Influence of biofertilizers and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) Link.

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In the present investigation, the effect of biofertilizers (*Azotobacter* and *Azospirillum*) and synthetic fertilizers (urea) were studied separately and in different combinations to establish morphological, biochemical, yield and biomass effects of *Echinochloa frumentacea*. Both bacterial inoculants at all levels and combination of chemical nitrogen show an increase in growth, yield and biochemical components when compared to the control (with out biofertilizers and chemical N_2). The result reveals that both morphology and yield parameter produces a better result during the combination of biofertilizers and chemical fertilizers than using either method alone. The highest yield of *Azospirillum* with 100% urea treatment obtained 8525.55 Kg/ha^{-1}. Biofertilizers with 100% urea treatment produced highest yields compared control. When compared the *Azospirillum* and *Azotobacter* combinations, *Azospirillum* along with 100% urea yielded better results than control.

Key words: biofertilizers, biochemical, inoculants, morphology, millet, urea

Introduction

India, the second largest populous country, mostly depends on agriculture for living. The population explosion has created a tremendous pressure on agriculture. The use of agricultural land for various industrial purposes reduces the area for production.

Most of Indian agricultural lands are deprived of some of the essential nutrients for growth and development of crop plants. One of the major essential elements for growth of plants is nitrogen. Nitrogen is required in large quantities for plants to grow, since it is the basic constituent of proteins, and nucleic acids. Nitrogen is provided in the form of synthetic chemical fertilizer

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(urea). Such chemical fertilizers pose a health hazard and microbial population problem in soil besides beings quite expensive and making the cost of production high. In such a situation the biofertilizers play a major role (Tiwary *et al*., 1998).

Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbiotically with plants (Subba Rao, 1993). Biofertilizers are inputs containing microorganisms which are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes (Tien *et al*., 1979). Biological nitrogen fixation is carried out by both symbiotic and free living bacteria and blue green algae. Symbiotic nitrogen fixation provides 80% of the biologically fixed nitrogen on land. Nitrogen fixing bacteria are very selective in choosing roots of particular legumes species to infect, invade and form root nodules (Subba Rao, 1993). A unique blend of organic manure using micro nutrients and some beneficial microorganisms with sugarcane press mud as base materials has been reported as useful (Arangarasan *et al*., 2000).

Echinochloa frumentacea (Robx.) Link is one of the important millets of India and, it is the quickest growing of all millets, in all conditions (Anonymous, 1952). The millet is cultivated in almost all the states in India as rain fed crop. It is grown as subordinate crop to sorghum or maize in the drier districts and, sometimes, as a fodder or green manure crop. The millet is consumed mostly by the poor classes either cooked in water like rice, or parched or boiled with milk and sugar. It is sometimes mixed with rice and fermented to produce a beer. The grains are also used for feeding cage birds. The grains have the follow contents (percentage): moisture 11.1; protein 6.2; other extracts 2.2; minerals 4.4; crude fibre, 9.8; carbohydrates, 65.5; Ca 0.002; and P 0.28; Fe 2.9 mg/100g; and a carotene trace. The principal protein is a prolamine rich in lysine, cystine and histideine, with a nutritive value markedly superior to that of polished rice. The presence of vitamin B_1 in sufficient amount to prevent vitamin B_1 deficiency (Anonymous, 1952).

In the present agricultural practices, there are number of microbial inoculants used as biofertilizers. They include *Azospirillum* and *Azotobacter*, and *Phosphobacterium*, which have been given much attention as they are responsible to plant growth and yield of crops under field inoculation. In the present investigation, the effect of biofertilizers (*Azotobacter* and *Azospirillum*) and synthetic fertilizers (urea) were studied separately and in different combinations to establish the morphological, biochemical, yield and biomass effects on *Echinochloa frumentacea*.

Materials and methods

Field and experimental design

The field experiments were conducted at research field of St. Joseph's college, Tiruchirappalli, Tamil Nadu during the Rabi seasons. Three random soil samples were taken from the field before starting the experiment and are tested in Government soil testing laboratory, Agricultural Department, Tiruchirappalli, to establsih the pH, soil texture and the total amount of nutrients present in the soil. The plants were spaced 10 × 10 cm apart and were supplied with the recommended dose of phosphorus (4.5 g P_2O_5 / Sq. m.) and potassium (4.5 g K_2O/ Sq. m.). The dose of nitrogen (9g N_2 / Sq. m.) was manipulated at various levels in combination with different biofertilizers as per the treatment schedule. The different treatment combination as follows:

R0 – control
R1 – 50% Urea (N_2)
R2 – 100% Urea (N_2)
R3 – *Azospirillum* alone
R4 – *Azospirillum* + 50 % Urea
R5 - *Azospirillum* + 100% Urea
R6 – *Azotobacter* alone
R7 – *Azotobacter* + 50% Urea
R8 – *Azotobacter* + 100 % Urea

The experiment was carried out in a randomized block design with nine treatments and three replications.

Plant materials and biofertilizers

Echinochloa frumentacea (Roxb.) Link., is a millet belonging to the family Graminaceae, Seeds were procured from Agricultural department, Ramanathapuram. The biofertilizers used as inoculums for seed treatment were *Azospirillum* and *Azotobacter*. The carrier based inoculum's packets were obtained from Stane's Company Ltd., Gundoor, Tiruchirappalli, Tamilnadu.

Morphological estimation

The morphological characters such as height of the plants, number of leaves, leaf length and leaf area were estimated at 20 days intervals (i.e., 20th, 40th, 60th and 80th days) are calculated.

Biochemical estimation

The biochemical constituents such as total Chlorophyll (Arnon method), total Sugar (Willis and Yemm method) and total Nitrogen (Nessler's method) were estimated using standardized protocols. Experiments for the determination of above mentioned cellular constituents were carried out using dried leaves except chlorophyll for which fresh leaves was used.

Biomass and yield parameters

After harvesting the biomass (fresh as well as dried) of the whole plant were estimated. Then the spike length and grain yield were estimated in square meter is calculated into hector.

Statistical analysis

Experiments were repeated at least two times each in three replicates. Significance of the 5% level was considered to demonstrate differences by using two ways ANOVA by new SPSS statistical software.

Results and discussion

Growth attributes

The maximal plant height, number of leaves, leaf area and leaf length were observed in the plot treated with *Azospirillum* along with 100% urea (N_2), followed by *Azotobacter* along with 100% urea. Both treatments resulted in significant increases in plant growth as compared to the control plant at 20 days. *Azospirillum* plus 100% urea treated plants had greater growth compared to plants treated with *Azotobacter* and 100% urea. Similar trends were also seen on the 40^{th}, 60^{th} and 80^{th} days.

The plants treated with *Azospirillum* plus 100% urea were 57% higher than that of the control, while the least difference was found in plants treated with *Azotobacter* alone, i.e. 14.6% higher than the control (Table 1). It is evident from the above results that the biofertilizers had a beneficial effect on growth attributes namely height of the plant by contributing nitrogen. Similar results were reported in maize and wheat plants where the inoculation with *Azospirillum* along with synthetic fertilizers mostly increased the height of shoots and roots (Rousta *et al*., 1998).

The number of leaves is an important factor, because the leaves are structures bearing photosynthetic machinery and an increase in leaf number, may promote the better root development better translocation of water uptake and deposition of nutrients and yield (Jevajyothi *et al*., 1993).

The increase in leaf length could be attributed to an increase in the length of the petiole which in turn is capable of assimilating maximum sunlight through the lamina. Plant growth regulating substance such as IAA, GA_3 and cytokines produced by *Azospirillum* are known to promote better growth (Tien *et al*., 1979; Tiwary *et al*., 1998).

The plants treated with *Azospirillum* along with 100% urea have maximum leaf area. Corresponding to the increased leaf number, an increment in leaf area index could be observed. It is well known that the leaf area index is related to primary productivity, which exhibit maximum efficiency in CO_2 assimilation and $C_6\ H_{12}\ O_6$ production. So through the application of biofertilizers it is obvious that the seed production and nutritive value of seeds should increase considerably. The benefits of *Azospirillum* inoculants have been shown to reduce N_2 requirement by 25% in paddy, sorghum and sunflower fields (Varma, 1993).

Biochemical attributes

Biofertilizers had a beneficial effect on growth attributes of *Echinochloa frumentacea* (Roxb.) Link. Biochemical parameters showed a good response to the bacterial inoculants. Treatments of *Azospirillum* and *Azotobacter* plus 100% urea resulted in significant increases in total chlorophyll, total sugar and total nitrogen as compared to the control treatment at the 20^{th}, 40^{th} and 60^{th} days. Decreases in biochemical content were observed after 60^{th} days in all the treatments (Table 2). The chlorophyll content was observed after the 60^{th} days, which is due to the loss of greenness as the plant matures. The beneficial effects of bacterial inoculation on increased chlorophyll content might have been due to the supply of higher amount nitrogen to the growing tissue and organs supplied by N_2 fixing *Azospirillum* and *Azotobacter*. Rukmani (1990) demonstrated the effect of *Azospirillum* on various growth and yield characters in Okra where the treatment with *Azospirillum* resulted in significant increase in total chlorophyll content.

The plants treated with bacterial inoculants show better results. The decrease in sugar content may attribute to the redistribution of sugar from the vegetative part to the reproductive part of the plant. This can be explained by the fact that the sugar transported from flowering parts is used by the developing

seeds. A fairly high amount of reducing sugar was recorded in banana inoculated with *Azospirillum* and *Azotobacter* (Tiwary *et al*., 1998). Improved total sugar content was also noted as a result of *Azospirillum* and *Azotobacter* inoculation in *Sorghum* (Kishore, 1998).

The effect of biofertilizers on total nitrogen content in leaf tissue was estimated at 20 days intervals. Leaves of the plant treated with *Azospirillum* together with 100% urea contained the highest amount of nitrogen throughout the period of growth. Inoculation of maize plants with *Azospirillum* showed an appreciable increase in the mean dry weight of shoots and roots, besides having higher NR activity in both leaves and roots (Ribaudo *et al*., 1998). El-Komy and Wahab (1998) reported that action of *Azospirillum* strain on Fababeen increased fixation of N_2 significantly. Application of cell free extract of *Azospirillum* also resulted in significant increase in plant growth and total N_2 yield. It is well known that the developing seeds utilize nitrogen from the vegetative parts for the synthesis of storage and non-storage seed proteins. As a consequence, the nitrogen content of vegetative parts decreased after the formation of spikes.

Biomass and yield attributes

The yield and biomass also increased significantly in plants treated with bacterial inoculants with chemical fertilizers. *Azospirillum* and *Azotobacter* with 50% and 100% urea resulted in a significant increase in 90 days after treatment (Table 3). Kishore (1998) reported an improved agronomic parameters viz., green fodder and yield in Sorghum inoculated with *Azospirillum*.

The effect of biofertilizers on ear length (Fig. 1) of the plant also caused significant increases. An increased ear length i.e. 56.91% over the control was found in *Azospirillum* along with 100% urea treated plants. Increase in ear length may increase the number of grains in spikes besides increasing the size of the grain. Kumar and Balasubramanian (1986) have shown an increase in grain size of rice as a result of biofertilizer application.

A highest increase in yield was found in the plot treated with *Azospirillum* along with 100% urea where 218.35g/sq.m. of grain was harvested. It is evident that the increases in plant height, leaf number and leaf area have contributed to increased yield plots supplied with *Azospirillum* along with 100% urea. Similar results were reported earlier by Jha and Mathur (1993) in *Pennisetum glaueum* inoculated with *Azospirillum*. Some other related results were obtained in general; the growth and yield attributes exhibited maximum values in treatments of bacteria inoculums and seedling treatments in

combination with 75% and 100% nitrogen application (Subba Rao *et al*., 1979; Panward *et al*., 1999; Hegde *et al*., 1999; Rethati *et al*., 2000; Selvakumari *et al*., 2000).

The plots supplied with 100% urea produced 245.20 g/ sq.m. of grain per plot whereas the same was found with 50% urea along with *Azotobacter* and *Azospirillum* as 243.10 and 246.23 g/ sq.m grain per plot was obtained respectively. *Azospirillum* inoculation with low level of chemical nitrogen (50%) markedly increased growth, yield and biochemical attributes over control. Similarly Vendan and Subramanian (1998) reported that a net amount of 50% fertilizers N_2 could be saved by inoculating *Azospirillum* and *Phosphobacteria* with rice. Therefore it is evident that a combination of biofertilizers with synthetic N_2 fertilizers could save 50% of urea aplication.

Figure 1. Details of treatments:

R_0-Control	R_1-50 %urea
R_2-100%urea	R_3- *Azospirillum* alone
R_4- *Azospirillum* + 50%urea	R_5- *Azospirillum* + 100%urea
R_6-*Azotobacter* alone	R_7-*Azotobacter*+50%urea
R_8- *Azotobacter*+100%urea	

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