Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotropicus* on available nutrients in soil

Abstract

The experiment on "Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotropicus* on available nutrients in soil" was carried out during Kharif 2022 in the experimental filed 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* 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. diazotropicus*, Arthrobacter, PSB (*Bacillus sp*.) and KSB (*Frateuria 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.diazotropicus* and Arthrobacter The data on stover yield ranged from 5618- 10729 kg/ha. All the treatments (except *G. diazotropicus*) increased significantly the grain yield over the control fertilized uninoculated (FUI). Among all the treatment combinations,

Keyword- Maize, *G. diazotropicus, Arthrobacter*, PSB (*Bacillus* sp.), KSB (*Frateuria aurantia*), FUI (Fertilized un-inoculated), UFUI (Unfertilized un-inoculated).

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". [18] "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". (Agriculture Statistics at a glance, Anonymous, 2016).

"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". **(**Sevilla *et al.,* 2001**). "**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" (Saravanan *et al*., 2007). , "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" (Dong *et al*., 1994). "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" (Bastian *et al*., 1999). "Co-inoculation with beneficial microorganisms improves growth characteristics, corn yield, and nutritional status of soil and plants" (Awasthi *et al*. 2011).

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 diazotropicus* 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 (*Frateuria aurantia*) on the growth and yield of corn as well as the main nutrients available in the soil.

Materials and Methods

The present investigation entitled "Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotropicus* 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).

Location of the experimental site

Jabalpur is situated in the south-eastern part of Madhya Pradesh at 23^{o}13' North latitude, 79^{o} 57' East longitudes at an altitude of 393 meter above mean sea level. Experimental field had even topography and proper drainage.

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

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 (Jakson, 1973)
Electrical conductivity (dS m^{-1} at 25 ^{0}C)	0.24	Electrical conductivity meter (Jackson, 1973)
Organic carbon (%)	6.70	Walkley and Black rapid titration method (1934)
Available nitrogen (kg/ ha)	223	Alkaline permanganate method (Subbiah and Asija, 1956)

Available phosphorus (kg/ ha)	15.3	Soils were extracted with 0.5 M $NaHCO_3$ and colour development by Ascorbic acid (Watanabe and Olsen's, 1965)
Available potassium (kg /ha)	278	Neutral normal ammonium acetate method by using flame photometer (Jackson, 1973)

Details of field experiment:

The experiment was conducted during Kharif 2022 in the experimental filed under Department of Soil Science & Agricultural Chemistry. The experiment contained 54 plots, each of 3m×2m (6 m^2) 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 maintined as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI) to measure the comparative effects of different bio-inoculants.

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.

Soil pH

Soil pH was determined in 1:2.5 soils-water suspensions (Piper, 1950) using Systronics pH meter.

Soil nitrogen

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

Available nitrogen

Available nitrogen in soil sample was determined by using alkaline permanganate method (Subbiah and Asija, 1956). 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 $KMnO_4$ 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.

Available phosphorus

The phosphorus content of soil was estimated by extraction procedure as described by Olsen *et al.* (1954). Soil available phosphorus was extracted using 0.5 M $NaHCO_3$ (pH 8.5) and determination was done by ascorbic acid method as described by (Page, A. L, Miller and Keeney 1982).

Available potassium

The available potassium was extracted by neutral 1 N ammonium acetate and it was estimated using flame photometer (Grasty, R. L. *et al.*, 1963).

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.

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.

Result and discussion

The experiment was conducted to the "Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotropicus* 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:

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. diazotropicus* on maize. Choudhry *et al*. (1981) 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.

Available soil nitrogen, phosphorus and potassium

The data regarding available N, P_2O_5 and K_2O 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. diazotropicus + Arthro + PSB + KSB outperformed all other treatments by 21.1%, followed by RDF + Arthro + PSB + KSB, RDF+G. diazotropicus + PSB + KSB, and RDF+G. diazotropicus+Arthro +PSB by 21.1, 19.6, and 19.5%, respectively, over fertilised uninoculated soil (197 kg N/ ha). However, RDF+G. diazotropicus + 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. diazotropicus*) showed significant results compared to the FUI control group. Among all the treatments, RDF+*G. diazotropicus* + Arthro + PSB + KSB responded best with an increase of 60.6%, followed by RDF + Arthro + PSB + KSB, RDF+*G. diazotropicus* + 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. diazotropicus* + 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 K_2O /ha varied from 206 to 286 kg /ha. All the treatments performed significantly over the control FUI plot. Among all the treatments, RDF+*G. diazotropicus* + *Arthro* + PSB + KSB responded the best by 38.8% increase and this was followed by RDF+*G. diazotropicus* + *Arthro* + *KSB*, RDF+*G. diazotropicus* + *Arthro* + PSB + KSB and RDF + *Arthro* + PSB + KSB by 37.8, 37.4 and 37.4% over the control of fertilized un-inoculated soil (206 kg K/ ha). However, the performance of RDF+*G. diazotropicus* + *Arthro* + PSB + KSB was at par to that of RDF+*G. diazotropicus* + *Arthro* + *KSB.*

The maximum available nitrogen in soil was increased with the treatment combinations of RDF+*G. diazotropicus* + *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. diazotropicus* + *Arthro* + PSB + KSB and RDF+*Arthro*+PSB+ KSB (by 60.6 and 45.5% increase, respectively) over the control (13.2 kg $P_2O_{5/}$ ha) and the respective results were mutually at par.

Available potassium in soil increased at maximum with the treatment combinations of RDF+*G. diazotropicus* + *Arthro* + PSB + KSB and RDF+*G. diazotropicus* +*Arthro* +KSB (by 38.8 and 37.8% increase, respectively) over the control (206 kg K_2O/ ha) and the respective results were mutually at par

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

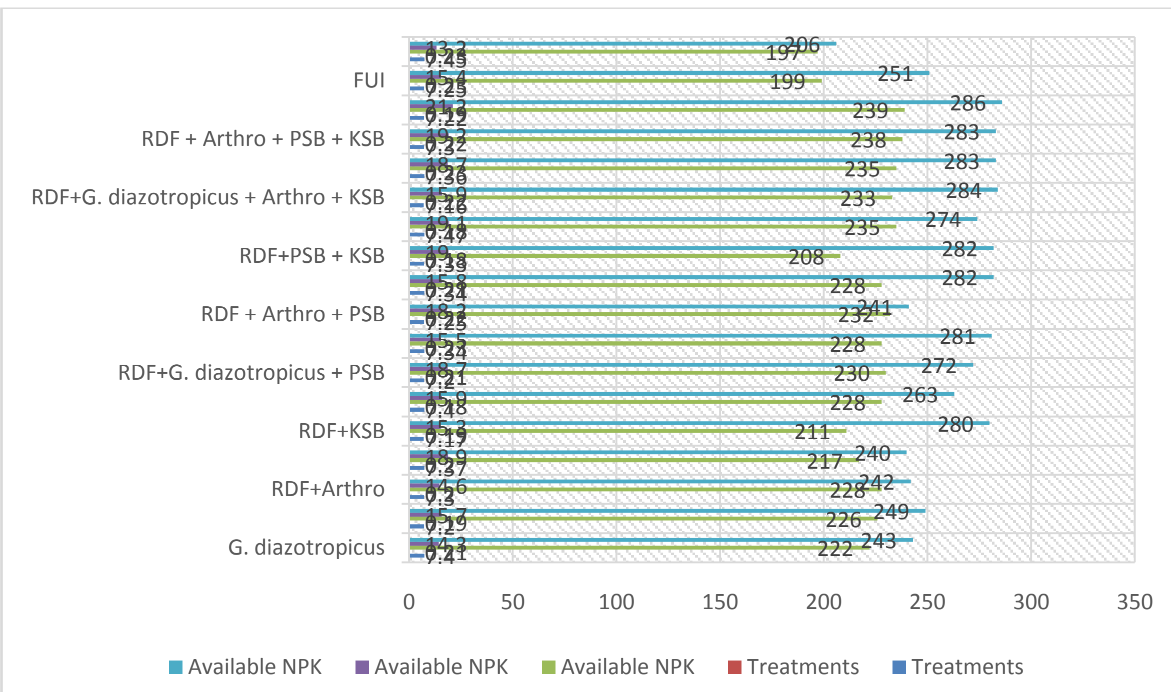

Table 2. Statistical analysis of microbial consortia as basal application and foliar spray of *G.diazotropicus* on soil pH, EC and available NPK in soil after harvest

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**

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. diazotropicus*) increased significantly the grain yield over the control fertilized un-inoculated (FUI).

Among all the treatment combinations, responded the best RDF+*G. diazotropicus* +*Arthro*+PSB + KSB by 33.4%, followed by the treatment combinations RDF+*G. diazotropicus* + *Arthro* + PSB, RDF+*G. diazotropicus* + *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. diazotropicus* + *Arthro* + PSB + KSB was at par to that of RDF+*G. diazotropicus* + *Arthro* + PSB.

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

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 to plants' mobilization and acquisition of nutrients (Kohler *et al*, 2007). Association of N_2-fixing bacteria with grasses, cereals, and other non-nodulating, aerobic crops, fixes N_2 in the presence of low partial pressure of oxygen. Identification of endophytic diazotroph bacteria potentially responsible for N_2 fixation may be possible to capture a significant contribution of BNF in several other grasses and perhaps also in root crops (Boddy *et al*.1995)

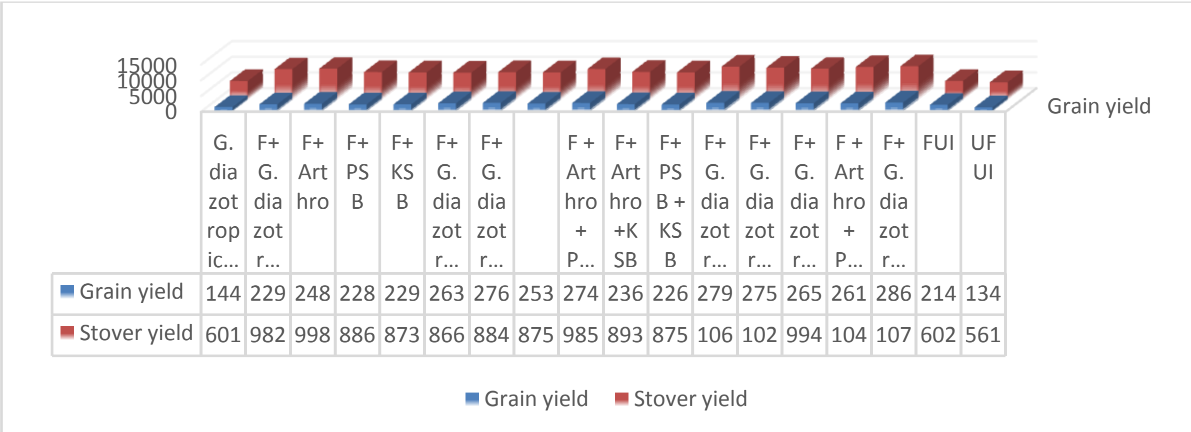

Fig-2. Effect of microbial consortia as basal application and foliar spray of *G. diazotropicus* on grain yield, stover yield

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

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

Conclusion

The experiment on "Studies on Stover yield of maize with basal application of fertilizer and foliar spraying of *Gluconacetobacter diazotropicus* on available nutrients in soil" was carried out during Kharif 2022 in the experimental filed 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. diazotropicus*) 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. diazotropicus*, Arthrobacter, PSB (Bacillus sp.), and KSB (*Frateuria aurantia*) bio-inoculants as seed treatment and foliar spray on maize (*Zea maize)* increased growth and accessible main nutrient in soil at harvest. All of these were the favourable effects of *G. diazotropicus* 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.

Reference

1. Anonymous, 2016. Agriculture statistics at a glance. Agricultural Statistics Division. Director of Economics and Statistics. Department of Agriculture and Cooperation, ministry of Agriculture, Govt. of India, New Delhi.
2. Awasthi R, Tiwari R and Nayyar H. 2011. Synergy between plant and phosphorous solubilizing microbes in soils: 60 Effects on Growth and Physiology of crops *International Research Journal of Microbiology* 2.12: 484-503.
3. Bastian F, Rapparini F, Baraldi R, Piccoli P, Bottini R. 1999. Inoculation with *Acetobacter diazotrophicus* increases glucose and fructose content in shoots of sorghum. *Symbiosis.*27.2: 147-156.
4. Boddey RM, Dobereiner J and Ahmed N. 1995. Nitrogen fixation associated with grasses and cereals : recent progress and perspectives for the future. *Fertilizer Research*. 4: 1-3, 241-250.
5. Choudhary ML, Singh JP and Narval RP. 1981. Effect of long term application of P, K and FYM on some soil chemical properties. *Journal of Indian Society Soil Science*. 29:81-85.
6. Dong Zhongmin DZ, Canny MJ and McCully ME. 1994. A nitrogen-fixing endophyte of sugarcane stems. A new role for the apoplast, *Plant Physiology* 105: 1139–1147.
7. Grasty, R. L., Miller, J. A., & Mohr, P. A. (1963). Preliminary results of potassium-argon age determinations on some Ethiopian Trap Series basalts. *Bull. Geophys. Obs. Addis Ababa*, *6*, 97-102.
8. Jackson ML. 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi. 214-221.
9. Kohler J, Carvaca F, Carrasco L and Roldan A. 2007. Interaction between a pgpr, an AM Fungus and a Phosphate-Solubilizing Fungus in the Rhizosphere of *Lactuca Sativa*. *Applied Soil Ecology* 35: 480-487
10. Olsen SR, Cole CV, Watanable JS, Dean LA. 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. USDA circular No. 939.
11. Page, A. L., Miller, R. H., & Keeney, D. R. 1982. Methods of soil analysis, part 2. *Chemical and microbiological properties*, 2, 643-698.
12. Piper CS.1950. Soil and Plant Analysis, Academic Press, New York.
13. Saravanan VS, Osborne J, Madhaiyan M, Mathew L, Chung J, Ahn K and Sa T. 2007. Zinc metal solubilization by *Gluconacetobacter diazotrophicus* and induction of pleomorphic cells, *Journal of Microbiology and Biotechnology* 55(1): 1477–1482.
14. Sevilla M, Burris RH, Gunapala N, and Kennedy C. 2001. Comparison of benefit to sugarcane plant growth and $^{15}N_2$ incorporation following inoculation of sterile plants with *Acetobacter diazotrophicus* wild-type and mutant strains. *Molecular Plant-Microbe Interact*. 14**:** 359-366.
15. Subbiah BV and Asija GL. 1956. A rapid procedure for the estimation of available nitrogen in soils. *Current Science*. 25:259-260.
16. Walkley A and Black IA. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science. 37:29-33.

17. Watanabe FS and Olsen SR. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Science Society of America Proceedings. 29:677-678.
18. Padwar G, Mitra NG, Chand T, Sahu RK, Padwar G. Effect of microbial consortia as basal application and foliar spray of Gluconacetobacter diazotropicus on growth, yield and nutrient uptake by maize. International Journal of Current Microbiology and Applied Sciences. 2020;9(6):2900-12.