



Studies on Stover Yield of Maize with Basal Application of Fertilizer and Foliar Spraying of *Gluconacetobacter diazotrophicus* on Available Nutrients in Soil

Garima Padwar ^{a*}, Gaurav Kumar Padwar ^b
and Sharda Dubey ^c

^a Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India.

^b Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G., India.

^c Mangalayatan University, Beswan, Aligarh, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experiment on "Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotrophicus* on available nutrients in soil" was carried out during Kharif 2022 in the experimental field under Department of Soil Science & Agricultural Chemistry. The experiment was carried out according to a randomized block design (RBD) with 3 replication including 18 treatments of the microbial consortium *Arthrobacter* sp., phosphate-solubilizing bacteria (*Bacillus* sp.) and soluble bacteria. There is also *Gluconacetobacter diazotrophicus*

*Corresponding author: E-mail: garimapadwar19@gmail.com;

sprayed on leaves at 25, 45 and 65 DAS of the plant growth stages. Maize seeds (cv. JM 216, duration 110 days, sowing 20 kg/ha) were sown in the specified plot (size 3mx2m) on June 28, 2022, with a distance of 60 and 20 cm. Plants are supplemented with the recommended dose of fertilizer 120:60:40 (Nitrogen:P2O5:K2O kg/ha) in basic fertilization. Additionally, two types of control plots were maintained: fertilized un-inoculated (FUI) control plots and un-inoculated (UFUI) control plots. Regarding the study of main nutrients in the soil at harvest time, the combination of similar treatments also increased N, P and K by 21.31, 21.31 and 60.6%, respectively. The most effective treatment was performed systematically by a combination of RDF+Arthro+PSB+KSB treatments for all parameters. Simultaneous inoculation of biological products *G. diazotrophicus*, Arthrobacter, PSB (*Bacillus sp.*) and KSB (*Frateruria aurantia*) when treating seeds and foliar fertilizing maize increased growth and the ability to provide important nutrients in the soil at harvest time. This is the positive impact of *G.diazotrophicus* and Arthrobacter. The data on stover yield ranged from 5618- 10729 kg/ha. All the treatments (except *G. diazotrophicus*) increased significantly the grain yield over the control fertilized uninoculated (FUI). Among all the treatment combinations.

Keywords: Maize; *G. diazotrophicus*; arthrobacter; PSB (*Bacillus sp.*); KSB (*Frateruria aurantia*); FUI (Fertilized un-inoculated); UFUI (Unfertilized un-inoculated).

1. INTRODUCTION

“Maize (*Zea mays* L.) is the most popular grain in the world. India ranks fifth in area and tenth in production and third after wheat and rice in total cereal production. Because of its adaptability and high productivity, corn cultivation quickly spread throughout the world and is now produced in almost every country in the world” [1]. “Corn is traditionally grown for food, mainly for domestic consumption, but demand for animal feed and industrial use has increased rapidly in recent times. In our country, more than 50% of corn output is used as animal feed. The state of Madhya Pradesh accounts for 13% of the total maize area and contributes equally to the total maize production of the country. Nutritionally, corn contains 60-68% starch, 1.2-5.7% cooking oil and 7-15% protein. In India, maize is grown on an area of 7.18 million hectares, contributing to an output of 14.1 million tons with a yield of 1959 kg ha⁻¹” [2].

“*Gluconacetobacter diazotrophicus* is a gram-negative, flagellated, microscopically dark brown or orange, aerobic, obligately endosymbiotic (endosymbiotic) and nitrogen-fixing endophytic bacterium of non-family plants” [3]. “In addition, it has many other useful properties in the field of agriculture, namely promoting plant growth, sugar metabolism pathways, excretion of organic acids and promoting increased phosphate solubility as well as zinc” [4], “auxin synthesis, antifungal and antibacterial properties and the appearance of bacteriocins (protein toxins that inhibit the growth of closely related bacterial strains). This bacterium is less plant/crop specific and is found in several unrelated plant species.

G. diazotrophicus requires large amounts of sucrose for full growth” [5]. “It is recommended as a biofertilizer for sugar crops. *G. diazotrophicus* is capable of fixing atmospheric nitrogen up to 300 kg/ ha. In addition to N₂ fixation, it is also capable of producing significant amounts of plant growth hormones such as IAA (indole acetic acid) and gibberellin in culture” [6]. “Co-inoculation with beneficial microorganisms improves growth characteristics, corn yield, and nutritional status of soil and plants” [7].

Considering the above facts, the present study was designed to studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotrophicus* on available nutrients in soil of co-inoculation of the endophytic bacterium *G. diazotrophicus* when sprayed on leaves with inoculation of seed colonies Arthrobacter, PSB (*Bacillus sp.*) and KSB (*Frateruria aurantia*) on the growth and yield of corn as well as the main nutrients available in the soil.

2. MATERIALS AND METHODS

The present investigation entitled “Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotrophicus* on available nutrients in soil” was carried out during kharif season 2022 at the Research field of Jawaharlal Nehru Krishi Vishwavidhyalaya, Jabalpur M.P. (India).

2.1 Location of the Experimental Site

Jabalpur is situated in the south-eastern part of Madhya Pradesh at 23°13' North latitude, 79° 57'

East longitudes at an altitude of 393 meter above mean sea level. Experimental field had even topography and proper drainage.

2.2 Soil Status of the Experimental Field

The soil, being dynamic natural body, affects profoundly the rate of growth, development and eventually the economic yield. The initial fertility status of soil before layout of experiment, a composite sample from 0-15 cm depth was randomly collected from the experimental field. All the possible precautions prescribed for standard soil sampling have been followed. Collected soil samples were, air-dried in the shade and grounded by wooden mortar pestle, thereafter sieved through 2 mm mesh and stored in polyethylene bags. The soil samples thus obtained were subjected to chemical properties analyses to assess the status of soil. The experiment was conducted on Vertisol belonging to fine montmorillonite, Hypothermic family of *Typic Haplustert* popularly known as “Black soil” its chemical properties were analyzed.

2.3 Details of Field Experiment

The experiment was conducted during Kharif 2022 in the experimental field under Department of Soil Science & Agricultural Chemistry. The experiment contained 54 plots, each of 3m×2m (6 m²) and total number of treatments 18 in three replications laid out under randomized block design (RBD). The treatments were tried on maize (cv. JM 216, duration 110 days, seed rate 20 kg/ha) as seed treatment (basal application) of microbial consortium containing Potash solubilizing bacteria- *Fraturia aurentia*, phosphate solubilizing bacteria (*Bacillus* sp.) and *Arthrobacter* sp. In addition, *Gluconacetobacter diazotrophicus* was applied as foliar application at 25, 45 and 65 DAS of the crop growth stages.

Besides these, two types of control plots were maintained as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI) to

measure the comparative effects of different bio-inoculants.

2.4 Soil Sampling

The surface (0-15 cm) soil samples were collected from the experimental site before sowing of soybean crop and after harvest. The soil samples were air dried and crushed with wooden pestle and mortar and sieved through 2 mm sieve. The material passed through the sieve was used for determination of various characters.

2.5 Soil pH

Soil pH was determined in 1:2.5 soils-water suspensions [12] using Systronics pH meter.

2.6 Soil Nitrogen

Soil nitrogen was estimated by using KEL PLUS Automatic Nitrogen Estimation System.

2.7 Available Nitrogen

Available nitrogen in soil sample was determined by using alkaline permanganate method [10]. A known weight of soil is mixed with excess of alkaline permanganate and distilled. Organic matter present in soils is oxidized by the nascent oxygen liberated by KMnO₄ in the presence of NaOH and thus ammonia is released. This released ammonia was absorbed in the boric acid (2%) containing double indicator and converted to ammonium borate. This ammonium borate was titrated with standard sulphuric acid.

2.8 Available Phosphorus

The phosphorus content of soil was estimated by extraction procedure as described by Olsen et al. [13]. Soil available phosphorus was extracted using 0.5 M NaHCO₃ (pH 8.5) and determination was done by ascorbic acid method as described by [14].

Table 1. Chemical properties of experimental soil at one soil depths

Particulars	Values	Method employed
Soil pH (pH 1:2.5)	7.42	Glass electrode pH meter [8]
Electrical conductivity (dS m ⁻¹ at 25°C)	0.24	Electrical conductivity meter [8]
Organic carbon (%)	6.70	Walkley and Black rapid titration method [9]
Available nitrogen (kg/ ha)	223	Alkaline permanganate method [10]
Available phosphorus (kg/ ha)	15.3	Soils were extracted with 0.5 M NaHCO ₃ and colour development by Ascorbic acid [11]
Available potassium (kg /ha)	278	Neutral normal ammonium acetate method by using flame photometer [8]

2.9 Available Potassium

The available potassium was extracted by neutral 1 N ammonium acetate and it was estimated using flame photometer [15].

2.10 Grain and Stover Yields

Crop was harvested and bundles were made plot wise, and allowed to dry in the plot for 2-3 days and then weighed. After threshing was done plot wise, stover and grain yield was recorded.

2.11 Statistical Analysis

The data recorded were statistically analyzed following randomized block design to test the statistical significance of variance among different treatment means as influenced with the application of the treatments on various attributes of maize.

3. RESULTS AND DISCUSSION

The experiment was conducted to the “Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotrophicus* on available nutrients in soil”. The analyses of variance (ANOVA) of the data on different parameters are also given in the tables and fig. The results have been presented and discussed and possible interpretations have been given under the following headings:

3.1 pH and EC

Table 2 presents the data on soil pH and EC in soil at harvest. Soil reactions (soil pH) and EC were statistically unaffected due to basal application and foliar spray of *G. diazotrophicus* on maize. Choudhry et al. [16] conducted a long term experiment at Hissar for 9 years and found that pH of the soil remained more or less unaffected under various fertility levels.

3.2 Available Soil Nitrogen, Phosphorus and Potassium

The data regarding available N, P₂O₅ and K₂O content in soil (0-15 cm) after harvest of the crop are presented in Fig. 1 and Statistical analysis Table 2.

Soil nitrogen availability ranged from 197 to 239 kg N /ha. Except for RDF+KSB and RDF+PSB + KSB, all treatments considerably enhanced soil accessible N content above the control FUI plot.

RDF+G. diazotrophicus + Arthro + PSB + KSB outperformed all other treatments by 21.1%, followed by RDF + Arthro + PSB + KSB, RDF+G. diazotrophicus + PSB + KSB, and RDF+G. diazotrophicus+Arthro +PSB by 21.1, 19.6, and 19.5%, respectively, over fertilised uninoculated soil (197 kg N/ ha). However, RDF+G. diazotrophicus + Arthro + PSB + KSB performed similarly to RDF + Arthro + PSB + KSB.

The easily digestible phosphorus content in soil ranges from 13.2 to 21.2 kg P₂O₅/ ha. All treatments (except *G. diazotrophicus*) showed significant results compared to the FUI control group. Among all the treatments, RDF+G. diazotrophicus + Arthro + PSB + KSB responded best with an increase of 60.6%, followed by RDF + Arthro + PSB + KSB, RDF+G. diazotrophicus + Arthro + PSB and RDF+PSB + KSB increased by 45.4, 44.6 and 43.9%, respectively, compared to the control (13.2 kg P₂O₅/ ha). RDF+G. diazotrophicus + Arthro + PSB + KSB are attached to RDF + Arthro + PSB + KSB. Yadav et al. (2009) reported that biological agents such as *Gluconacetobacter diazotrophicus* and *Trichoderma viride* have great potential to restore soil fertility and promote sugarcane growth. Therefore, field experiments were carried out to study the synergistic effect of biological agents (*G.diazotrophicus* and *T. viride*), FYM and N fertilizer on sugarcane rhizomes and crop yield.

The data on available potassium in soil kg K₂O /ha varied from 206 to 286 kg /ha. All the treatments performed significantly over the control FUI plot. Among all the treatments, RDF+G. diazotrophicus + Arthro + PSB + KSB responded the best by 38.8% increase and this was followed by RDF+G. diazotrophicus + Arthro + KSB, RDF+G. diazotrophicus + Arthro + PSB + KSB and RDF + Arthro + PSB + KSB by 37.8, 37.4 and 37.4% over the control of fertilized uninoculated soil (206 kg K/ ha). However, the performance of RDF+G. diazotrophicus + Arthro + PSB + KSB was at par to that of RDF+G. diazotrophicus + Arthro + KSB.

The maximum available nitrogen in soil was increased with the treatment combinations of RDF+G. diazotrophicus + Arthro + PSB + KSB and RDF+Arthro+PSB+ KSB (by 21.3 and 21.1% increase, respectively) over the control (197 kg N /ha).

Available phosphorus in soil increased at maximum with the treatment combinations of RDF+G. diazotrophicus + Arthro + PSB + KSB and RDF+Arthro+PSB+ KSB (by 60.6 and 45.5%

increase, respectively) over the control (13.2 kg P₂O₅/ ha) and the respective results were mutually at par.

Available potassium in soil increased at maximum with the treatment combinations of RDF+G. *diazotrophicus* + *Arthro* + PSB + KSB and RDF+G. *diazotrophicus* +*Arthro* +KSB (by 38.8 and 37.8% increase, respectively) over the control (206 kg K₂O/ ha) and the respective results were mutually at par.

3.3 Grain Yield and stover Yield

Fig. 2. Exhibits the data on yields of grain and stover. The data on grain yield ranged from 1348 to 2863 kg /ha. All the treatments (except *G. diazotrophicus*) increased significantly the grain yield over the control fertilized un-inoculated (FUI).

Among all the treatment combinations, responded the best RDF+G. *diazotrophicus*

+*Arthro*+PSB + KSB by 33.4%, followed by the treatment combinations RDF+G. *diazotrophicus* + *Arthro* + PSB, RDF+G. *diazotrophicus* + *Arthro* + KSB and RDF + *Arthro* + PSB, by 30.1, 28.3 and 25.4%, respectively over the control (1348 kg ha⁻¹). However, the performance of RDF+G. *diazotrophicus* + *Arthro* + PSB + KSB was at par to that of RDF+G. *diazotrophicus* + *Arthro* + PSB.

The data on stover yield ranged from 5618-10729 kg/ha. All the treatments (except *G. diazotrophicus*) increased significantly the grain yield over the control fertilized uninoculated (FUI). Among all the treatment combinations, RDF+G. *diazotrophicus* + *Arthro* + PSB + KSB responded the best by 77.9%, followed by, RDF+G. *diazotrophicus* + *Arthro* + PSB, RDF + *Arthro* + PSB + KSB and RDF+G. *diazotrophicus*+*Arthro*+ KSB by 76.0, 74.7 and 70.5%, respectively over the control (6029 kg ha⁻¹). However, the performance of F+G. *diazotrophicus* + *Arthro* + PSB + KSB was at par to that of RDF+G. *diazotrophicus* + *Arthro* + PSB.

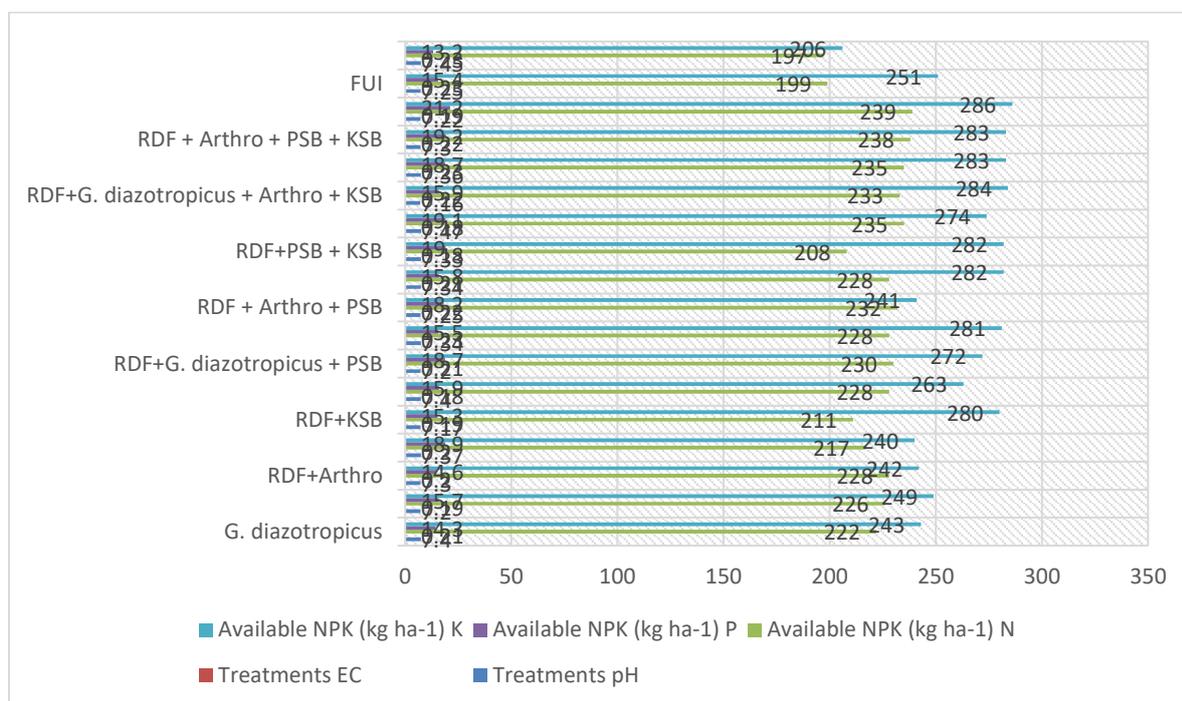


Fig. 1. Effect of microbial consortia as basal application and foliar spray of *G. diazotrophicus* on soil pH, EC and available NPK in soil after harvest

Table 2. Statistical analysis of microbial consortia as basal application and foliar spray of *G. diazotrophicus* on soil pH, EC and available NPK in soil after harvest

CV (%)	0.24	0.06	18.1	1.42	21.2
S.Em (+)	0.08	0.02	6.12	0.48	7.20
CD (p=0.05)	NS	NS	10.6	8.94	8.52

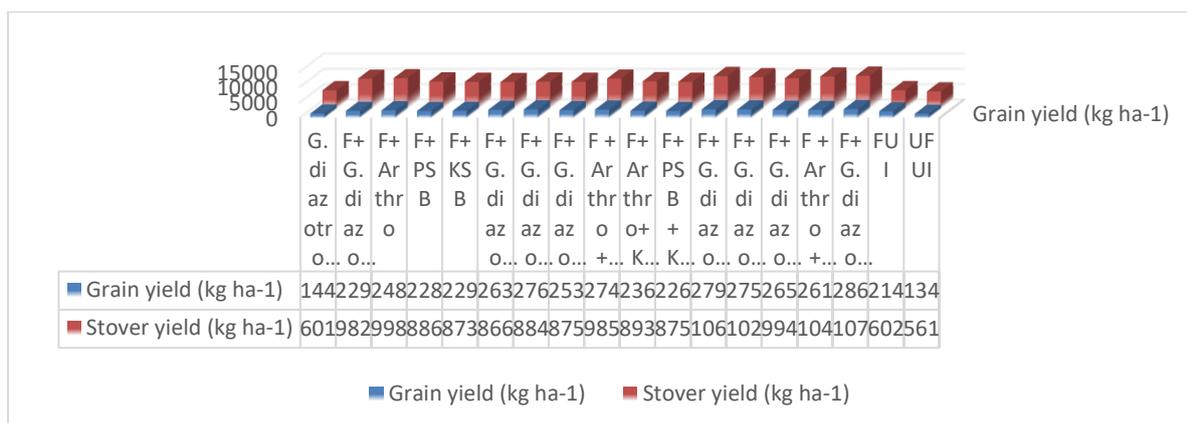


Fig. 2. Effect of microbial consortia as basal application and foliar spray of *G. diazotrophicus* on grain yield, stover yield

Table 3. Statistical analysis of microbial consortia as basal application and foliar spray of *G. diazotrophicus* on grain yield, stover yield

CV (%)	126.1	424.1
S.Em (+)	2404	8940
CD (p=0.05)	42.8	143.9

Several studies have shown that inoculation with rhizobacteria improves plant growth, increases the activity of soil enzymes (solubilization of inorganic compounds and mineralization of organic compounds), leading to plants' mobilization and acquisition of nutrients [17]. Association of N₂-fixing bacteria with grasses, cereals, and other non-nodulating, aerobic crops, fixes N₂ in the presence of low partial pressure of oxygen. Identification of endophytic diazotroph bacteria potentially responsible for N₂ fixation may be possible to capture a significant contribution of BNF in several other grasses and perhaps also in root crops [18].

4. CONCLUSION

The experiment on “Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotrophicus* on available nutrients in soil” was carried out during Kharif 2022 in the experimental field under Department of Soil Science & Agricultural Chemistry. For all criteria, The data on stover yield ranged from 5618- 10729 kg/ha. All the treatments (except *G. diazotrophicus*) increased significantly the grain yield over the control fertilized uninoculated (FUI). Among all the treatment combinations, the top performing treatment was constantly followed by the treatment combination of RDF+Arthro+PSB+KSB. Co-inoculation of *G. diazotrophicus*, *Arthrobacter*, PSB (*Bacillus* sp.),

and KSB (*Frateuria aurantia*) bio-inoculants as seed treatment and foliar spray on maize (*Zea mays*) increased growth and accessible main nutrient in soil at harvest. All of these were the favourable effects of *G. diazotrophicus* and *Arthrobacter* when the crop was supplemented with sufficient accessible nutrients, notably phosphorus using PSB, as the soil was relatively phosphorus deficient. However, because the soil was sufficiently potash-rich, KSB response was not observed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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