



## SYNERGISTIC EFFECT OF *AZOSPIRILLUM* AND PSB INOCULATION ON GROWTH AND YIELD OF FOXTAIL MILLET

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**ABSTRACT:** Studies were conducted to know the effect of inoculation of *Azospirillum* sp. and phosphate-solubilizing bacteria (PSB) on the yield of foxtail millet [*Setaria italica* (L.) Beauv] (cv. Lepakshi) during Kharif and Rabi seasons, grown in Black soil of sandy loam type in earthen pots by the selected isolates of *Azospirillum* A1 (45 C2) and A2 (45 L1) and one isolate of PSB (45 LR3). Pots were inoculated separately with *Azospirillum* A1 alone, PSB alone, *Azospirillum* A1 and PSB in combination. The same was also repeated with A2 isolate. Pots which received heat killed inoculum served as control. Triplicates were maintained for each treatment. Inoculation of foxtail millet (cv. Lepakshi) with two strains of *Azospirillum* sp. and one strain of a Phosphate Solubilizing Bacterium (PSB) individually and in combination significantly increased plant height, dry weight of shoot and root over control plants. Yield increased due to inoculation with *Azospirillum* and PSB in combination. Dual inoculation with *Azospirillum* and PSB improved the yield.

**Key words:** *Azospirillum*, PSB, Dual inoculation, Foxtail millet, Lepakshi, growth, yield

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### INTRODUCTION

The existence of life on earth is favored by the cycling of biological elements wherein the complex biological systems after their decay are converted into simpler forms. These cycles like carbon, sulphur, phosphorus and nitrogen are essential for biology and the role played by microorganisms in these cycles is important. Nitrogen is an essential and key element in improving crop productivity throughout the world. In recent years, chemical fertilizers are used to boost the agricultural production. About 42 million tons of fertilizer N is being used annually on global scale for the production of three major cereal crops viz., wheat, rice and maize. Crop plants are able to use about 50% of the applied fertilizer N while 25% is lost from the soil-plant system through leaching, volatilization and denitrification [1]. Even though molecular nitrogen is abundant in atmosphere, it is generally deficient in soils resulting in decreased yield of the crops. As a result of its role and its low supply, the management of nitrogen resources is an important aspect in agricultural productivity. Although increasing nitrogen demand of crops is mainly satisfied by the application of mineral fertilizers, biological nitrogen fixation, a process involving the reduction of atmospheric nitrogen to ammonia by microorganisms, which accounts for nearly 60% of earth's newly fixed nitrogen [2], has assumed great importance in maintaining soil fertility status. Unless the molecular nitrogen is converted into the suitable form (ammonia), the plants are unable to utilise it. This ability of reduction of atmospheric nitrogen to ammonia, known as biological nitrogen fixation, is confined to microorganisms. The well known asymbiotic diazotrophic bacteria belong to the genera like *Acetobacter*, *Azotobacter*, *Azospirillum*, *Azoarcus*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Pseudomonas*, *Klebsiella* etc., which are able to exert positive effect on plants by producing and secreting plant growth regulators (PGRs) and/ or by supplying biologically fixed nitrogen [3].

The nitrogen gain through non-symbiotic systems is low compared to symbiotic systems. However, the contribution of heterotrophic nitrogen fixers can be of considerable importance under conditions of high organic matter and moisture availability. *Azospirillum* is an associative microaerophilic diazotroph isolated from the roots and above ground parts of a variety of crop plants. Further, some of the investigations emphasize significant contribution of N with inoculation of diazotrophic bacteria like *Azospirillum* to foxtail millet [4, 5].

Phosphorus is also a vital nutrient for plants and microorganisms next only to nitrogen. Phosphorus is one of the major essential macronutrients for biological growth and for proper plant development [6, 7]. Organic matter derived from dead and decaying plant debris is rich in organic sources of phosphorus. The deficiency of phosphorus may occur in crop plants growing in soils containing adequate phosphates. This may be partly due to the fact that the plants are able to absorb phosphorus only in available form. Soil phosphates are rendered available either by plant roots or by soil microorganisms through their secretion of organic acids. Therefore, phosphate-solubilizing soil microorganisms play some role in correcting phosphorus deficiency of crop plants. These microorganisms may also release soluble inorganic phosphates into soil through decomposition of phosphate-rich organic compounds. The recent trend of nutrient supply approach for improving agricultural production has given greater emphasis on the use of biofertilizers along with chemical fertilizers. Organic phosphorus constitutes 20-40% of total phosphorus. Several soil bacteria particularly *Bacillus*, *Pseudomonas* and fungi possess the ability to solubilize insoluble phosphates into soluble forms. Phosphate-solubilizing bacteria (PSB) and nitrogen-fixing bacteria attracted many workers for their commercial utilization in agriculture. One way to correct the deficiency of phosphorus in plants is to inoculate seeds or soils with phosphate-solubilizing microorganisms (PSMO) along with the use of phosphatic fertilizers.

Small millets, which are produced mainly by subsistence farmers as rain fed crops, play an important role in the diets of people living in interior rural and tribal areas in semi-arid tropics [8]. Small millets, borne on short slender grass plants, are also called as minor millets but they are not unimportant as they have wide adaptations. They can withstand certain degree of soil acidity and alkalinity, stress due to moisture and temperature variations in soils from heavy to sandy infertile soils. Anantapur district of Andhra Pradesh receives a poor annual rain fall of 34.4 mm and a temperature of  $31 \pm 9^\circ\text{C}$  resulting in frequent droughts. Under these conditions, foxtail millet, one of the short duration crops (75 to 90 days), is cultivated as a minor millet mixed crop. Foxtail millet is chosen for the present study because it is one of the staple food crops of this area which is nutritious (125 mg protein  $\text{g}^{-1}$ ) and contains all essential amino acids. Further, it not only resists drought conditions but also withstands delayed monsoons and is suitable for light black and red soils of Anantapur district. Hence the present study was aimed to investigate the synergistic effect of inoculation of the bacteria (*Azospirillum* and PSB) on growth, yield, and yield components during Kharif and Rabi seasons.

## MATERIALS AND METHODS

Black soil (5 Kg) of sandy loam type (pH 7.69, organic matter 1.24%, total N- 0.21% and P 14.2 mg P/Kg) was placed in earthen pots. Seeds of foxtail millet cultivar Lepakshi were surface sterilized and sown in the earthen pots. One week after germination, thinning was made such that only one plant remained per pot. Experiments were conducted to study the effect of inoculation (10 ml at 15-day intervals from 15 to 60 DAS (days after sowing) of diazotrophic isolates of *Azospirillum* and phosphate-solubilizing bacteria (PSB) individually and in combination. Two selected isolates of *Azospirillum* A1 (45 C2) and A2 (45 L1) and one isolate of PSB (45 LR3) were chosen for inoculation studies. One set of the pots was inoculated separately with *Azospirillum* A1 alone, PSB alone, *Azospirillum* A1 and PSB in combination and another set of pots was inoculated with *Azospirillum* A2 alone, PSB alone, *Azospirillum* A2 and PSB in combination. Pots which received heat killed inoculum served as control. Triplicates were maintained for each treatment. These studies were conducted during Rabi season and Kharif season.

### Preparation of inoculum

*Azospirillum* sp. was isolated from the rhizosphere of foxtail millet at different days (30, 45, 60 and 75 DAS) on semi-solid nitrogen-free bromothymol blue malate (Nfb) medium [9]. Pure cultures of the two selected strains of *Azospirillum* were grown in malate broth with the following composition (g/l): DL-Malic acid -5.0,  $\text{K}_2\text{HPO}_4$  - 0.5,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.2, NaCl - 0.1,  $\text{CaCl}_2$  - 0.02, Trace element solution-2 ml, Fe EDTA - 4 ml, Yeast extract - 0.5, KOH - 4.0, Distilled water -1000 ml. (Trace element solution contained 200 mg of sodium molybdate, 235 mg of manganous sulphate, 280 mg of boric acid, 8 mg of copper sulphate and 24 mg of zinc sulphate in 200 ml distilled water). The medium was supplemented with  $\text{NH}_4\text{Cl}$ . The pH of the medium was adjusted to 6.8 using potassium hydroxide. Similarly, one strain of PSB isolated from rhizosphere of foxtail millet was grown in Pikovskaya's broth modified by Sundara Rao and Sinha [10] with the following composition (g/l): Glucose - 10.00,  $\text{Ca}_3(\text{PO}_4)_2$  - 5.0,  $(\text{NH}_4)_2\text{SO}_4$  - 0.50, KCl - 0.20,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.10,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  - Traces,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  - Traces, Yeast extract - 0.50, Agar agar - 20.00.

The log phase cultures were used for inoculation. The cells were harvested by centrifugation at 5000 g at 4°C for 20 min. The supernatant was discarded and the pellet was washed two times with saline (5 g of NaCl and 0.12 g of MgSO<sub>4</sub>.7H<sub>2</sub>O in 1l distilled water) and resuspended in saline at a concentration of 10<sup>8</sup> colony forming units (CFU) per ml. Ten ml of the bacterial suspension was inoculated to each plant. Similarly the inoculum containing both *Azospirillum* and PSB was also inoculated to each plant and were harvested 75 DAS.

### Growth parameters

Growth parameters of the plants were recorded. Plant height (from base to tip of the plant) was measured on 30, 45, 60 and 75 DAS. The data recorded for three plants for each treatment were analyzed statistically. The dry weight of shoot, root and grain was determined by drying the plant material at 80°C for 72 h in a hot air oven at 75 DAS.

### Yield

The yield was estimated by taking into account the weight of the panicle and also weight of 1000 seeds.

### Statistical analyses

In all the cases, analyses of significant differences of ( $P \leq 0.05$ ) between values of each sampling and treatment were performed using Duncan's New Multiple Range (DMR) test [11].

## RESULTS AND DISCUSSION

### Inoculation studies

To study the effect of inoculation on growth and yield of foxtail millet two efficient isolates of diazotrophic *Azospirillum* and an efficient isolate of phosphate-solubilizing bacterium were inoculated to Lepakshi variety of foxtail millet during Rabi and Kharif seasons of 2005.

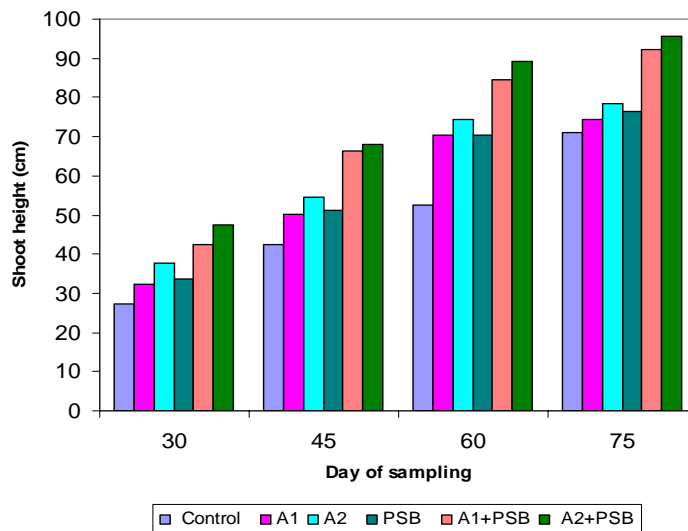
### Shoot height

Shoot height was recorded at 30, 45, 60 and 75 days after sowing during Rabi and Kharif seasons. Effect of inoculation on shoot height of foxtail millet in Rabi season was shown in Figure 1. A1 isolate exhibited considerable effect on shoot height. However A2 isolate exhibited more pronounced effect on plant height. The PSB isolate also enhanced shoot height significantly. Coinoculation with A2 and PSB isolates resulted in maximum stimulation in plant height followed by A1 and PSB isolates. The stimulatory effect on shoot height produced by A1 and PSB isolates as individual inoculants is almost at par. Data on shoot height as influenced by the inoculants reveal that both the diazotrophic and the PSB isolates had pronounced effect on shoot height in Kharif season. Mixed inocula produced maximum effect on shoot height which followed the order: A2+PSB > A1+PSB. Similarly, the isolate A2 was more effective in increasing the shoot height when compared with A1 isolate. The increase in shoot height following inoculation with the PSB was also significant and the effect is on par with the isolate A1, Figure 2. The data indicate that the shoot height varied with the season, where Rabi season recorded higher shoot height compared to Kharif season. The results were in agreement with the reports of other workers. Significant increase in plant height of *Setaria italica* was observed due to inoculation with four isolates of *Azospirillum brasilense* over uninoculated control plants [12]. A varied response in increase in plant height of pearl millet due to inoculation with five different strains (viz., S7, S14, S51, S54 and S59) of *A. brasilense* was reported [13]. Similarly increase in shoot length in rice and finger millet was noticed [14, 15]. The effect of PSB inoculation on plant height was significant over control plants. Very few reports are available on the effect of phosphate-solubilizing bacteria on *Setaria italica*. A strain of *P. putida* a PSB stimulated the growth of shoot and roots and increased <sup>32</sup>P-labeled phosphate uptake in *Canola* [16]. Similar results were also reported in winter maize elsewhere [17]. Coinoculation of *Azospirillum* and PSB in the present study significantly increased the shoot height of foxtail millet over the control and the individual inoculants. Virtually very little information is available on the effect of *Azospirillum* inoculation along with PSB on *Setaria italica*. However a few reports are available with regard to other crops. *Azospirillum* and phosphate-solubilizing microorganisms significantly increased plant height over control in rice at flowering stage [14]. Coinoculation of *Azospirillum* and PSB increased plant height significantly over control in shola tree species [18]. Similar results were reported in pearl millet [19]. Since diazotrophs were coinoculated with phosphobacteria, increased P use efficiency in addition hormone secretion might have resulted in higher crop yield.

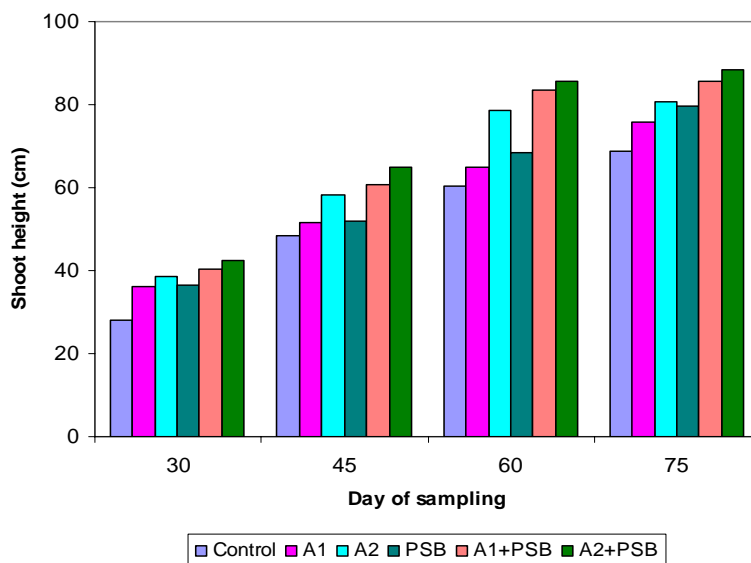
### Dry weight of shoot

Dry weight of shoot of foxtail millet was estimated at the time of harvest as described earlier during the two seasons (Rabi and Kharif). Dry weight of shoot samples was estimated at 75<sup>th</sup> day and expressed in terms of mg per plant. All the inoculants increased the shoot dry weight significantly during Rabi season. Lesser increase in shoot dry weight was observed in the plants inoculated with PSB isolate. A2 isolate was more effective in increasing the shoot dry weight compared to A1 isolate. The increase in dry matter was maximum and more pronounced with dual inoculation of A2 and PSB isolates when compared with A1 and PSB isolates Figure 3. Results on the effect of inoculants on shoot dry weight in Kharif season are shown in Figure 4.

Significant increase in shoot dry weight was observed with all the inoculants used. A maximum and pronounced increase in shoot dry weight was exhibited by the dual inoculants. A perusal of the data reveals that there was seasonal variation in shoot dry matter production. Similar results were also reported in *Setaria italica* and *Zea mays* due to inoculation with *A. brasilense* [20, 21]. An increase in shoot dry weight of *S. italica* due to inoculation with *A. brasilense* and *A. lipoferum* respectively was reported [22, 23]. Increased dry weight of finger millet upon inoculation with PSB over control plants was reported [24]. Similarly, dry matter increase was also reported in pearl millet inoculated with PSB [25]. PSB inoculation resulted in increased dry matter in maize [17]. Very few reports are available on the effect of dual inoculants on dry matter production of foxtail millet. Results from the present study indicate that dual inoculation with *Azospirillum* and PSB increased dry weight of plants over other treatments. Similarly, increased dry matter production in pearl millet with dual inoculants was reported [25]. A positive synergistic effect of *Azospirillum* and PSB is responsible for higher uptake of nutrients and yield.



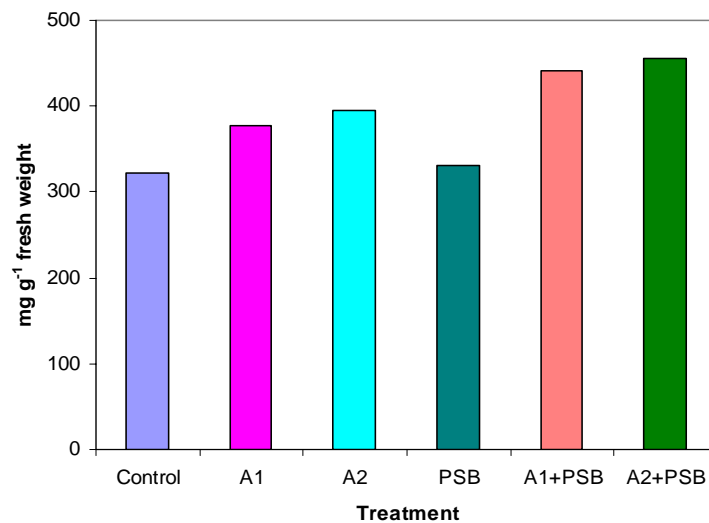
**Figure-1. Effect of inoculation of *Azospirillum* spp. and PSB on shoot height of foxtail millet variety Lepakshi in Rabi season**



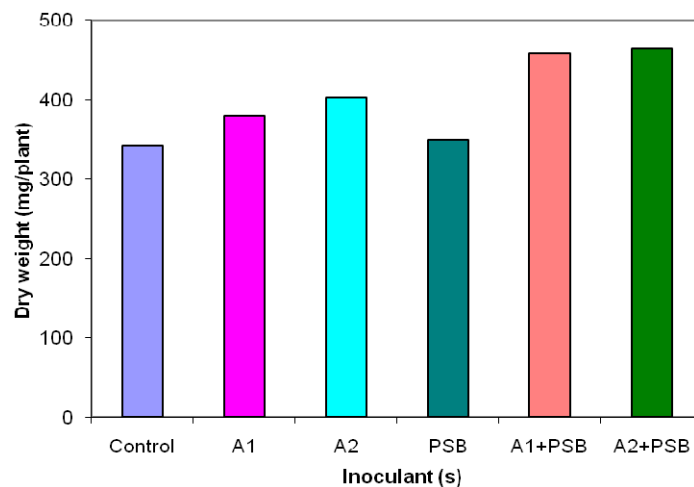
**Figure-2. Effect of inoculation of *Azospirillum* spp. and PSB on shoot height of foxtail millet variety Lepakshi in Kharif season**

**Dry weight of root:**

All the inoculants increased root dry matter significantly in Rabi season and maximum increase in dry matter production was noticed with the dual inoculants combination A2 and PSB isolates followed by A1 and PSB isolates, Figure 5. A comparison of data on root dry weight of foxtail millet in both the seasons showed that root dry matter production was less in Kharif season. All the treatments resulted in higher root dry matter yield over the control plants. Among plants receiving combined inoculation, the root dry weight of plants inoculated with A2+PSB was more than A1+PSB, Figure 6. PSB isolate in Rabi season resulted in slightly higher root dry matter when compared with A1 isolate whereas in Kharif season the increase in root dry weight was almost the same with A1 and PSB isolates. Similar results were also reported by other workers due to inoculation on root dry matter production. A significant increase in root dry weight due to inoculation with 2 strains of *A. brasilense* (Cd and Cd-1) alone over control plants in *S. italica* were reported [20,21,22]. Similarly, increased dry weight of roots and shoot due to inoculation with PSB (*B. circulans*) was reported in finger millet [24], in pearl millet [25]. Root weight was generally more responsive to inoculation. Dual inoculation with *Azospirillum* and PSB increased the dry weight of roots, which are in accord with the results reported in pearl millet [25].



**Figure-3. Effect of inoculation of *Azospirillum* spp. and PSB on shoot dry weight of foxtail millet variety Lepakshi in Rabi season**



**Figure-4. Effect of inoculation of *Azospirillum* spp. and PSB on shoot dry weight of foxtail millet variety Lepakshi in Kharif season**

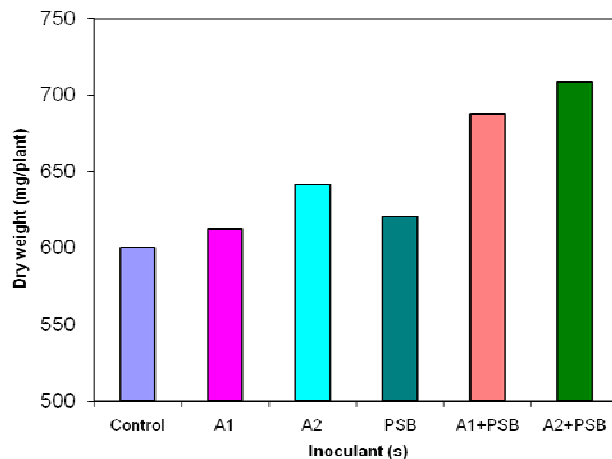


Figure-5. Effect of inoculation of *Azospirillum* spp. and PSB on root dry weight of foxtail millet variety Lepakshi in Rabi season

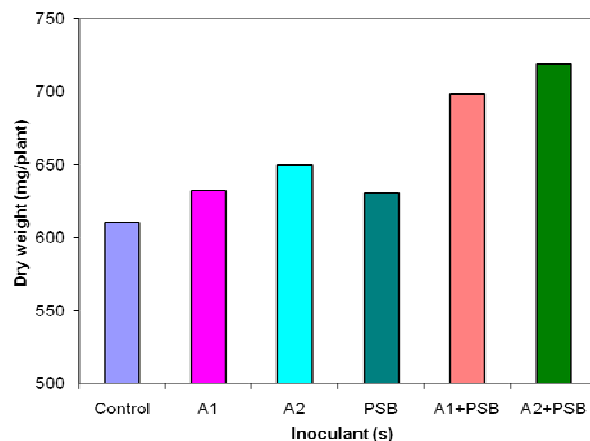


Figure-6. Effect of inoculation of *Azospirillum* spp. and PSB on root dry weight of foxtail millet variety Lepakshi in Kharif season

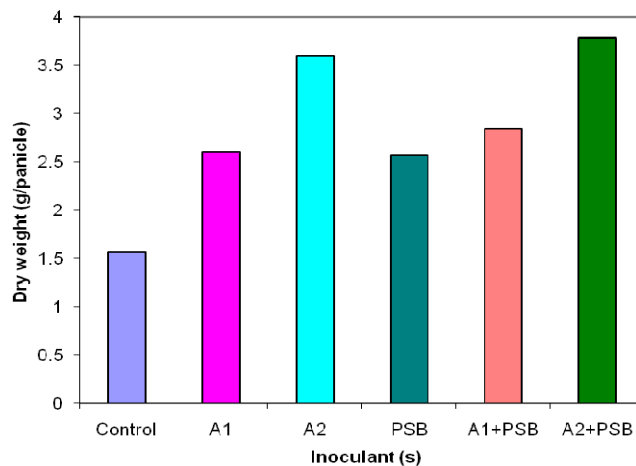
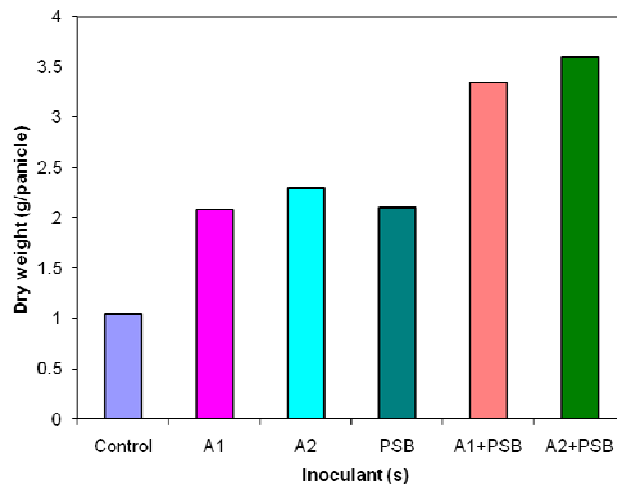
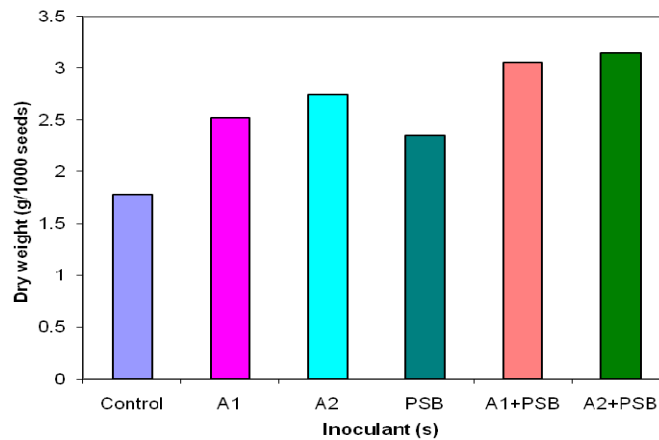


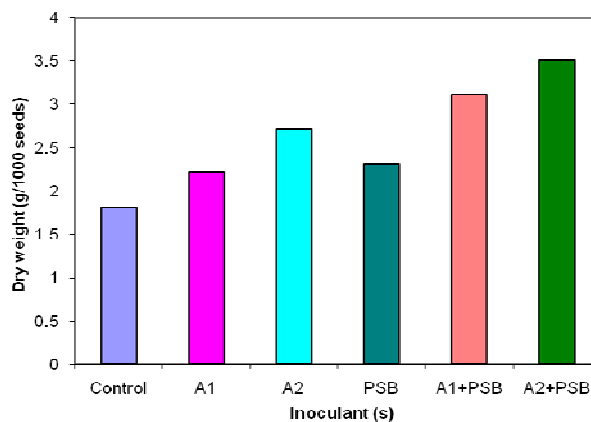
Figure-7. Effect of inoculation of *Azospirillum* spp. and PSB on panicle weight of foxtail millet variety Lepakshi in Rabi season



**Figure-8. Effect of inoculation of *Azospirillum* spp. and PSB on panicle weight of foxtail millet variety Lepakshi in Kharif season.**



**Figure-9: Effects of inoculation of *Azospirillum* spp. and PSB on weight of 1000 seeds of foxtail millet variety Lepakshi in Rabi season**



**Figure-10: Effects of inoculation of *Azospirillum* spp. and PSB on weight of 1000 seeds of foxtail millet variety Lepakshi in Kharif season**

**Effect of inoculation on yield**

Yield attributes like panicle weight and weight of 1000 seed was recorded at the time of harvest. At the time of harvest the panicle weight was determined during the two seasons. Examination of data on the effect of inoculum on panicle weight during Rabi revealed that the per cent increase in yield over control was significantly higher. Maximum per cent increase was observed in respect of plants inoculated with A2+PSB as shown in figure 7. Significant increase in panicle weight was observed in inoculated plants over control plants. Moreover comparison of the data revealed that plants responded more to inoculum in Kharif season than Rabi in respect of yield (Figure 8).

**Weight of 1000 seed**

Perusal of the data on the effect of inoculum on 1000 seed in foxtail millet revealed that the per cent increase in yield (42 to 77%) over control was significantly higher. Among inoculations there is a significant increase in 1000 seed weight in inoculated plants. Maximum per cent increase was observed in respect of plants inoculated with A2+PSB during Rabi (Figure 9). The data on the percentage increase in 1000 seed weight during Kharif was shown in Figure 10. Significant increase in seed weight (45 to 94%) was observed in inoculated plants over control plants. A significant increase in grain yield and 1000 seed weight in wheat upon inoculation with *Azospirillum* was observed by others [26, 27]. Significant increase in grain yield due to *Azospirillum* inoculation was noticed in Rabi sorghum [28], in rice [14, 29]. Similarly, increased grain yield in pearl millet with *Azospirillum* inoculation over control was observed [25]. Inoculation of foxtail millet with *Azospirillum* sp. alone increased the yield significantly [30]. Seed bacterization with *B. polymyxa* and *P. striata* showed a significant increase of yield and uptake of N and P in wheat over uninoculated control plants [31]. The enhanced growth and yield of the plants in response to dual inoculations might be due to better nitrogen fixation by *Azospirillum* coupled with availability of phosphorus made by phosphate-solubilizing bacteria. These results are in agreement with those reported in pearl millet [25]. Co-inoculation of *B. polymyxa* and *P. striata* strains showing phosphate-solubilizing ability along with a strain of *A. brasilense* resulted in significant improvement of grain and dry matter yield with a concomitant increase in N and P uptake, compared with individual inoculations in sorghum [32]. Improved grain yield in barley compared with single inoculations, when inoculated with *Agrobacterium radiobacter* (PSB) and *A. lipoferum* together was observed in pot and field experiments [33]. A positive synergistic effect of *Azospirillum* and PSB is responsible for the higher accumulation of N and higher yield. Synergistic effect of *Azospirillum* and PSB on growth and yield of foxtail millet was pronounced and are in agreement with the views expressed by other workers earlier in different crops elsewhere [18,19, 34,35,36,37].

**CONCLUSION**

Inoculation of *Azospirillum* sp. and PSB showed synergistic effect which resulted in better and increased yield in Lepakshi variety of foxtail millet.

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