



Effect of Different Levels of Fertilizers in Combination with Biofertilizers on Biological Properties of Soil under Maize

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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

A field experiment entitled "Effect of different levels of fertilizers in combination with biofertilizers on biological properties of soil under maize" was conducted at Agriculture College Farm, Bapatla, during both *kharif* 2020 and 2021. The experiment was laid out in randomized block design (RBD) with seven treatments and replicated thrice. The treatments consisted of T₁- Control; T₂- 100% RDF; T₃- 125% RDF; T₄ – 100% RDF + VAM; T₅- 100% RDF +VAM + *Azospirillum* + PSB; T₆- 75% RDF + VAM; T₇- 75 % RDF + VAM + *Azospirillum* + PSB. The results revealed that microbial activity (Bacteria, Fungi and Actinomycetes) and enzyme activity (Urease, Dehydrogenase, Acid phosphatase and Alkaline phosphatase) of maize were significantly higher with 100% RDF +VAM + *Azospirillum* + PSB (T₅) at kneehigh, tasseling and harvest stages of maize crop during *kharif* 2020 and 2021. Lowest microbial activity and enzyme activity were recorded with the T₁(control) which received no fertilizers at all the three stages of crop growth during both the years of study. Present study highlights the need of use of biofertilizers along with inorganic fertilizers to enhance microbial activity and improve soil health.

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1. INTRODUCTION

Maize (*Zea mays* L.) is one of the important cereal crops next only to wheat and rice in the world. In India, it ranks fourth after rice, wheat and sorghum. Maize is principal staple food in many countries, particularly in the tropics and subtropics and it is being consumed both as food and fodder and also required by the various industries. The crop has high genetic yield potential hence, it is called Miracle crop and "Queen of Cereals. It is a nutrient exhaustive crop than other cereals and absorbs large quantity of nutrients from the soil during its different growth stages. Maize responds well to fertilizer but under field conditions due to over reliance on nitrogenous fertilizers and no or negligible used of organic manure its yield potential is difficult to exploit. biofertilizers not only help to provide balanced nutrient but also support sustainable production due to their pivotal role in soil health enhancement.

Biofertilizers are products containing live or latent microorganisms that are capable of mobilizing nutrients from unavailable form to available form through biological processes [1]. Biofertilizers are type of organic fertilizers which provide an ecologically safe means of fertilization. Biofertilizers are preparations of specific microorganisms which, when applied to seed, plant surfaces (such as leaves), or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the availability of nutrients to the host. Commonly used microorganisms as biofertilizer are *Azospirillum*, Phosphate Solubilizing Bacteria (PSB) and *Vesicular Arbuscular Mycorrhiza* (VAM). Soil fertility is influenced by the biofertilizers, which play an important role in fixing atmospheric nitrogen, solubilizing insoluble form of phosphorous, potash and mobilizes the immobile nutrients in soil. Biofertilizers act as an alternative which help to reduce the consumption of chemical fertilizers, to solve the environmental issues, reduce the damage to human health and enhance microbiological activity of the soil [2].

2. MATERIALS AND METHODS

2.1 Site Description

The field experiment were carried out during both *kharif* seasons of 2020-2021 at Agricultural

College Farm, Bapatla. The experimental soil was clay loam in texture, slightly alkaline in reaction (pH 7.56), non- saline (0.64 dS m^{-1}), medium in organic carbon (5.4 g kg^{-1}) and soil biological properties viz; microbial activity (bacterial population ($32.0 \times 10^6 \text{ CFU g}^{-1} \text{ soil}$), fungal population ($10.0 \times 10^3 \text{ CFU g}^{-1} \text{ soil}$); actinomycetes population ($14.0 \times 10^4 \text{ CFU g}^{-1} \text{ soil}$) and enzyme activity (urease activity ($24.67 \mu\text{g NH}_4\text{-N g}^{-1} \text{ soil } 2 \text{ hrs}^{-1}$), dehydrogenase activity ($46.37 \mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ hrs}^{-1}$), acid phosphatase activity ($51.12 \mu\text{g PNP g}^{-1} \text{ hr}^{-1}$) and alkaline phosphatase activity ($76.33 \mu\text{g PNP g}^{-1} \text{ hr}^{-1}$) at initial soil characters of the experimental field.

2.2 Experimental Design and Treatments

The experiment was laid out in randomized block design (RBD) with seven treatments and replicated thrice. The experimental treatment details are as following T₁- Control; T₂- 100% RDF; T₃- 125% RDF; T₄ – 100% RDF + VAM; T₅- 100% RDF +VAM + *Azospirillum* + PSB; T₆- 75% RDF + VAM; T₇- 75% RDF + VAM + *Azospirillum* + PSB.

Bacterial count was determined by using serial dilution pour plate method [3], Fungi count was determined by using serial dilution pour plate method [4], Actinomycetes count was determined by using serial dilution pour plate method [5]. Urease activity was measured by estimating the ammoniacal nitrogen in soil suspension by steam distillation method [6], Dehydrogenase activity in the soil sample was determined by following the procedure as described by Klein et al. [7], Phosphatase activity was measured by estimating concentration of paranitrophenol (PNP) produced due to the hydrolysis of the substrate p-nitrophenol phosphate by phosphatase enzyme [8], respectively.

3. RESULTS AND DISCUSSION

3.1 Microbial Activity

3.1.1 Bacterial population

Close perusal of the data pertaining to bacterial population (Table 1) indicated that various levels of inorganics and biofertilizers treatments imposed under maize crop have shown significant effect on bacterial population at all the

crop growth stages during 2020-2021. The results revealed that, during both the years of study and at all the three stages viz., at kneehigh, tasseling and harvest stages of maize, the treatment T₅ i.e., 100% RDF + VAM + *Azospirillum* and PSB (57.67,62.67,55.33 x 10⁶ CFU g⁻¹ soil in 2020 and 60.33, 65.33, 58.67 x 10⁶ CFU g⁻¹ soil in 2021) recorded significantly highest bacterial population in soil and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (53.00, 57.33, 51.33 x 10⁶ CFU g⁻¹ soil and 55.33, 60.33, 53.67 x 10⁶ CFU g⁻¹ soil), T₃ (125% RDF) (48.33, 54.00, 49.67 x 10⁶ CFU g⁻¹ soil and 52.67, 57.00, 51.00 x 10⁶ CFU g⁻¹ soil) and T₄ (100% RDF+VAM) (47.33, 54.00, 49.33 x 10⁶ CFU g⁻¹ soil and 50.33, 55.00, 49.00 x 10⁶ CFU g⁻¹ soil) at kneehigh, tasseling and harvest stages of maize crop. They were significantly superior over other treatments during 2020-2021, respectively. The lowest bacterial population was recorded in the treatment T₁ i.e., control (26.33, 30.00, 26.87 x 10⁶ CFU g⁻¹ soil in 2020 and 29.33, 35.33, 27.00 x 10⁶ CFU g⁻¹ soil in 2021) which received no fertilizers at all the three stages of crop growth.

Combined application of inorganics and biofertilizers significantly influenced the bacterial population. Increase in bacterial population was due to increased supply of nutrients and more organic matter content [9]. This may be due to the rhizospheric effect, which provides a rich source of energy and nutrients to the bacteria resulting in higher bacterial populations. It also enhances availability of root exudates and growth promoting substances during growth of crop. The effect of treatments on bacterial population was gradually increased from kneehigh to tasseling and later decreased after harvest of the crop. The results were in corroboration with those of Selvi et al. [10] who attributed it to the release of more root exudates during active growth of the plant.

3.1.2 Fungal population

The data presented in the Table 2 indicated that various levels of inorganics and biofertilizers treatments imposed under maize crop have shown significant effect on fungal population at all the crop growth stages during 2021-2021 of experimentation. Irrespective of the growth stages of maize and both the years study, the treatment T₅ i.e., 100% RDF + VAM + *Azospirillum* and PSB (18.67, 21.67, 16.33 x 10³ CFU g⁻¹ soil in 2020 and 20.67,23.67,19.67 x 10³ CFU g⁻¹ soil in 2021) recorded significantly highest fungal population in soil and it was on

par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (18.33, 19.67, 14.67 x 10³ CFU g⁻¹ soil and 19.33, 21.67, 18.33 x 10³ CFU g⁻¹ soil), T₃ (125% RDF) (17.33, 18.67,15.67 x 10³ CFU g⁻¹ soil and 19.33,21.00,17.33 x 10³ CFU g⁻¹ soil) and T₄ (100% RDF+VAM) (15.67, 17.33, 13.67 x 10³ CFU g⁻¹ soil and 17.33, 20.33, 16.67 x 10³ CFU g⁻¹ soil) at kneehigh, tasseling and harvest stages of maize crop. They were significantly superior over other treatments during 2020-2021, respectively. The lowest fungal population was recorded in the treatment T₁ i.e., control (8.00, 10.33, 8.00 x 10³ CFU g⁻¹ soil in 2020 and 9.33, 12.67, 9.67 x 10³ CFU g⁻¹ soil in 2021) which received no fertilizers at all the three stages of crop growth.

Combined application of inorganics and biofertilizers significantly influence the fungal population at different stages of maize. The fungal population increased from kneehigh to tasseling and decreased harvest stage of crop. Yevdokimov et al., [11] found that the addition of increasing amounts of N fertilizer increased soil fungal biomass significantly and a high sensitivity of fungi to other nutrients, such as N, than to carbon. This phenomenon explained the increase in fungal biomass with the increase in recommended dose of N fertilizer [12]. An improvement in fungal populations with combined application of biofertilizers and inorganics might be due to maintenance of microbial population in soil with the help of organics Ravishankar et al. [13]. Significant increase in fungal population with the biofertilizer application was due to availability of sufficient food and energy source in the form of organic carbon [14].

3.1.3 Actinomycetes population

Close observation of data revealed that significant difference in actinomycetes population was noticed in the soils with application of different levels of fertilizers and biofertilizer treatments (Table 3). Significantly higher actinomycetes population was recorded in the treatment T₅ i.e., 100% RDF + VAM + *Azospirillum* and PSB (21.33,23.67,19.67 x 10⁴ CFU g⁻¹ soil in 2020 and 23.67,25.33,21.67 x 10⁴ CFU g⁻¹ soil in 2021) and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (19.67, 22.33, 18.33 x 10⁴ CFU g⁻¹ soil and 22.67, 24.33, 19.67 x 10⁴ CFU g⁻¹ soil), T₃ (125% RDF) (19.00, 21.33, 17.00 x 10⁴ CFU g⁻¹ soil and 21.00, 22.67, 18.33 x 10⁴ CFU g⁻¹ soil) and T₄ (100% RDF+VAM) (18.67, 20.67, 16.67 x 10⁴ CFU g⁻¹ soil and 20.33, 22.33, 18.00 x 10⁴ CFU g⁻¹ soil).

CFU g⁻¹ soil) at kneehigh, tasseling and harvest stages of maize crop. They were significantly superior over other treatments during 2020-2021, respectively. The lowest actinomycetes population was recorded in the treatment T₁ i.e., control (12.33, 13.67, 10.00 x 10⁴ CFU g⁻¹ soil in 2020 and 12.67, 15.00, 10.33 x 10⁴ CFU g⁻¹ soil in 2021) which received no fertilizers at all the three stages of crop growth.

Application of biofertilizers in combination with inorganic fertilizers to maize crop resulted in significant increased in actinomycetes population during the study. Rani and Sanoria, [15] also reported that total bacterial population was positively correlated with actinomycetes and PSB population at all the growth stages of crop. The increase in microbial population could be attributed to ample supply of carbon which encourages the heterotrophic organisms compared to the inorganic fertilizer application. Addition of biofertilizers and organic manure might have also resulted in increased secondary and micronutrients required for the buildup of body tissue. Similar observations were demonstrated by Krishnakumar et al. [16] and Rajrushi et al. [17].

3.2 Enzyme Activity

3.2.1 Urease ACTIVITY

Close observation of data revealed that significant difference in urease activity was noticed in the soils with application of different levels of fertilizers and biofertilizer treatments (Table 4). Among the different treatments applied, the treatment T₅ i.e., 100% RDF + VAM + *Azospirillum* and PSB (37.64, 42.62, 36.46 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ in 2020 and 41.03, 45.63, 38.13 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ in 2021) recorded significantly highest urease activity in soil and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (34.84, 38.46, 33.60 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ and 37.85, 41.47, 35.92 µg NH₄-N g⁻¹ soil 2 hrs⁻¹), T₃ (125% RDF) (32.47, 35.67, 31.48 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ and 35.48, 38.70, 33.81 µg NH₄-N g⁻¹ soil 2 hrs⁻¹) and T₄ (100% RDF+VAM) (31.87, 34.90, 31.32 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ soil and 34.47, 37.92, 32.65 µg NH₄-N g⁻¹ soil 2 hrs⁻¹) at kneehigh, tasseling and harvest stages of maize crop. They were significantly superior over other treatments during 2020-2021, respectively. The lowest urease activity was recorded in the treatment T₁ i.e., control (19.12, 21.22, 17.55 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ in 2020 and 20.43, 22.30, 18.41 µg NH₄-N g⁻¹ soil 2 hrs⁻¹ in 2021) which

received no fertilizers at kneehigh, tasseling and harvest stages of maize crop.

Application of different levels of fertilizers and biofertilizers significantly influenced by the urease activity. Enhancement of urease activity with increased rate of nitrogen application along with biofertilizers to soil which acted as sole source of carbon and energy for microbes by which their population increased resulting in increased enzymatic activity [18]. They also documented that urease enzyme activity was highly related to soil organic matter content and microbial activity of the soils. The urease activity was observed to be positively correlated with bacterial population and available nitrogen in soil. This might be due to the reason that urease enzyme is responsible for the hydrolysis of urea fertilizer thus its activity increases with increase in the level of N-fertilizer in the form of urea. On the other hand, urease activity increased with advancement of crop stage i.e., from kneehigh to tasseling stage and then decreased at harvest stage of the crop. Lowest urease activity was obtained at harvest stage which might be due to the continuous depletion of nitrogen in the soil.

3.2.2 Dehydrogenase activity

The data presented in the Table 5 revealed that dehydrogenase activity in soil at different stages of maize was significantly influenced by different levels of fertilizers and biofertilizer treatments during both the years of study. The highest dehydrogenase activity was recorded in the treatment T₅ i.e., 100% RDF + VAM + *Azospirillum* and PSB (66.65, 74.13, 64.68 µg TPF g⁻¹ soil 24 hrs⁻¹ in 2020 and 70.51, 76.44, 68.01 µg TPF g⁻¹ soil 24 hrs⁻¹ in 2021) at kneehigh, tasseling and harvest stages of maize crop during 2020-2021 and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (62.83, 70.32, 60.56 µg TPF g⁻¹ soil 24 hrs⁻¹ and 67.48, 72.30, 63.89 µg TPF g⁻¹ soil 24 hrs⁻¹), T₃ (125% RDF) (60.75, 68.16, 58.76 µg TPF g⁻¹ soil 24 hrs⁻¹ and 64.55, 70.88, 61.42 µg TPF g⁻¹ soil 24 hrs⁻¹) and T₄ (100% RDF+VAM) (57.97, 65.64, 56.34 µg TPF g⁻¹ soil 24 hrs⁻¹ soil and 62.32, 67.96, 59.36 µg TPF g⁻¹ soil 24 hrs⁻¹). They were significantly superior over other treatments during both the years of study, respectively. The lowest dehydrogenase activity was recorded in the treatment T₁ i.e., control (39.52, 42.02, 38.07 µg TPF g⁻¹ soil 24 hrs⁻¹ in 2020 and 42.16, 44.51, 41.07 µg TPF g⁻¹ soil 24 hrs⁻¹ in 2021) which received no fertilizers at all the three stages of crop growth.

The treatments having application of biofertilizer and inorganic fertilizers were observed with higher dehydrogenase activity as compared to control. Dehydrogenase activity was recorded highest in the treatments which received inorganics and biofertilizers this might be due to the enhanced level of soil enzyme activity which promotes the recycling of nutrients in the soil ecosystem. Similar results were also reported by Rai and Yadav, [19]. This may be due to stimulation of bacterial population by inoculation of biofertilizers. Significant positive correlation was recorded between bacterial population and dehydrogenase enzyme. They also showed positive correlation between microbial biomass and soil dehydrogenase activity. Masto et al. [20] also documented that mineral N fertilizers increased the dehydrogenase activity in soil. The increase in dehydrogenase activity was due to increase in population of anaerobic microorganism in soils. However, the dehydrogenase activity gradually increased with advancement of crop stage *i.e.*, from kneehigh to tasseling stage and but later decreased at harvest of the crop. This might be due to the continuous depletion of organic matter in the soil.

3.2.3 Acid phosphatase activity

The data presented in the Table 6 revealed that the acid phosphatase activity in soil significantly increased with combined application of inorganics and biofertilizers in the maize during *kharif* 2020 and 2021. Significantly higher acid phosphatase activity in soil was recorded in the treatment T₅ *i.e.*, 100% RDF + VAM + *Azospirillum* and PSB (72.72, 77.19, 70.02 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2020 and 75.39, 80.20, 72.37 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2021) and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (68.87, 74.37, 66.71 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 71.52, 77.03, 68.74 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$), T₃ (125% RDF) (66.56, 72.17, 64.53 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 69.22, 75.16, 66.54 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$) and T₄ (100% RDF+VAM) (64.84,71.16, 63.57 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 67.18, 73.15, 65.25 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$) at kneehigh, tasseling and harvest stages of maize crop. The lowest acid phosphatase activity was recorded in the treatment T₁ *i.e.*, control (43.35, 49.81, 41.27 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2020 and 46.02, 53.38, 44.29 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2021) which received no fertilizers at kneehigh, tasseling and harvest stages of maize crop. However, the acid phosphatase activity gradually increased with advancement of crop stage *i.e.*, from kneehigh to tasseling stage and later decreased at harvest. This might be due to the continuous depletion of phosphorus in the soil.

The treatments having application of biofertilizer and inorganic fertilizers were observed with higher acid phosphatase activity as compared to control. Higher acid phosphatase activity might be ascribed to the increased population of microorganisms due to availability of substrate which in turn release this enzyme of extracellular origin. These findings are in agreement with the studies of Rama Krishnaiah and Vijaya [21]. Plant response to these PSB could be associated with other mechanisms, rather than only P solubilization. However, PSB can facilitate growth and development of plants by producing essential nutrients or by varying the concentration of plant growth promoting substances including phytohormones, such as indole acetic acid [22]. Phosphatase enzymes are believed to be involved in mineralizing organic phosphorus to inorganic phosphorus. The increased activity of phosphatase enzyme in soil might be due to increased solubilization and mobilization of soil through the activity of phosphatase enzyme which was increased when PSB was used. PSB strains were reported to produce significant amount of organic acids and showed enormous potential for increasing available P to the plant vicinity [23].

3.2.4 Alkaline phosphatase activity

The data presented in Table 7 revealed that alkaline phosphatase activity in soil at different stages of maize was significantly influenced by different levels of fertilizers and biofertilizer treatments during *kharif* 2020 and 2021. The results revealed that significantly higher values of alkaline phosphatase activity was recorded with the treatment T₅ *i.e.*, 100% RDF + VAM + *Azospirillum* and PSB (111.64, 119.75, 108.66 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2020 and 114.67, 122.38, 110.65 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2021) recorded and it was on par with the treatments T₇ (75% RDF+ VAM + *Azospirillum* and PSB) (105.88, 113.59, 103.20 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 108.89, 118.95, 105.17 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$), T₃ (125% RDF) (102.93, 109.20, 99.82 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 104.04, 111.88, 100.82 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$) and T₄ (100% RDF+VAM) (98.26, 106.51, 96.66 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ and 101.63, 108.84, 99.69 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$) at kneehigh, tasseling and harvest stages of maize crop. The lowest alkaline phosphatase activity was recorded in the treatment T₁ *i.e.*, control (71.05, 78.11, 71.28 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2020 and 74.38, 82.51, 73.47 $\mu\text{g PNP g}^{-1} \text{hr}^{-1}$ in 2021) which received no fertilizers at all the three stages of maize crop.

Table 1. Effect of different levels of fertilizers in combination with biofertilizers on bacteria population ($X 10^6$ CFU g^{-1} soil) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	26.33	34.00	26.87	29.33	35.33	27.00
T ₂ : 100% RDF	41.67	49.67	40.67	45.33	52.33	44.00
T ₃ : 125% RDF	48.33	54.00	49.67	52.67	57.00	51.00
T ₄ : 100% RDF + VAM	47.33	54.00	49.33	50.33	55.00	49.00
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	57.67	62.67	55.33	60.33	65.33	58.67
T ₆ : 75% RDF + VAM	37.67	44.33	34.00	40.33	47.67	38.00
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	53.00	57.33	51.33	55.33	60.33	53.67
SEm (\pm)	3.40	2.95	2.21	3.28	3.72	3.42
CD (P=0.05)	10.47	9.09	6.81	10.10	11.45	10.53
CV (%)	13.08	9.90	8.78	11.72	11.81	12.66

Table 2. Effect of different levels of fertilizers in combination with biofertilizers on fungi population ($X 10^3$ CFU g^{-1} soil) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	8.00	10.33	8.00	9.33	12.67	9.67
T ₂ : 100% RDF	14.33	16.33	13.00	15.67	18.67	14.33
T ₃ : 125% RDF	17.33	18.67	15.67	19.33	21.00	17.33
T ₄ : 100% RDF + VAM	15.67	17.33	13.67	17.33	20.33	16.67
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	18.67	21.67	16.33	20.67	23.67	19.67
T ₆ : 75% RDF + VAM	12.33	14.00	11.00	13.67	16.33	13.67
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	18.33	19.67	14.67	19.33	21.67	18.33
SEm (\pm)	1.12	1.03	0.95	1.17	1.15	1.12
CD (P=0.05)	3.42	3.17	2.93	3.60	3.54	3.44
CV (%)	12.63	10.65	12.38	12.04	10.58	12.34

Table 3. Effect of different levels of fertilizers in combination with biofertilizers on actinomycetes population ($\times 10^4$ CFU g^{-1} soil) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	12.33	13.67	10.00	12.67	15.00	10.33
T ₂ : 100% RDF	18.33	19.67	15.33	19.67	21.67	16.67
T ₃ : 125% RDF	19.00	21.33	17.00	21.00	22.67	18.33
T ₄ : 100% RDF + VAM	18.67	20.67	16.67	20.33	22.33	18.00
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	21.33	23.67	19.67	23.67	25.33	21.67
T ₆ : 75% RDF + VAM	16.00	17.33	13.33	16.33	19.00	14.33
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	19.67	22.33	18.33	22.67	24.33	19.67
SEm (\pm)	0.95	1.12	0.99	1.16	1.02	1.20
CD (P=0.05)	2.94	3.45	3.04	3.59	3.14	3.65
CV (%)	9.22	9.87	10.64	10.20	8.25	11.88

Table 4. Effect of different levels of fertilizers in combination with biofertilizers on urease activity (μg NH₄⁺ N g⁻¹ 2hrs⁻¹) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	19.12	21.22	17.55	20.43	22.30	18.41
T ₂ : 100% RDF	27.85	33.20	26.32	30.50	35.19	28.33
T ₃ : 125% RDF	32.47	35.67	31.48	35.48	38.70	33.81
T ₄ : 100% RDF + VAM	31.87	34.90	31.32	34.47	37.92	32.65
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	37.64	42.62	36.46	41.03	45.63	38.13
T ₆ : 75% RDF + VAM	25.30	29.29	24.13	26.83	30.31	25.12
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	34.84	38.46	33.60	37.85	41.47	35.92
SEm (\pm)	1.92	2.57	2.03	1.90	2.54	2.10
CD (P=0.05)	5.91	7.93	6.27	5.85	7.83	6.48
CV (%)	10.97	12.97	12.09	10.02	11.98	11.83

Table 5. Effect of different levels of fertilizers in combination with biofertilizers on dehydrogenase activity ($\mu\text{g TPF g}^{-1} 24 \text{ hrs}^{-1}$) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	39.52	42.02	38.07	42.16	44.51	41.07
T ₂ : 100% RDF	54.47	58.57	53.10	58.29	60.71	55.75
T ₃ : 125% RDF	60.75	68.16	58.76	64.55	70.88	61.42
T ₄ : 100% RDF + VAM	57.97	65.64	56.34	62.32	67.96	59.36
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	66.65	74.13	64.68	70.51	76.44	68.01
T ₆ : 75% RDF + VAM	51.09	56.38	49.44	53.56	58.08	53.45
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	62.83	70.32	60.56	67.48	72.30	63.89
SEm (\pm)	3.59	4.71	3.51	2.86	4.33	3.69
CD (P=0.05)	11.06	14.22	10.83	8.82	13.34	11.36
CV (%)	10.99	12.99	11.13	8.22	11.53	11.12

Table 6. Effect of different levels of fertilizers in combination with biofertilizers on acid phosphatase ($\mu\text{g PNP g}^{-1} \text{ hr}^{-1}$) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	43.35	49.81	41.27	46.02	53.38	44.29
T ₂ : 100% RDF	60.03	65.12	57.21	63.51	68.12	60.66
T ₃ : 125% RDF	66.56	72.17	64.53	69.22	75.16	66.54
T ₄ : 100% RDF + VAM	64.84	71.16	63.57	67.18	73.15	65.25
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	72.72	77.19	70.02	75.39	80.20	72.37
T ₆ : 75% RDF + VAM	56.98	62.12	53.38	60.33	65.48	57.39
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	68.87	74.37	66.71	71.52	77.03	68.74
SEm (\pm)	3.76	3.51	3.66	3.39	3.50	3.65
CD (P=0.05)	11.59	10.82	11.29	10.45	10.79	11.23
CV (%)	10.15	8.80	10.22	8.85	8.51	9.88

Table 7. Effect of different levels of fertilizers in combination with biofertilizers on alkaline phosphatase ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$) at different stages of maize

Treatments	Kharif (2020)			Kharif (2021)		
	Kneehigh	Tasseling	Harvest	Kneehigh	Tasseling	Harvest
T ₁ : Control	71.05	78.11	71.28	74.38	82.51	73.47
T ₂ : 100% RDF	92.37	102.40	91.16	96.06	105.12	93.49
T ₃ : 125% RDF	102.93	109.20	99.82	104.04	111.88	100.82
T ₄ : 100% RDF + VAM	98.26	106.51	96.66	101.63	108.84	99.69
T ₅ : 100% RDF + VAM+ <i>Azospirillum</i> + PSB	111.64	119.75	108.66	114.67	122.38	110.65
T ₆ : 75% RDF + VAM	90.92	97.11	89.46	93.69	101.81	90.49
T ₇ : 75% RDF + VAM + <i>Azospirillum</i> + PSB	105.88	113.59	103.20	108.89	118.95	105.17
SEm (\pm)	5.75	5.04	5.23	5.15	4.97	5.18
CD (P=0.05)	17.73	15.53	16.11	15.88	15.33	15.97
CV (%)	10.04	8.23	9.51	8.90	7.88	9.26

The alkaline phosphatase activity gradually increased with advancement of crop stage *i.e.*, from kneehigh to tasseling stage and but later decreased at harvest. This might be due to the continuous depletion of phosphorus in the soil. This might be due to the reason that microbes continuously produce and secrete the enzyme necessary for degradation of their substrate (food). Roots might have secreted organic acids and carbohydrates, which stimulate higher soil enzyme activities. Soil alkaline phosphatase was found to be positively correlated with soil PSB population at each time intervals. They also recorded increase in enzyme activity with corresponding increase in microbial population. Higher alkaline phosphatase activity was observed with conjunctive use of PSB, which increase soil microflora resulting more soil enzyme activity [24]. Increases dose of inorganic phosphorus increased the activity of alkaline phosphatase activity. The results are in conformity with the findings of Venkatarao et al. [25].

4. CONCLUSIONS

Combined application of different levels of fertilizers and biofertilizers significantly improved soil microbial and enzyme activity in soil. The integrated use of biofertilizers and along with inorganic fertilizers will help in improving nutrient use efficiency, protect nutrients against losses such as leaching and volatilization losses and reduce soil and environmental degradation. Biofertilizers are eco- friendly, non-toxic and relatively cheaper natural products. The application of biofertilizers and inorganics not only improving nutrient availability but also improves the protect the soil against degradation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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