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APPRAISAL OF MICROBIAL INOCULANT ON GROWTH, YIELD AND QUALITY ATTRIBUTES OF FORAGE SORGHUM

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SUMMARY

The field experiments were conducted during *kharif* season of 2016 and 2017 at Punjab Agricultural University, Regional Research Station, Bathinda, Punjab and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The experiment was conducted in RCBD and replicated thrice. Biofertilizers, *viz. Azotobacter* sp., *Sphingobacterium* sp., *Burkholderia* sp. and *Streptomyces badius* were used for seed treatment of sorghum before sowing. Total nine treatments were evaluated, T_1 : RDF, T_2 : RDF + *Azotobacter* sp., T_3 : RDF + *Azotobacter* sp.+ *Sphingobacterium* sp., T_4 : RDF + *Azotobacter* sp.+ *Burkholderia* sp, T_5 : RDF + *Azotobacter* sp.+ *Streptomyces badius* , T_6 : 75% of RDF + *Azotobacter* sp., T_7 : 75% of RDF + *Azotobacter* sp.+ *Sphingobacterium* sp., T_8 : 75% of RDF + *Azotobacter* sp.+ *Burkholderia* sp., T_9 : 75% of RDF + *Azotobacter* sp.+ *Streptomyces badius*. The result revealed that nonetheless, in all the treatments in which microbial inoculants were applied along with chemical fertilizer, numeric increases were observed. The highest growth and yield attributes were observed in T4 treatment (RDF + *Azotobacter* sp.+ *Burkholderia* sp.) at both the locations. The percentage increase in plant height, leaves per plant, tillers per plant, leaf/root ratio, green fodder yield and dry fodder yield in T4 treatment over control was 3.89, 11.28,9.6,11.2,4.11,4.28 and 4.72, 12.44.8.22,20.71,6.4,7.37, respectively at Bathinda and Ludhiana. This might be because of matching beneficial bacteria with their preferred crops, leading to optimized root colonization. Further, HCN content in all the treatments with microbial inoculants were slightly more than control but it was below permissible limit. In light of the results, it is concluded that the application of RDF + *Azotobacter* sp. + *Burkholderia* sp. recorded maximum green forage yield with good quality of forage sorghum.

Key words : Sorghum, *Azotobacter*, *Sphingobacterium*, *Burkholderia*, *Streptomyces*, fodder yield

Livestock is one of the most economically important sub sectors of agriculture. It contributes 25 per cent to the total agricultural income. India supports 15 per cent of the world's livestock population with only 2.5 per cent of the world's geographical area. Since the beginning of 1980; milk production has shown an average compound annual growth rate of nearly 4.5 per cent (Ravi Shankar, 2008). India has a huge livestock population of 512.1 million. Nonetheless, the production of milk and other livestock products are about the lowest in the world because of vast gap between demand and supply of all kind of feed and fodders.

Forages are the mainstay of animal wealth and their production. The scarcity of green forages and grazing resources in the country has made the livestock to suffer continuously with malnutrition resulting in their production potentiality at sub optimum level as compared to many developed nations (Crawford *et al.*, 2018).The projected shortages of dry and green fodder are 40 and 36 per cent compared with the requirement of 416 and 222 million tonnes for the current livestock population, respectively (Agriculture Statistics, 2012). The scope of increasing the area cultivated for forages is rather limited, because of mounting pressure and preferential need for food and commercial crops (Backiyavathy *et al.*, 2006). Thus, improving the yield and nutritional quality of forage crops can help mitigate the unsustainable negative impacts of livestock production (Capstaff* and Miller, 2018).

Sorghum (*Jowar*) is a very important *kharif* fodder cultivated on 2.70 lakh hectares (2016-17) and remains palatable and green over an extended period than bajra and maize fodders (Anonymous, 2018). Sorghum crop exhausts more nutrients than other forage crops and being a cereal crop, its requirement is in higher amount of nitrogen (Crawford *et al.*, 2018). Disparity in the use of fertilizers has been one of the key factors in shrinking crop productivity and depleting the soil fertility. Best nutrition is required for getting the maximum forage yield and quality. Organic manures are good complimentary sources of nutrients which further enhance the efficiency of the applied mineral nutrients in addition to improving biological and physical properties of soil on other hand (Chaudhary *et al.*, 2004). A sensible and amalgamated use of inorganic and organic sources of plant nutrients is crucial to maintain soil health and manage the efficiency of nutrients. Integration of nutrients plays an essential role in better establishment and penetration of sorghum roots, which helps the plant to take up water from deeper layers and to maintain high relative plant water content under soil moisture deficit condition (Jadhao *et al*., 2002).

Integrated nutrient management (INM) plays a key role in modern agriculture in increasing the productivity of crops and sustained management of soil fertility (Singh, *et al*., 2016). Microbial inoculants can be important components of INM. Keeping foresaid points in view, present research was carried out for appraisal of microbial inoculant on quality and yield attributes of forage sorghum under field conditions.

MATERIAL AND METHODS

The field experiments were conducted during *kharif* season of 2016 and 2017 at Punjab Agricultural University, Regional Research Station, Bathinda, Punjab (30^o 09''36' N latitude, 74^o 55''28' E longitude; 211m above sea level) and Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The soil of the experimental site at Bathinda is sandy loam in texture having alkaline pH (8.40) and low in organic carbon (0.29%). The nutrient status of the soil during start of the experiment was low in available nitrogen (110 kg ha^{-1}), medium in available phosphorus (15.9 kg ha-1) and high in available potassium (326.0 kg ha^{-1}). Similarly, the soil of Ludhiana Research farm is loamy sand in nature. The organic carbon content and pH of surface soil layer (0-15 cm) were 0.38% and 8.30 respectively. The available nitrogen, phosphorus and potash in the surface layer were 171.5, 19.2 and 145.2 kg ha^{-1}, respectively. Single cut sorghum variety SL 44 was sown at the rate of 25 kg ha^{-1} with a spacing of 22 cm. The field experiment was laid out in RCBD and replicated thrice. Nitrogen and phosphorus were applied as per the treatments through urea and single super phosphate. Biofertilizers, viz. *Azotobacter* sp., *Sphingobacterium* sp., *Burkholderia* sp. and *Streptomyces badius* were used for seed treatment of sorghum before sowing. Total nine treatments were tried T_1 : RDF, T_2 : RDF + *Azotobacter* sp., T_3 : RDF + *Azotobacter* sp.+ *Sphingobacterium* sp., T_4 : RDF + *Azotobacter* sp.+ *Burkholderia* sp, T_5 : RDF + *Azotobacter* sp.+ *Streptomyces badius* , T_6 : 75% of RDF + *Azotobacter* sp., T_7 : 75% of RDF + *Azotobacter* sp.+ *Sphingobacterium* sp., T_8 : 75% of RDF + *Azotobacter* sp.+ *Burkholderia* sp., T_9 : 75% of RDF + *Azotobacter* sp.+ *Streptomyces badius*. Other cultural operations and plant protection measures were followed as per the recommendations. At 70 days after sowing (DAS), green fodder yield from net plot was recorded. A representative green fodder sample from each net plot was oven dried and then dry fodder yield was calculated based on loss of moisture percentage. Data were recorded on plant height, number of leaves per plant and leaf stem ratio from five random plants/ plot in the net plot area at harvesting. The HCN content in forage plants was determined by the Picric acid method of Hogg and Ah gren (1942). The data of two years were pooled and statistically analyzed for interpretation of results.

RESULTS AND DISCUSSION

Inoculation of *Sorghum bicolor* (C4 plant) with selected microbial inoculants can enhanced plant growth through nutrient uptake and biological nitrogen fixation (BNF). Therefore, the objective of the current study was to evaluate the ability of microbial inoculants to improve sorghum growth under field conditions. The results obtained from the present investigation as well as pertinent discussion have been summarized under following heads.

Plant Height : The data on plant height of forage sorghum recorded at harvest as influenced by different integrated nutrient management treatments are presented in Table 1 and 3. The result revealed that the effect of integrated nutrient management treatments on plant height at harvest was non significant. Nonetheless, in all the treatments in which microbial inoculants were applied along with chemical fertilizer, numeric increases have been noted. However, highest plant height was observed in T4 treatment (RDF + *Azotobacter* sp.+ *Burkholderia* sp.) at both the locations. The percentage increase in height with T4 treatment over control was 3.89 per cent and 4.72 percent at Bathinda and Ludhiana respectively. This might be because of matching beneficial bacteria with their preferred crops might have optimized root colonization and biocontrol (Raaijmakers & Weller, 2001).Further, enhanced height by dual inoculation of *Azotobacter* sp. + *Burkholderia* sp might be because of production of phytohormones by *Azotobacter* sp. + *Burkholderia* sp. The term "phytohormone" is confined to naturally occurring substances and includes mainly cytokinins, auxins and gibberellins. These are activators of growth and enlargement processes (Kukreja *et al.*, 2004). Ahmad *et al.* (2008) demonstrated quantitative production of indole acetic acid by seven isolates of *Azotobacter*. Baral and Adhikari (2013) also reported production of

TABLE 1
Effect of various biofertillizers on yield attributes of sorghum at Bathinda location

Treatment	Plant height (cm)	Leaves/ plant (no.)	Tillers/ plant	Leaf/roof ratio
T_1	153.8	11.05	19.4	0.399
T_2	155.8	11.18	20.1	0.407
T_3	159.8	11.13	20.6	0.424
T_4	161.2	11.28	22.7	0.443
T_5	155.7	11.25	21.4	0.438
T_6	155.0	11.06	18.3	0.407
T_7	158.1	11.11	20.8	0.410
T_8	160.8	11.25	21.2	0.427
T_9	154.7	11.15	20.0	0.408
C. D. (P=0.05)	NS	NS	NS	NS

TABLE 2
Effect of various biofertillizers on green and dry fodder yield, HCN content in sorghum at Bathinda location

Treatment	Green fodder yield (q/ha)	Dry fodder yield (q/ha)	HCN content at 70 Days after sowing
T_1	413	100.4	77.0
T_2	424	101.7	78.8
T_3	425	103.0	79.1
T_4	430	104.7	79.4
T_5	422	101.2	79.8
T_6	420	100.8	77.4
T_7	421	101.3	77.7
T_8	427	103.3	78.0
T_9	419	98.3	78.4
C. D. (P=0.05)	NS	NS	NS

gibberellins, indole acetic acid, riboflavin and thiamin besides nitrogen fixation by Azotobacter. The genus Azotobacter includes 6 species of *Azotobacter* and *A. chroococcum* most commonly inhabiting in diverse soils all over the world (Mahato *et al.*, 2009). Likewise, Tallapragada *et al.* (2015) isolated and identified strain of *Burkholderia seminalis* (*B.seminalis*) using 16s rDNA and further reported production of IAA from *B. seminalis* in nutrient broth (supplemented with tryptophan) and was established through thin layer chromatography.

Further, Meena *et al.* (2010) also carried out a field experiment and indicated that application of RDF through inorganic fertilizer along with biofertilizer gave maximal plant height of 301.7 cm, green fodder yield 490.42 q ha^{-1} and dry matter yield 179.25 q ha^{-1}. Bouthaina *et al*. (2010) demonstrated that the plant height, shoot and root fresh and dry weights, leaf area, root length, enhanced significantly with treatments of biofertilizer.

Leaves per plant : Leaves play a predominant role in producing and furnishing of food material synthesized during photosynthesis. Thus decrease or increase in number of leaves per plant has an undeviating effect on the green forage yield of forage crops (Shivprasad and Singh, 2017). Leaves per plant with recommended dose of fertilizers (T_1) was statistically at par with all the recommended dose of fertilizers treatments along with microbial inoculants and 75 % of recommended dose along with microbial inoculants. Nevertheless, the numbers of leaves per plant were numerically higher in all the treatment with microbial inoculants. Further highest no. of leaves per plant was observed with RDF+ *Azotobacter* sp. + *Burkholderia* sp i.e 11.28 and 12.44 leaves per plant at Bathinda and Ludhiana, respectively. The percentage increase in no. of leaves with RDF+ *Azotobacter* sp. + *Burkholderia* sp treatment over RDF only (control) was 2.0 per cent and 2.13 per cent at Bathinda and Ludhiana respectively .The beneficial effects of microbial inoculants might be due to increase in the availability of phosphorus (P) and nitrogen (N) due to biological nitrogen fixation (BNF) and phosphate solublization that has resulted in enhanced vegetative growth of the plant. Further, increase availability of P by microbial inoculants results in augmented cell division, cell elongation as well as healthy root

TABLE 3
Effect of various biofertillizers on yield attributes of sorghum at Ludhiana location

Treatment	Plant height (cm)	Leaves/plant (no.)	Tillers/plant (no.)	Leaf/root ratio
T_1	163.3	12.18	21.9	0.420
T_2	165.7	12.31	22.1	0.427
T_3	168.3	12.29	22.3	0.490
T_4	171.0	12.44	23.7	0.507
T_5	167.7	12.38	22.0	0.440
T_6	167.3	12.21	19.9	0.423
T_7	166.3	12.28	19.7	0.450
T_8	169.2	12.35	22.4	0.457
T_9	166.3	12.31	18.6	0.433
C. D. (P=0.05)	NS	NS	NS	NS

TABLE 4
Effect of various biofertillizers on green and dry fodder yield, HCN content in sorghum at Ludhiana location

Treatment	Green fodder yield (q/ha)	Dry fodder yield (q/ha)	HCN content at 70 Days after sowing
T_1	545	135.6	77.2
T_2	567	141.4	79.3
T_3	572	145.3	79.8
T_4	580	145.6	79.7
T_5	571	143.5	80.1
T_6	569	141.2	77.8
T_7	571	143.7	78
T_8	579	144.6	78.5
T_9	565	142.5	78.9
C. D. (P=0.05)	NS	NS	NS

development, which might have enhanced the uptake of nutrients and which in turn promoted more no. of leaves per plant.

Enhanced growth parameters viz. length, stem girth and number of leaves due to biofertilizer or microbial inoculants application of cashew grafts var. Chintamani-1 was studied by Shankarappa *et al.* (2017) under greenhouse condition. Similarly, Rana and Chandel (2003) noticed that *Azotobacter* inoculated plants had significantly higher plant height, number of leaves per plant and leaf area in strawberry. Ingle *et al*. (2008) also recorded that growth parameters like plant height, number of branches, number of leaves, number of internodes and length of third internodes per plant were significantly improved in okra with 100 per cent nitrogen, *Azotobacter* and phosphate solubilizing bacteria.

Tillers per plant : Number of tillers/plant is a predominant yield contributing parameter. Higher the number of tillers, higher will be the fodder yield. The numbers of tillers per plant were not affected significantly at harvest in all the treatments. However, data presented in Table 1 and 3 showed all the microbial inoculants irrespective of their application as single or dual produced more number of tillers per plant. The elevated no. of tillers per plant was observed with RDF+ *Azotobacter* sp. + *Burkholderia* sp i.e 22.7 and 23.7 leaves per plant at Bathinda and Ludhiana respectively. The percentage increase in no. of tillers per plant with RDF+ *Azotobacter* sp. + *Burkholderia* sp treatment over RDF only (control) was 9.6 per cent and 8.22 per cent at Bathinda and Ludhiana respectively The increase in number of tillers per plant with inoculation of microbial inoculant may be due to production of plant growth hormones such as IAA, cytokinins and gibberellin which further enhance the uptake of nutrients. Moreover, nutrients like Phosphorous (P) is a prime nutrient required for normal growth and metabolic process occurring in plants (Singh and Satyanarayana, 2011). Phosphorus effects many plant operations like seed germination, seed maturity and plant growth rate, which comprises root development of stalk and stem of the plants, flower and seed formation, N_2- fixation, energy metabolism, synthesis of nucleic acid, photosynthesis, respiration, crop quality and resistance against various biotic and abiotic stresses (Singh and Prasad, 2014). Saleem *et al* (2015) demonstrated significant effect of inoculation with nitrogenous strains (*Azotobacter* and *Azospirillum*) on number of tillers m^{-2}

Leaf to Stem Ratio : The leaf: stem ratio was worked out by weight of leaves divided by the weight of stem, the mean values were worked out and recorded for each plot. Treatment did not differ significantly regarding the data in Table 1 of leaf to stem ratio. However, maximum leaf to stem ratio was recorded in the treatment T_4: RDF+ *Azotobacter* sp. + *Burkholderia* sp at both the locations i.e Bathinda and Ludhiana. The inoculation might have enhanced nitrogen availability to vegetative parts especially leaves thus increasing the size and fresh weight of leaves which resulted in wider leaf to stem ratio. This might be also due to favourable influence of nitrogen on cell elongation and cell division, which could have made more functional leaves for a longer period of time (Nabooji *et al.*, 2018). The results were in harmony with those of El-Tookhy *et al.* (2000) who reported significant promoting effect of seed inoculation on leaf to stem ratio of barley (forage).

Green fodder yield (q/ha) and Dry fodder yield : Forage yield is a function of genetic and environmental factors which plays a predominant role in plant growth as well as Development (Karthika and R. Kalpana, 2017). Higher green and dry forage yield was recorded by the application of RDF + *Azotobacter* sp.+ *Burkholderia* sp. at harvest of forage sorghum (Table 2 and 4). Green forage yield obtained in this treatment was 4.11 per cent and 6.4 per cent higher over application of RDF only at Bathinda and Ludhiana at harvest respectively. The increase in total green forage yield might be due to the positive effect of nitrogen on plant height, number of tillers per Plant. This further might be due to biological nitrogen fixation. Similar results have been reported by Farboodi *et al.*, 2011 who also observed significant increase in plant biomass of maize cultivars with seed inoculation and nitrogen fertilization at recommended dose.

Dry forage production is basically a measure of photosynthetic efficiency of assimilatory system in plants. Dried stalk yield refers to the function of maximum nutrients accumulation in plant biomass, the genetic makeup of a crop, soil nutrient status and management strategies (Saleem *et al.*, 2015. Similar trend was also observed for dry matter yield with the application of microbial inoculants as in green forage. Dry forage yield was also higher by 4.28 and 7.37 per cent over control (RDF only) at harvest. This might be attributed to balanced nutrients provided to the crop which resulted into significantly increase in green forage yield and hence dry forage. This may also be due to increased availability and absorption of nutrients mostly nitrogen and phosphorus to plant which eventually resulted in additional vegetative growth due to improvement in plant height and tillers on the account of enlargement of cells.

Moreover, Nitrogen is required by plants in greater amount, it comprises about 1.5–2.0% of plant dry matter, besides approximately 16% of total plant protein (Alvarez *et al*., 2012). Studies have revealed that nitrogen is also an indispensable constituent of chlorophylls, which is closely associated with photosynthetic process (Nursu'aidah *et al*., 2014). Whereas, Phosphorus is abundantly present in the form of phosphate is in cell membranes of the plant, where it plays vital roles in being the constituent of DNA, RNA, and ATP (Brown and Weselby, 2010).Thus, it is regarded as a key component for growth and development of plants.

The improvement in growth and yield parameters from inoculum treatments was also demonstrated in earlier studies conducted on various crops (Bashan *et al*., 2004, Shaalan, 2005, Abd El-Ghany *et al*., 2010). These findings were similar with those of Gadhethariya *et al.* (2000) who reported that inoculation either with *Azotobacter* or *Azospirillum* produced significantly higher green forage (481 and 495 q ha^{-1}, respectively) and dry forage yields (221 and 231 q ha^{-1}, respectively) of forage sorghum during first cut over the uninoculated crop. The results are supported by the findings of Dos Santos *et al*. (2017) observed significant increase in the biomass of grass and grain sorghum inoculated with *Burkholderia* sp. In other study, increased forage yield of sorghum by inoculation of seeds by *Azotobacter* and *Azospirillum* reported by Singh *et al*., 2005. This organism is capable of producing antifungal compounds, antibacterial, hormones and siderophore (Sharma 2002).These results are in line with those of [Mandal *et al.*, 2000] who reported an increase of 10% **-** 15% in green and dry forage yield of oat through seed inoculation. Increase in fodder yield with integrated nutrient management was also reported by Das *et al*. (2008). Similarly, Mahdi *et al*. (2010) showed that bio fertilizer being crucial components of organic farming play necessary role in maintaining long term fertility and sustainability by fixing atmospheric nitrogen.

HCN content at 70 days after sowing : HCN is readily absorbed into the blood stream of grazing ruminants and results in cellular asphyxiation leading to illness of cattle finally resulting in the end of animals and even at doses as little as 0.5 g are sufficient to kill a cow. Hydrocyanic acid content in excess of 500 ppm (on wet weight basis) in the forage sorghum is toxic to the animal health (Karthika and Kalpana, 2017).The perusal of the data revealed that non significant impact of INM on HCN content, nevertheless lowest HCN was noted in RDF only i.e control at both the location. In all the treatment with microbial inoculants numeric increase in HCN content was observed at Bathinda and Ludhiana respectively but it was with in the permissible limit. The numeric increase in HCN content with microbial inoculants might be due to enhanced availability of nitrogen.

To recapitulate, it is concluded that the application of RDF + *Azotobacter* sp.+ *Burkholderia* sp. recorded maximum green forage yield with good quality of forage sorghum. These microbial inoculants could play a significant role in increasing the productivity per unit area which is need of hour in neoteric era due to burgeoning pressure on natural resources.

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