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Influence of different biofertilizers and their mode of application on nitrogen saving and maize crop performance

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Abstract

A study was conducted on "Influence of different biofertilizers and their mode of application on nitrogen saving and maize crop performance" during *rabi* 2020-21 at College Farm, College of Agriculture, Rajendranagar, Hyderabad, to study the effect of biofertilizers and their method of application and utilize it in the integrated nutrient management to study their effect on soil properties, growth, yield and nutrient uptake in maize crop. Soil of the experimental site was indicated that soil was slightly alkaline (pH 7.55) in reaction, non-saline (0.55 dS m⁻¹), low in available nitrogen (185.36 kg ha⁻¹), medium in available P₂O₅ (43 kg ha⁻¹) and high in available K₂O (302.68 kg ha⁻¹) with medium (5.9 g kg⁻¹) organic carbon content.

Keywords: pH, EC, organic carbon, nitrogen uptake, phosphorous uptake, potassium uptake

1. Introduction

In the present day agriculture, where indiscriminate use of fertilizers and pesticides have resulted in soil pollution and other alterations in the soil environment (Panagos *et al.*, 2013, Kurrey *et al.*, 2018) [7, 5]. These problems have led to an increased interest in the sustainable and eco-friendly agricultural practices with the aim of reducing the cost (Salantur *et al.*, 2005) [9] and also to improve soil environment. Sustainable soil health aims to overcome the problems of heavy metal accumulation, improvement in physico chemical, chemical and populations of beneficial soil microorganisms.

Maize is the third most important cereal crop after Rice and Wheat with highest requirement of Nitrogen for the production of increasing yields (Amin, 2011) [1]. Many hybrids released having high yield potential and require higher doses of fertilizers especially nitrogenous fertilizers and these higher doses of fertilizers have resulted in increased input cost and also causing detrimental effects on soil health and environmental pollution.

Bio fertilizers are the products that contain live or latent microorganisms that are capable of mobilizing nutrients from unavailable form to available forms through biological processes (Gaur, 2010) [2]. Since the increasing cost and diminishing returns especially in Maize crop production is uneconomical particularly for small and marginal farmers, it is found essential to opt for integrated nutrient supply by using a combination of inorganic fertilizers, biofertilizers and organic manures (Talwar *et al.*, 2017) [10]. The use of nitrogen fixing microbes helps in reducing the dependence on inorganic nitrogen fertilizers. The use of bacterial consortium which also include phosphate solubilizers tend to increase the availability of phosphorus from unavailable forms. Thus, increases availability of phosphorus to plants.

Azotobacter and *Azospirillum* are free living bacteria which colonize near the root zone and enhance the availability of nitrogen in soil through nitrogen fixation. While, the bacterial consortium which also consists of phosphate solubilizing bacteria helps in solubilizing the fixed forms of phosphorus and make them available for crop growth (Kachari and Korla, 2009, Talwar *et al.*, 2017) [4, 10]. Hence the present study on integrated application of biofertilizers and nitrogen fertilizers on growth and yield, nitrogen use economy in Maize crop and their effect on chemical and biological properties of soil.

2. Materials and Methods

2.1 Study area and Treatments

The experiment was conducted at College Farm, College of Agriculture, Rajendranagar, Hyderabad during *rabi* 2020-21. The soil of the experimental site was sandy clay loam in texture, medium in organic carbon, low in available nitrogen, medium in available P_2O_5 and high in available K_2O . Soil was slightly alkaline and non-saline in nature. The treatments included T_1 - 100% RDN (240 kg ha^{-1}) without biofertilizers, T_2 - 80% RDN + seed treatment with *Azotobacter* @ 500 g ha^{-1} , T_3 - 80% RDN + seed treatment with *Azospirillum* @ 500 g ha^{-1} , T_4 - 80% RDN + seed treatment with *Azotobacter* @ 250 g ha^{-1} + *Azospirillum* @ 250 g ha^{-1} , T_5 - 80% RDN + seed treatment with bacterial consortium @ 500 g ha^{-1} , T_6 - 80% RDN + soil application of *Azotobacter* @ 5 kg ha^{-1} , T_7 - 80% RDN + soil application of *Azospirillum* @ 5 kg ha^{-1} , T_8 - 80% RDN + soil application of *Azotobacter* @ 2.5 kg ha^{-1} + soil application of *Azospirillum* @ 2.5 kg ha^{-1} , T_9 - 80% RDN + soil application of bacterial consortium @ 5 kg ha^{-1} , T_{10} - 80% RDN (192 kg ha^{-1}) without biofertilizer.

2.2 Analysis of soil properties

Soil pH was determined by glass electrode pH meter in 1: 2.5 soil water suspensions after equilibrating soil with water for 30 minutes with intermittent stirring as described by Jackson (1967) [3]. The soil sample used for pH determination was allowed to settle down and the clear supernatant was taken out and the electrical conductivity of the supernatant liquid was determined using conductivity bridge as described by Jackson (1967) [3]. Organic Carbon in soil sample was analysed by wet chromic acid digestion method as outlined by Walkley and Black (1934) [12]. To a 0.5 g of 0.5 mm sieved soil in 500 mL conical flask, 10 ml of 1 N potassium dichromate and 20 mL of conc. H_2SO_4 were added and mixed gently for a minimum and allowed the mixture for reaction to take place on asbestos sheet for 30 min. At the completion of 30 min, 10 mL of orthophosphoric acid, 200 mL distilled water and 1 mL of diphenylamine indicator were added. Then the solution was back titrated against 0.5 N Ferrous ammonium sulphate till the appearance of green colour. A blank was run without soil simultaneously.

For the estimation of nitrogen content, the finely powdered plant material of 0.2 g was digested with 2 mL of concentrated H_2SO_4 in a test tube by keeping it overnight. Then the contents in the test tubes were heated on hot plate and added 30% H_2O_2 drop wise. It was heated intermittently till the contents become colourless. The digested material was distilled by micro kjeldahl method with 10 mL of 40% sodium hydroxide. The ammonia thus released was collected in 4% boric acid mixed with bromocresol green and methyl red mixed indicator. This was titrated against 0.02 N sulphuric acid and nitrogen content of samples were calculated and expressed in percent. The diacid wet digestion was used for the determination of P and K. The phosphorus content in the aliquot of the digested sample was determined by Vanado-molybdo phosphoric acid method using Barton's reagent which forms a yellow coloured phospho - vanado molybdate complex. The intensity of yellow colour solution was read after 30 minutes at 420 nm with spectrophotometer using blue filter and expressed in percent (Piper, 1966) [8]. Potassium in the digested plant material was estimated with flame photometer. Feed the clear filtrate directly to the flame photometer after adjusting the instrument to the higher

concentration and expressed in percent. (Muhr *et al.*, 1965) [6].

3. Results and Discussion

Soil physico chemical properties

The data on effect of biofertilizers and their method of application along with nitrogen fertilizers on soil reaction (pH), electrical conductivity (EC) and Organic carbon (g kg^{-1}) are presented in Table 1.

Application of chemical fertilizers alone or in combination with biofertilizers did not show any significant effect on pH and EC of the post harvested soil. The soil pH values ranged from 7.17 (80% RDN + seed treatment with *Azospirillum* @ 500 g ha^{-1}) to 7.47 (80% RDN + seed treatment with bacterial consortium @ 500 g ha^{-1}). Similar non significant values were reported for total soluble salt content with the maximum EC of 0.49 dSm^{-1} being recorded in treatment receiving 100% RDN without biofertilizers. While, minimum 0.36 dSm^{-1} in treatment receiving 80% RDN + seed treatment with *Azospirillum* @ 250 g ha^{-1} + *Azotobacter* @ 250 g ha^{-1} and regarding soil organic carbon content the treatment T_5 receiving 80% RDN + seed treatment with bacterial consortium @ 500 g ha^{-1} resulted in significant higher organic carbon content (6.7 g kg^{-1}) and it was on par with treatments T_3 , T_9 , T_4 and T_1 in recording higher organic carbon content. While the treatments T_{10} , T_2 , T_7 and T_8 were on par with each other and followed the above treatments in recording low organic carbon content and were statistically differed from the above treatments.

Though the effect of treatments on soil pH was non significant, the addition of biofertilizers to soil is known to bring about changes in pH *i.e.*, pH decreased with the application of biofertilizers. This might be due to release of H^+ ions from the biofertilizers during mineralization and the increase in organic carbon content of soil indicates that the bacteria applied either as seed treatment or soil application utilized the atmospheric nitrogen for their cell protein synthesis. While the treatments receiving 100% RDN without biofertilizer resulted in robust crop growth adding root biomass to soil thereby resulting in higher organic carbon content (Kurrey *et al.*, 2018) [5]. Verma, (2011) [11] has suggested soil organic matter content of soil as one of the factors favourable for increased microbial population.

Nutrient content of Maize crop

The percent nitrogen content of maize as affected by treatments at tasselling and at harvest and the phosphorus and potassium contents were determined at harvest both in grain and stover presented in Tables 2 to 4 and Fig.1 to 3.

The high nitrogen content was observed at tasseling and at harvest in grain and stover but total nitrogen content in grain and stover increased slightly at harvest. At tasselling, highest nitrogen content (1.89 percent) was recorded by application of 80% RDN + seed treatment with bacterial consortium @ 500 g ha^{-1} (T_5) and it was on par with treatments T_3 and T_4 with (80% RDN + seed treatment with *Azospirillum* @ 500 g ha^{-1}) and (80% RDN + seed treatment with *Azotobacter* @ 250 g ha^{-1} and *Azospirillum* @ 500 g ha^{-1}). Lowest nitrogen content (1.02) was recorded by the treatment T_{10} (80% RDN). Among grain and stover at harvest, highest amounts of nitrogen were recorded in grain over stover. 80% RDN + seed treatment with bacterial consortium @ 500 g ha^{-1} (T_5) recorded highest nitrogen concentration (1.23 percent) which was on par with treatments 80% RDN + seed treatment with *Azospirillum* @

500 g ha⁻¹ (T₃) and 80% RDN + seed treatment with *Azotobacter* @ 250 g ha⁻¹ and *Azospirillum* @ 250 g ha⁻¹ (T₄) and 100% RDN (T₁). Lowest nitrogen content (0.79 percent) was observed in treatment with 80% RDN (T₁₀). The data further revealed that the nitrogen concentration in stover increased with incremental increase of different levels of inorganic and organic sources. Application of 80% RDN + seed treatment with bacterial consortium @ 500 g ha⁻¹ (T₅) recorded significantly higher nitrogen content (0.84 percent) in stover which is on a par with treatment 80% RDN + seed treatment with *Azotobacter* @ 250 g ha⁻¹ and *Azospirillum* @ 250 g ha⁻¹ (T₄). Lowest nitrogen content (0.39 percent) was observed in treatment with 80% RDN (T₁₀). The lowest (0.19 percent) and highest (0.31 percent) values of phosphorus content were recorded in treatment receiving 80% RDN (T₁₀) and 80% RDN + seed treatment with bacterial consortium @ 500 g ha⁻¹ (T₅). However the values of

phosphorus content showed, were significantly higher and on par in treatments T₃ (80% RDN + seed treatment with *Azospirillum* @ 500 g ha⁻¹) and T₄ (80% RDN + seed treatment with *Azotobacter* @ 250 g ha⁻¹ and *Azospirillum* @ 250 g ha⁻¹) in grain. The percent phosphorus in stover also followed similar trends. However the percent phosphorus in grain was higher (0.31 percent) than in stover (0.17 percent) in T₅. Similar trends were observed in all other treatments. Potassium content varied from (0.36 percent) in treatment T₁₀ with 80% RDN to (0.54 percent) in treatment T₅ with 80% RDN + seed treatment with bacterial consortium @ 500 g ha⁻¹ (T₅) in grain and it was on par with T₄ (0.53 percent) and T₃ (0.51 percent). In stover, potassium content varied from (1.07 percent) to (1.39 percent) in treatments receiving 80% RDN alone and 80% RDN + seed treatment with bacterial consortium @ 500 g ha⁻¹ (T₅).

Table 1: Effect of biofertilizers, their method of application along with nitrogen fertilizer on physico- chemical properties of soil

Treatments	pH	EC (dS m ⁻¹)	OC (g kg ⁻¹)
T ₁ - 100% Recommended dose of N without biofertilizers	7.39	0.49	6.30
T ₂ - 80% RDN + Seed treatment with <i>Azotobacter</i> 500 g / ha	7.32	0.38	6.00
T ₃ - 80% RDN + Seed treatment with <i>Azospirillum</i> 500 g / ha	7.17	0.42	6.40
T ₄ - 80% RDN + Seed treatment with 250 g / ha of <i>Azotobacter</i> + 250 g/ha <i>Azospirillum</i>	7.36	0.36	6.20
T ₅ - 80% RDN + Seed treatment with <i>Bacterial consortium</i> 500 g / ha	7.47	0.39	6.70
T ₆ - 80% RDN + Soil application of <i>Azotobacter</i> 5 kg / ha	7.45	0.45	6.00
T ₇ - 80% RDN + Soil application of <i>Azospirillum</i> 5 kg / ha	7.29	0.44	6.10
T ₈ - 80% RDN + Soil application of 2.5 kg / ha <i>Azotobacter</i> + 2.5 kg / ha <i>Azospirillum</i>	7.42	0.48	5.90
T ₉ - 80% RDN + Soil application of <i>Bacterial consortium</i> 5 kg / ha	7.50	0.45	6.50
T ₁₀ - 80% RDN without biofertilizer	7.37	0.43	5.80
S.Em ±	0.02	0.08	0.01
CD (P=0.05)	NS	NS	0.05

Bacterial consortium: *Azotobacter* + PSB +ZNSB + KSB

Table 2: Effect of biofertilizers, their method of application along with nitrogen fertilizer on nitrogen content (%)

Treatments	Tasseling	Harvest	
		Grain	Stover
T ₁ - 100% Recommended dose of N without biofertilizer	1.62	1.15	0.58
T ₂ - 80% RDN + Seed treatment with <i>Azotobacter</i> 500 g / ha	1.09	0.82	0.42
T ₃ - 80% RDN + Seed treatment with <i>Azospirillum</i> 500 g / ha	1.67	1.19	0.76
T ₄ - 80% RDN + Seed treatment with 250 g / ha of <i>Azotobacter</i> + 250 g/ha <i>Azospirillum</i>	1.72	1.20	0.82
T ₅ - 80% RDN + Seed treatment with <i>Bacterial consortium</i> 500 g / ha	1.89	1.23	0.84
T ₆ - 80% RDN + Soil application of <i>Azotobacter</i> 5 kg / ha	1.39	0.96	0.56
T ₇ - 80% RDN + Soil application of <i>Azospirillum</i> 5 kg / ha	1.18	0.88	0.45
T ₈ - 80% RDN + Soil application of 2.5 kg / ha <i>Azotobacter</i> + 2.5 kg / ha <i>Azospirillum</i>	1.58	1.08	0.59
T ₉ - 80% RDN + Soil application of <i>Bacterial consortium</i> 5 kg / ha	1.65	1.12	0.64
T ₁₀ - 80% RDN without biofertilizer	1.02	0.79	0.39
SEm ±	0.08	0.05	0.03
CD (P=0.05)	0.23	0.14	0.09
CV (%)	7.28	8.46	9.11

Bacterial consortium: *Azotobacter* + PSB +ZNSB + KSB

Table 3: Effect of biofertilizers, their method of application along with nitrogen fertilizer on phosphorus content (%)

Treatments	Harvest	
	Grain	Stover
T ₁ - 100% Recommended dose of N without biofertilizer	0.24	0.14
T ₂ - 80% RDN + Seed treatment with <i>Azotobacter</i> 500 g / ha	0.20	0.13
T ₃ - 80% RDN + Seed treatment with <i>Azospirillum</i> 500 g / ha	0.29	0.16
T ₄ - 80% RDN + Seed treatment with 250 g / ha of <i>Azotobacter</i> + 250 g/ha <i>Azospirillum</i>	0.30	0.17
T ₅ - 80% RDN + Seed treatment with <i>Bacterial consortium</i> 500 g / ha	0.31	0.17
T ₆ - 80% RDN + Soil application of <i>Azotobacter</i> 5 kg / ha	0.24	0.13
T ₇ - 80% RDN + Soil application of <i>Azospirillum</i> 5 kg / ha	0.25	0.12
T ₈ - 80% RDN + Soil application of 2.5 kg / ha <i>Azotobacter</i> + 2.5 kg / ha <i>Azospirillum</i>	0.26	0.15
T ₉ - 80% RDN + Soil application of <i>Bacterial consortium</i> 5 kg / ha	0.29	0.16

T ₁₀ - 80% RDN without biofertilizer	0.19	0.10
SEm ±	0.01	0.01
CD (P=0.05)	0.04	0.02
CV (%)	8.61	7.42

Bacterial consortium: *Azotobacter* + PSB +ZNSB + KSB

Table 4: Effect of biofertilizers, their method of application along with nitrogen fertilizer on potassium content (%)

Treatments	Harvest	
	Grain	Stover
T ₁ - 100% Recommended dose of N without biofertilizer	0.46	1.24
T ₂ - 80% RDN + Seed treatment with <i>Azotobacter</i> 500 g / ha	0.39	1.12
T ₃ - 80% RDN + Seed treatment with <i>Azospirillum</i> 500 g / ha	0.51	1.35
T ₄ - 80% RDN + Seed treatment with 250 g / ha of <i>Azotobacter</i> + 250 g/ha <i>Azospirillum</i>	0.53	1.36
T ₅ - 80% RDN + Seed treatment with <i>Bacterial consortium</i> 500 g / ha	0.54	1.39
T ₆ - 80% RDN + Soil application of <i>Azotobacter</i> 5 kg / ha	0.42	1.19
T ₇ - 80% RDN + Soil application of <i>Azospirillum</i> 5 kg / ha	0.49	1.24
T ₈ - 80% RDN + Soil application of 2.5 kg / ha <i>Azotobacter</i> + 2.5 kg / ha <i>Azospirillum</i>	0.46	1.25
T ₉ - 80% RDN + Soil application of <i>Bacterial consortium</i> 5 kg / ha	0.51	1.34
T ₁₀ - 80% RDN without biofertilizer	0.35	1.07
SEm ±	0.02	0.06
CD (P=0.05)	0.06	0.16
CV (%)	7.86	8.32

Bacterial consortium: *Azotobacter* + PSB +ZNSB + KSB

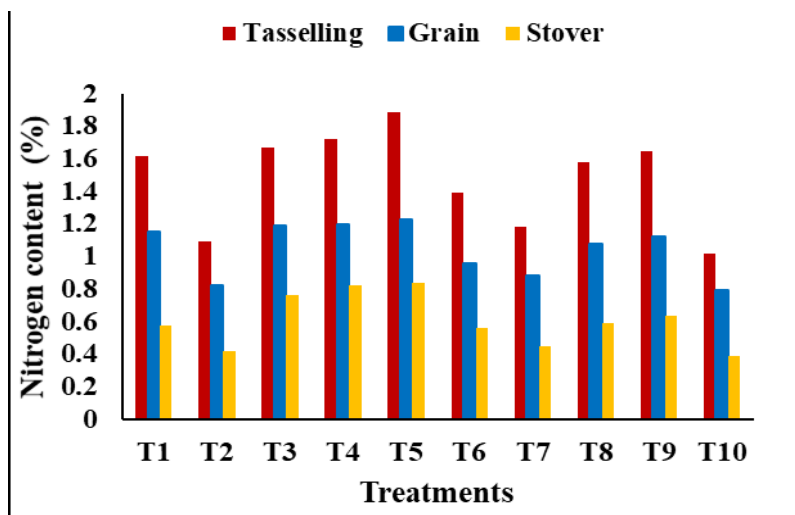


Fig 1: Effect of biofertilizers, their method of application

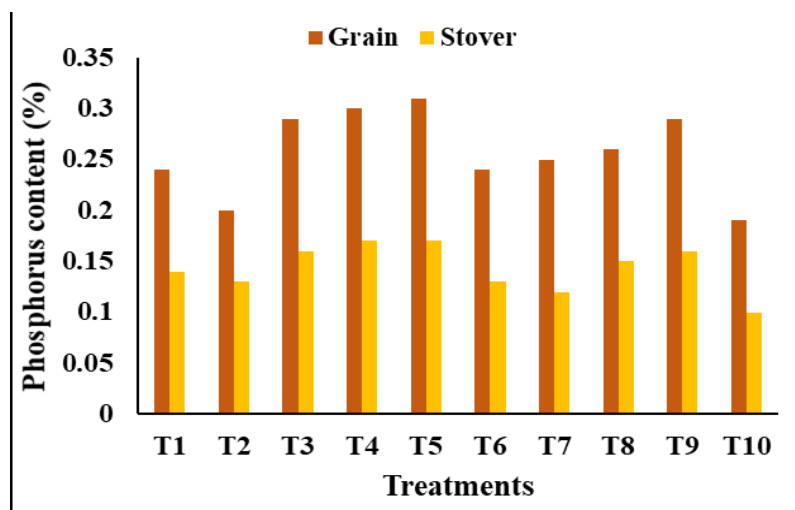


Fig 2: Effect of biofertilizers, their method of application along with nitrogen fertilizers on nitrogen content (%)

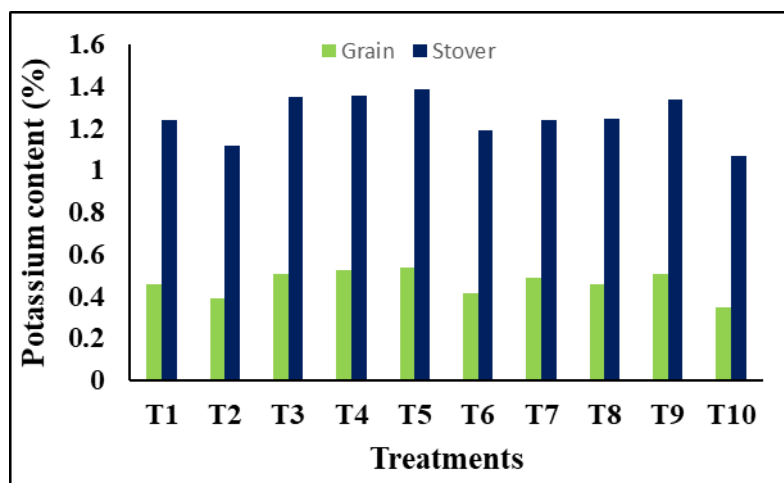


Fig 3: Effect of biofertilizers, their method of application along with nitrogen fertilizers on potassium content (%)

Conclusion

The results are clear by the fact that the application of 80% RDN along with seed application of *Azotobacter*, *Azospirillum* and Bacterial consortium have resulted in obtaining maximum nutrient content which were followed by 80% RDN in combination with soil application of *Azotobacter*, *Azospirillum* and *Bacterial consortium* and 100% RDN alone.

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