an increased number of phosphobacteria population which recorded 41 x 10^3 cfu/g of seed immediately after seed treatment. Its population declined subsequently. The same trend was observed when phosphobacteria alone was seed treated. The biofertilizer-treated seeds showed a higher population of phosphobacteria as compared to control. Soil temperature recorded during the period of experiment in soil ranged from 31-42ºC. The results showed that the inoculated phosphobacteria survived on seed despite the dryness and high soil temperature. The results showed the feasibility of biofertilizer treatment to seeds in pre-monsoon sowing as the bacterial inoculants survived on seeds up to 21 days even though they were sown in dry soil and remained there until receipt of showers (O'Callaghan et al 2022).

Calcium carbonate content of soil

The results on the calcium carbonate content of soils collected from different locations viz Aruppukottai black cotton soil (2.5%), Kovilpatti black cotton soil (1.0%) and Madurai loamy soil (0.7%). Calcium carbonate content was higher in Aruppukottai black cotton soil with 2.5 per cent followed by Kovilpatti black cotton soil with 1 per cent. The loamy soil of Madurai recorded 0.5 per cent of carbonate.

Effect of biofertilizer application on the vesicular arbuscular mycorrhizal (VAM) spores and root infection in sorghum

The results on the effect of biofertilizer application on VAM spores in soil and VAM root infection on sorghum plants are presented in Table 3.

The VAM spores present in soil were assessed on 60 and 90 days after growth (DAG). At 60 DAG, VAM spores were observed only in treatments where mycorrhiza was a component. However, at 90 DAG, VAM spores were observed in all treatments. Azophos + mycorrhiza treatment showed an increased number of spores with 17 spores/100 g of soil while phosphobacteria + mycorrhiza treatment showed a higher number of spores as compared at 90 DAG. VAM root infection was higher in azophos + mycorrhiza-treated plants which was 76.0 per cent followed by mycorrhizal treatment with 60.0 per cent at 60 DAG. Phosphobacteria + mycorrhiza-treated plants showed a higher root infection followed by azophos + mycorrhiza treatment at 90 DAG.

Effect of biofertilizer application on the yield attributes and yield of sorghum

The results on the effect of biofertilizer application on yield attributes and yield of sorghum are presented in Table 4.

Stover yield (fresh and dry weight): Fresh stover weight was higher in azophos + mycorrhiza with 70 tonnes/ha followed by mycorrhiza (53 tonnes/ha) and azophos (50 tonnes/ha), the latter two being at par. Dried stover weight was higher in the treatment azophos + mycorrhiza with 25 tonness/ha followed by recommended NPK and control (20 tonnes/ha each).

Earhead length and yield: The earhead length in Sorghum APK 1 ranged from 23.7 to 26.9 cm. It was higher in all the biofertilizer-treated plants compared to control. Inoculation of *Azospirillum* was found to be more efficient in increasing the earhead length with 26.9 cm at par with azophos with 26.7 cm as against 23.7 cm in uninoculated control. Earhead yield was maximum in azophos (13.00 kg/plot) and phosphobacteria + mycorrhiza (12.80 kg/plot), the two being at par and lowest in recommended dose of NPK (7.00 kg/plot).

1,000-grain weight: The 1,000-grain weight of sorghum APK 1 ranged from 22.5 to 24.7 g. All the biofertilizers augmented the 1,000-grain weight compared to uninoculated control and azophos treatments (22.5 g each).

Grain yield: Azophos + mycorrhiza-treated plants showed a higher grain yield of 3,536 kg/ha followed by mycorrhiza alone treatment with 3,094 kg/ha and phosphobacteria + mycorrhiza treatment (3,000 kg/ha), the two being at par.

DISCUSSION

The appearance of the soil and hotness prevailing in southern Tamil Nadu during summer gives an impression that this soil might not have any microbiological activity particularly in summer. In the present study, it was found that the soil here was dry and the ambient temperature ranged from 31-42ºC. Besides, the survival of inoculated organisms, it was likely that soil adhering to the seeds when removed for testing might also have contributed to the presence of *Azospirillum* and phosphobacteria as these were also observed in uninoculated seeds. Phosphobacteria-treated seeds showed a higher population of phosphobacteria. The phosphobacterial population was higher in the treatment receiving phosphobacteria + mycorrhiza in combination which recorded a population of 18 x 10^3 cfu/g of seed followed by azophos treatment with 14 x 10^3 cfu/g. Combined inoculation of azophos with mycorrhiza and phosphobacteria also recorded a relatively higher phosphobacterial population. In the uninoculated control also, phosphobacteria were present.

The *Azospirillum* population was higher in the treatment receiving *Azospirillum* with 14 x 10^5 cfu/g of seed. The treatment of azophos + mycorrhiza, azophos also recorded a population of 9 x 10^3 cfu/g+5 × 10^7) of seed. It was negligible in the uninoculated seed. It is not only the bacterial inoculants adhered to seed when inoculated but also survived when the treated seeds were sown in a dry soil subjected to hot dry condition until the showers drenched the soil enabling the seeds to germinate. In the present study, under a

Table 3. Effect of biofertilizer application on the vesicular arbuscular mycorrhizal spores and root infection in sorghum

Treatment	VAM spores (number/100 g of soil) after DAG		VAM root infection (%) after DAG	
	60	90	60	90
Azospirillum	-	2	28.0	12
Phosphobacteria	-	4	11.6	36
Azophos	-	5	36.0	32
Mycorrhiza	5	21	60.0	12
Azophos + Mycorrhiza	17	19	76.0	48
Phosphobacteria + Mycorrhiza	14	26	10.4	25
Recommended NPK (40:20:0 kg/ha)	-	7	8.0	18
Control (uninoculated)	-	1	16.0	24

DAG = Days after growth; Treatments 1 to 6 applied with 75% of the recommended N and P

Table 4. Effect of biofertilizer application on the yield attributes and yield of sorghum

Treatment	Fresh stover weight (tonnes/ha)	Dried stover weight (tonnes/ha)	Earhead length (cm/plant)	Earhead yield (kg/plot)	1000-grain weight (g)	Grain yield (kg/ha)
Azospirillum	23	12	26.9	11.50	24.0	2,725
Phosphobacteria	45	12	25.6	9.50	24.7	2,765
Azophos	50	13	26.7	10.50	22.5	2,727
Mycorrhiza	53	13	25.9	11.75	24.2	3,094
Azophos + Mycorrhiza	70	25	24.9	13.0	23.8	3,536
Phosphobacteria + Mycorrhiza	30	12	25.9	12.8	24.1	3,000
Recommended NPK (40:20:0 kg/ha)	41	20	25.9	7.00	24.1	2,652
Control (uninoculated)	35	20	23.7	10.5	22.5	2,595
SEd	2.40	0.83	0.17	0.20	1.38	51.05
$CD_{0.05}$	5.16	1.77	0.36	0.40	0.97	109.50

Treatments 1 to 6 applied with 75% of the recommended N and P

simulated dry condition in pots containing the black cotton soil, the sorghum seeds treated with biofertilizers established survival of inoculated bacteria for 3 weeks.

Biofertilizers were found to induce early germination and augmented higher germination, seedling vigour and enhanced root proliferation conferring the drought tolerance (Nosheen et al 2021). In the present experiment, the adherence of the inoculated bacteria was seen in all the treatments.

In the phosphobacteria + mycorrhiza-treated combination, the VAM spores were comparatively higher (26/100 g soil) in 90 days after crop growth. In summer, activation of the bacterial cells was reported by Barnard et al (2013). Higher soil phosphorus content of 11.2 kg/ha was recorded by Ramalakshmi et al (2008) with azophos and mycorrhizal treatment in black cotton soil of Aruppukottai while 11.1 and 11.0 kg/ha was observed in the treatment of phosphobacteria and mycorrhiza in 90 days after crop growth. The results of the present study are in conformity with the above findings.

VAM-inoculated plants showed that the rate of photosynthesis in rice plants under drought and saline conditions which was reported by Ruiz-Sanchez et al (2010). Rani et al (2019) reported that in rabi sorghum, seed treatment of sorghum with liquid formulation of *Azospirillum* @ 4 ml + phosphobacteria @ 4 ml/kg seed, proved to be optimal for obtaining higher grain yield of 2.33 tons/ha with net return of Rs 51,217/ha and B-C ratio of 2.10. In pot and field conditions, combined inoculation of azophos and mycorrhiza increased the seed germination. Enhancement of the seed germination due to biofertilizer has already been reported in several crops like cardamom, coffee and rice (Sherpa et al 2021). The results of the current field study showed that VAM root infection was comparatively higher in azophos + mycorrhiza treatment combination of 76 per cent than the mycorhizal treatment alone, especially at 60 days after crop growth.

In dry land agriculture, any technology that enhances seed germination would be a boon to the farmer. The seeds have to germinate with meager moisture of the soil or before the soil gets dried up. Germination failure is the first constraint in an arid climate. Bareke (2018) observed that for germination to occur, seeds of each species must attain specific moisture content. In arid climates, inadequate moisture is a frequent cause of germination failure. Reddy et al (2020) reported that a poor seed-soil moisture contact reduced the rate of water uptake and thus caused delayed or poor germination. Abdelhameid (2020) reported that mycorrhizal inoculation and potassium fertilization in sweet sorghum cultivated under water stress in calcareous soil significantly increased leaves, stalks and grains nitrogen content by 9.49, 21.54 and 2.83 per cent compared to uninoculated treatment. Sorghum APK 1 raised in the present study also reported higher earhead yield of 13.00 kg per/plot in treatment azophos + mycorrhiza alongwith phosphobacteria + mycorrhiza with 12.8 kg per plant.

The increased germination observed due to biofertilizers might be due to elaboration of auxins and growth promoting substances. Patel and Saraf (2017) also reported production of gibberellic acid by *Azospirillum*. Plant growth promotion of plants at seedling stage and at subsequent growth stages could be mainly due to the plant growth promoting substances produced by *Azospirillum brasilense* (Backer et al 2018). Although auxin production by the biofertilizers tested has not been studied in the present investigations, the *Azospirillum*, phosphobacteria and azophos might have elaborated such substances that might have induced higher germination (Cortivo et al 2020). In the present experiment, azophos + mycorrhiza-treated plants showed a higher grain yield of 3,536 kg/ha followed by mycorrhizal treatment alone with yield of 3,094 kg/ha and phosphobacteria + mycorrhiza with yield of 3,000 kg/ha.

CONCLUSION

Conventional methods of agriculture play a significant role in meeting the food demands of a growing human population which has also led to an increasing dependence on chemical fertilizers and pesticides. Chemical fertilizers namely urea, DAP, SSP and potash are industrially-manipulated substances composed of known quantities of N, P and K and their exploitation causes air and groundwater pollution by eutrophication of water bodies. Hence, an appropriate method of biofertilizer (individual or in combination) application can reduce the recommended dose of fertilizers. The results of the present study established the beneficial effect of biofertilizers in pre-monsoon sowing of sorghum in black soil. The combined inoculation of azophos @ 3 packets (200 g/packet) with mycorrhiza @ 2 per cent (w/w) of seed treatment followed by soil application of azophos @ 10 packets (200 g/packet/ha). This treatment enhanced the seed germination, plant growth, root length and soil available N, P and K content in plants. The farmers can save 25 per cent of N and P by resorting to a low cost biofertilizer treatment without sacrificing the yield.

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