

RHIZOBACTERIAL INOCULANTS COMBINED WITH INORGANIC AND ORGANIC AMENDMENTS AFFECTED WHEAT ATTRIBUTES, SOIL PROPERTIES AND SOIL ENZYME ACTIVITIES

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Abstract

A field experiment to investigate the impact of mineral fertilizers, organic manure along with microbial inoculant on agronomic attributes, soil properties and enzyme activities on wheat quantitative and qualitative attributes. The experiment was laid in Randomized Complete Block Design with split plot arrangement. Bio-inoculants (Biozote Max and Biozote) were assigned to the main plots, while organo – chemical amendments including press mud @ 5 t ha⁻¹ + ½ RDF, Poultry Manure @ 5 t ha⁻¹ + ½ RDF, FYM @ 5 t ha⁻¹ + ½ RDF, NPK @ 90: 120: 150 kg ha⁻¹ were allocated to sub plots. Soil phosphate contents, growth, physiology, and yield of wheat and enzyme activities were studied. Result of the study showed that biozote max gave higher value for various parameters namely plant height (91.3 cm), number of tillers per plant (9.96), thousand grain weight (42.15g) and straw yield (37.33 t ha⁻¹). While the grain yield (5.33 t ha⁻¹) and harvest index (51.33) were recorded highest in the NPK treated plots along with biozote max. Biochemical parameters showed highest chlorophyll content (2.86 SPAD value) and P – uptake (9.88 mg kg⁻¹) in FYM integrated with biozote max plots, while the percent protein content (12%) was found maximum in NPK along biozote max. Soil Parameters including soil pH, Electrical conductivity and Bulk density were significantly reduced 8.17, 1.24 µScm⁻¹ and 1.41 g cm⁻³ respectively by the application of different amendments. The Nutrients content was also significantly changed by the application of organic amendments with Rhizobacterial inoculation. The Acid and Alkaline Phosphatase activity was significantly changed, acid phosphatase was recorded higher in treatment where inorganic fertilizer was applied, while Alkaline Phosphatase was higher in Poultry manure treated plots along with inoculants. These results reflect that organic amendments, inorganic fertilizer and inoculants have significant role in improvement of soil physico – chemical properties, nutrients content and activity of enzyme phosphatase.

Key words: Enzymes, Fertilizers, Manures, Phosphorus.

Introduction

Phosphorus is an important major nutrient required by plants in large quantities for various functions including as a vital component of DNA and RNA, crucial in energy storage and transfer and important for plant growth and development. Phosphorus deficiency has become limiting factors for plant growth due to P fixation by clay, alkaline and calcareous soils. Additionally, significant P turnover takes place at the surface layers of soil, and the number of microbes decreases with increasing soil depth. To ensure that agricultural areas had an appropriate supply of labile P, many P management systems were put into action (Withers *et al.*, 2018). Because of this, many ecosystems across the globe are currently being exposed to mineral and organic phosphate fertilizer inputs, leading to the accumulation of stable phosphate minerals and organic compounds in soil through biological assimilation as well as chemical processes of precipitation and sorption that are involved in the process of phosphate fixation (Luo *et al.*, 2022). The fertility of the soil is directly related to the availability of these pools for utilization by plants (Simpson *et al.*, 2011).

There are different alternatives that are being used instead of fertilizers, include compost, manures from livestock and plant waste products (Chojnacka *et al.*, 2020). These organic supplements influence the biomass and variety of microorganisms, which may result in an increase in the rate of nutrient cycling in the soil as well as other ecological activities, such as extracellular enzyme activity

(e.g., phosphatase synthesis) (Steinauer *et al.*, 2015). Agricultural practices can have a significant impact on nutrient dynamics, such as the amount of phosphorus that is available to plants for growth (Singh *et al.*, 2012; Purakayastha *et al.*, 2019). Inoculation of Plant Solubilizing Bacteria (PSBs) with or without organic or inorganic fertilizers has been a concern for research on P dynamics, as it results in varying crop yields and P availability (Manzoor *et al.*, 2017). The mineral-associated phosphorus content of poultry manure is significantly higher than other manures (Samson *et al.*, 2020). Diverse soil organisms have different responses to the availability of phosphorus. These differences can be attributed to differences in nutritional requirements and economic strategies. A significant amount of phosphorus is absorbed into the biomass of rhizosphere bacteria from manure. The length of time that phosphorus spends residing in microbial biomass determines how the reduced availability of phosphorus affects plant and microbial absorption. The chemical sorption of phosphorus in soil can be reduced when microbe's breakdown orthophosphate in soil solutions and then release it at the appropriate moment (Jones & Oburger, 2011). The amount of time it takes for microbial biomass to release phosphorus into the environment can range anywhere from ten days to one year, depending on the kind of soil and the amount of fertilizer that is added (Richards, 2012). This is the potential source of available phosphorus (P) for plants, particularly under conditions where P is in short supply. It has been reported by many workers that Phosphate-solubilizing

bacteria boost plant growth and increase the availability of phosphorus to plants (Timofeeva *et al.*, 2022). A synergistic and complimentary microbial consortium, based on a variety of functional groups of strains has the potential to increase plant development and the impacts of organic fertilizer (Santoyo *et al.*, 2021). Phosphorus-mineralizing bacteria are responsible for the production of organic acids and enzymes, both of which are necessary for the hydrolysis of inorganic complexes (Wan *et al.*, 2021).

Crop available phosphorus is affected by the inoculation of *Bacillus* and *Pseudomonas* (Kumar *et al.*, 2017). An increase in the proportion of labile phosphorus in soil can be attributed, in parts to the production of hydrolysing enzymes and organic acids during the mineralization and solubilization of organic molecules and P mineral complexes (Afzal *et al.*, 2014). A variety of integrated soil fertility management techniques can be used in addition to employing organic waste as a source of nutrients. The current study aims to utilize microbial inoculants along with various manures to increase the amount of phosphorus available to plants, improve soil health, properties and nutrient content and above all increase wheat yield.

Material and Methods

Experimental site: A field experiment was conducted at research area of Department of Soil Science, Faculty of Agriculture, Gomal University, Dera Ismail Khan to assess the impact of integrated application of inorganic fertilizer and organic manure with and without bio-inoculants. Soil phosphate content, growth, physiology and yield of wheat were determined after the harvest of wheat crop.

Experimental design: The experiment was laid out in Randomized complete block design (RCBD) with split plot arrangement with two inoculants (Biozote and Biozote Max) in main plots and organo-mineral amendments in sub plots. Wheat Var. Hashim-2008 was sown after inoculation with inoculants Biozote and Biozote Max. The Organo-mineral amendments included (T₁: Control + ½ RDF, T₂: Press Mud @ 5 t ha⁻¹ + ½ RDF, T₃: Poultry Manure @ 5 t ha⁻¹ + ½ RDF, T₄: FYM @ 5 t ha⁻¹ + ½ RDF, T₅: NPK @ 90: 120: 150 kg ha⁻¹). Complete dosage according to each treatment of P and K was applied as basal using DAP and K₂SO₄ as sources alongwith urea in two splits at 1st and 2nd irrigations. Each treatment was replicated thrice to minimize error. Field samples were collected before sowing of the crop for pre-sowing soil analysis. Before sowing seeds were treated with bio-inoculants. Land was prepared using standard cultural practices and during crop period weeds were controlled manually. At the time of harvest different agronomic parameters including plant height, No. of tillers per plant, 1000 grain weight, grain and straw yield and harvest index were determined.

Biochemical analysis

Biochemical analysis was carried out by determining the SPAD Chlorophyll using portable SPAD meter at heading stage, while plant phosphate and protein content were determined by digestion of plant material in block digester and phosphorus in the extract was measured by

spectrophotometer. While protein content was determined by Kjeldhal apparatus.

Soil analysis: Pre-sowing soil parametric status is presented in (Table 1). After crop harvest composite soil samples from each plot were collected, grounded and passed through 2 mm sized mesh. The core samplers were used to collect undisturbed soil samples and oven dried to quantify bulk density (Blake & Hartage, 1986) of soil. The sieved soil samples were processed to measure soil organic matter using titrimetric technique (Nelson & Sommers, 1996), total nitrogen through Kjeldhal apparatus (Bremner & Mulvaney, 1982), extractable phosphorus by using NaHCO₃ extractant determined on UV-Vis spectrophotometer (Olsen & Watanabe, 1957) and extractable potassium using flame photometer (Richards, 2012). The assay of alkaline and acid phosphatases was carried out in a fresh sample using the procedure given by Tabatabai (1994).

Table 1. Pre-sowing soil characteristics.

Characteristics	Value	Unit
Textural class	Clay Loam	
pH	8.34	
EC	1.56	dSm ⁻¹
Organic matter	0.61	%
Bulk Density	1.08	gcm ⁻³
SAR	7.87	
Nitrogen (N)	0.051	%
Phosphorus (P)	6.03	mg kg ⁻¹
Potassium (K)	4.05	mg kg ⁻¹

Statistical analysis

Statistical analysis was carried using statistical package Statistics 9.1 and the means were compared at 5% level of significance using the least significance difference (LSD) (Steel *et al.*, 1997).

Results

Agronomic parameters: Different plant parameters were determined during study including plant height, number of tiller per plant, 1000 grain weight, grain yield, straw yield and harvest index.

The height of wheat plants was significantly affected by the different organic/synthetic amendments and microbial inoculants (Fig. 1a). The tallest plant (91.3 cm) was recorded in the treatment receiving FYM along with Biozote Max. It was statistically at par with the treatment receiving FYM with Biozote inoculant. The least height was found in non-treated plants that were significantly shorter than others.

The effect of various organic manure and synthetic fertilizers inoculation with microbes on tillers count of wheat crop is presented in (Fig. 1b). The maximum number of tillers were calculated in farmyard manure along with biozote max treated plots which were statistically at par with biozote and farm manure treated plots but significantly greater in number compared to all other treatments. The least number of tillers were measured in treatments receiving press mud without any inoculation of seed, it was statistically at par with control.

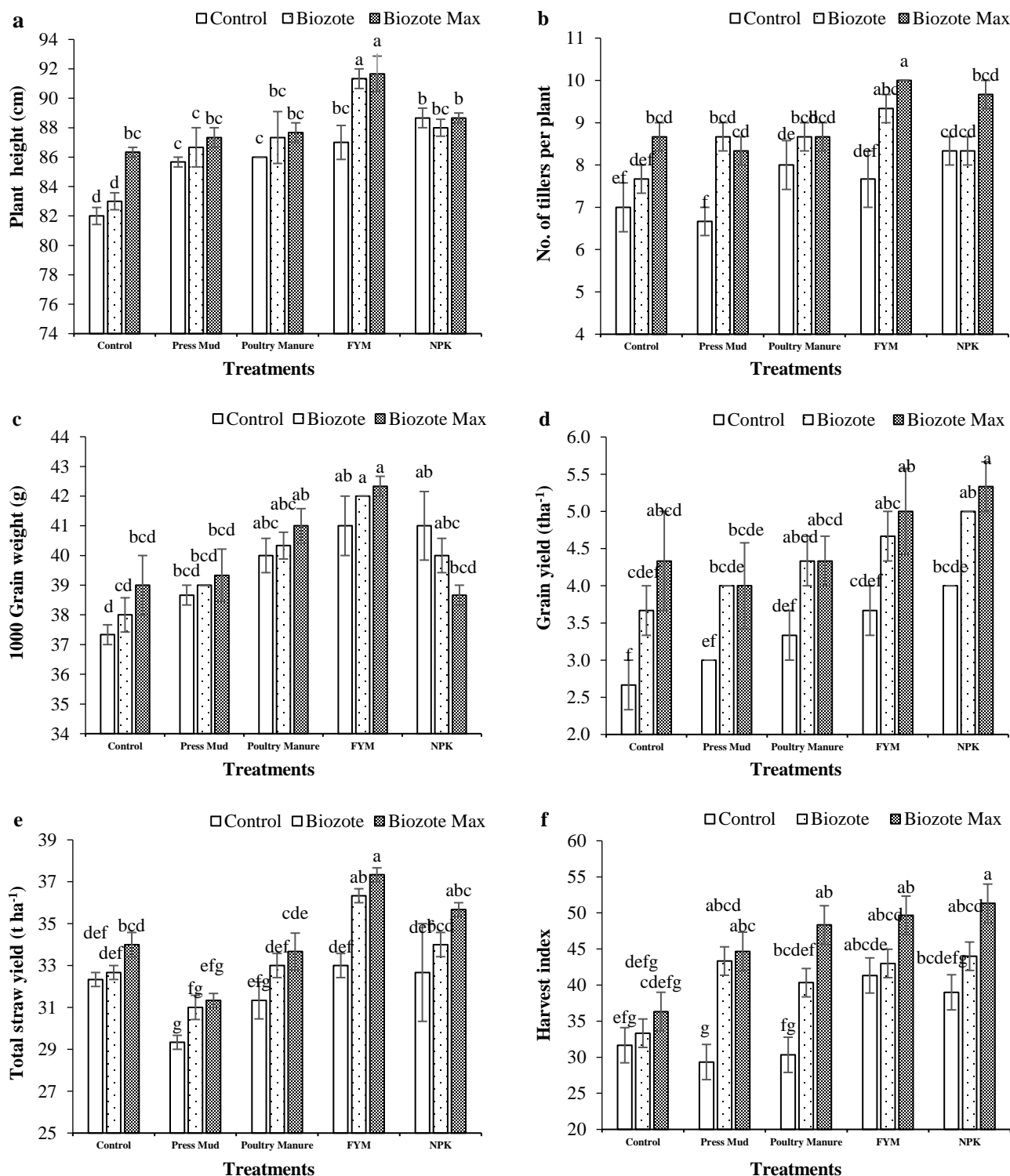


Fig. 1. Effect of Organic manures and inorganic fertilizers, inoculated with microbes on soil physiochemical parameters a) Plant height b) No. of tillers per plant c) Thousand grain weight d) Grain yield e) Straw Yield f) Harvest Index.

Thousand grain weight is an important yield contributing parameter. The highest 1000 grain weight was recorded in the treatment receiving FYM along with biozote max. It was statistically at par with the same treatment inoculated with biozote (Fig. 1c) followed by the rest of the treatments. The least 1000 grains weight were measured in the control where neither organic amendment nor inoculation was carried out.

The grain yield of wheat was significantly affected by organic manure and synthetic fertilizer with microbial

inoculants (Fig. 1d). Maximum grains yield was recorded 5.33 t ha⁻¹ in plots receiving NPK and with biozote max. Statistically similar results for Biozote max along with FYM, poultry manure was recorded for grain yield. The minimum grain yield was recorded in the control without inoculation of seed.

The straw yield of wheat was significantly increased by the application of organic manure and inorganic fertilizers along with microbial treatments (Fig. 1e). Maximum straw

yield was (37.33 t ha^{-1}) produced in farmyard manure and biozote max treated plots that was statistically at similar with biozote max and NPK treated plots but significantly lower straw yield was found in the control.

The results pertaining to the wheat harvest index showed significant effect by application of organic and inorganic amendments in combination with microbial inoculants (Fig. 1f). The highest harvest index (51.33) was observed in biozote max and NPK treated plots which was statistically at par with biozote max along with Farmyard and poultry manure and press mud treated plots followed by the rest of the treatment, with least found in control.

Biochemical parameters: Chlorophyll contents of wheat leaves were significantly affected by inorganic fertilizers and organic manure inoculated with microbial strains (Fig. 2a). The highest chlorophyll contents (2.86 SPAD value) were measured in farmyard manure and biozote max treated plant leaves that were statistically at par with all the microbe and fertilizer/manure amalgamated treatments.

Protein contents of wheat grains produced from soils treated with different treatments of organic and inorganic fertilizers and microbes showed significant difference at 5% level of significance (Fig. 2b). The grains with higher protein content were recorded in the plots where biozote max was applied with NPK which was 12%. Least protein content was found in plots where no microbial inoculants were used.

The Phosphorus uptake by wheat crop showed significant effect of organic amendments and inorganic fertilizers (Fig. 2c). The highest P- uptake was recorded in the plots receiving FYM along with biozote max as inoculant (9.88 mgkg^{-1}). It was statistically at par with other farmyard manure treated plots receiving Biozote and NPK+biozote max treated plots. The least uptake (4.33 mgkg^{-1}) was observed in non-treated plants that was significantly lower than all other treatments.

Soil physico – chemical properties: A significant variation in mean of soil reaction by the addition of organic manure, inorganic fertilizer and microbial inoculants was observed (Fig. 3a). Maximum pH (8.31) was recorded for the soils solely treated with biozote max, it was statistically at par with treated and non-treated soils and the least was measured 8.17 in soils treated solely with NPK that was suggestively lower than all others.

The data pertaining to soil electrical conductivity showed significant effect of organic manure and inorganic fertilizers along with microbial treatments (Fig. 3b). The highest electrical conductivity was recorded in the control without inoculation. The treatments of FYM and Poultry manure along with biozote and biozote max reduced the electrical conductivity respectively.

Figure 3c illustrates the significant variation in means of sodium adsorption ratio (SAR) of soil due to the addition of organic manures and synthetic fertilizer supplemented with microbial inoculation. SAR of soil treated with biozote max was the highest (7.57) that was statistically at par with sole biozote treated and manure + microbe treated plots. The control without microbial inoculation showed lower value for SAR.

Bulk density of soil also exhibited significant decrease due to addition of inorganic and organic amendments along with microbial inoculants (Fig. 3d). Maximum compactness (bulk density) 1.48 g cm^{-3} was observed in untreated control treatment that declined to 1.47 and 1.46 g cm^{-3} with application of biozote max and biozote. The value recorded for bulk density 1.41 g cm^{-3} was lowest in the soils which were treated with farmyard manure alone and in combination with biozote.

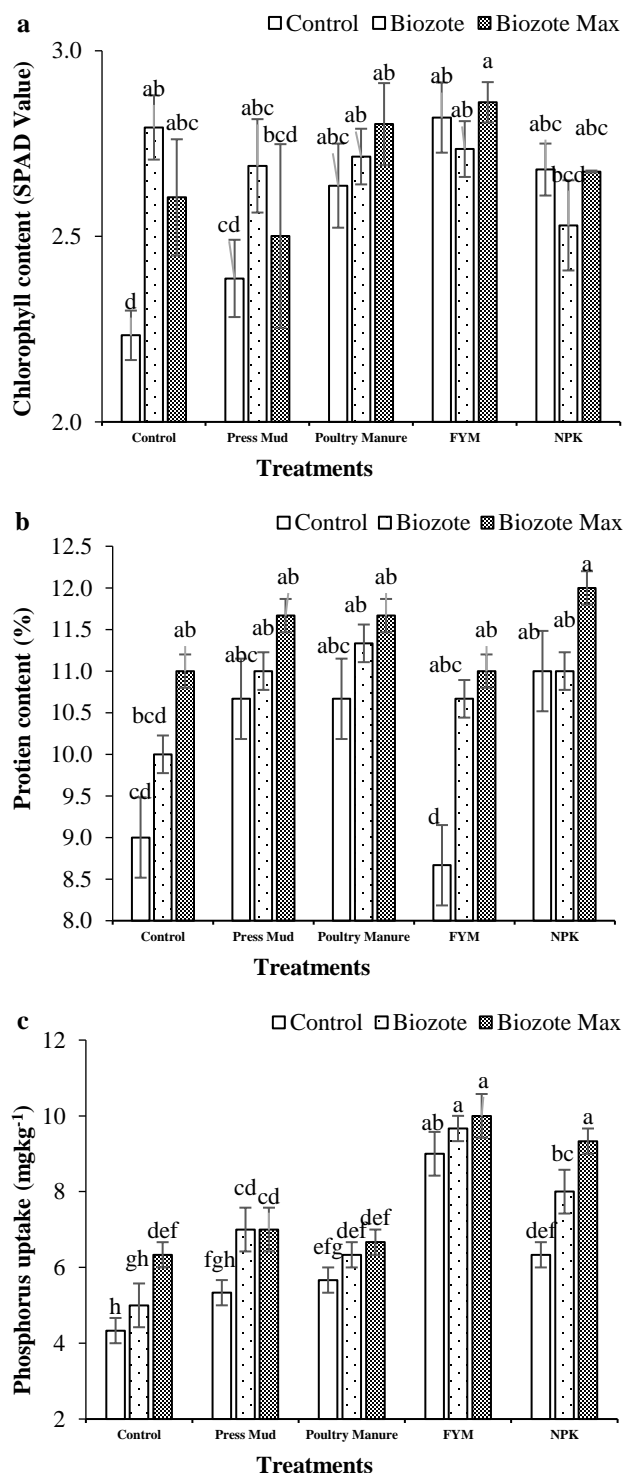


Fig. 2. Effect of Organic manures and inorganic fertilizers, inoculated with microbes on soil physiochemical parameters a) Chlorophyll content b) Protein content and c) Phosphorus uptake.

Soil macro – nutrients: The significant variation in nutrition capacity of soil is illustrated in figure 4. Figure 4a explains that a gradual increase in the nitrogen content of soil from 0.04% in non-treated soils to 0.048% in soil where manures viz. poultry manure, FYM and press mud was added along with the bio - inoculants biozote and biozote max. The least value for nitrogen was recorded in control without inoculation of microbes.

The impact of inorganic and organic amendments along with microbe on extractable soil phosphorus was significant with maximum value of 6.09 mgkg⁻¹ in non-treated control to 6.37 mgkg⁻¹ in poultry manure + biozote max treated soils (Fig. 4b). The microbial inoculant with manure have increase the soil phosphorus due to decomposition of manure and least adsorption of Phosphate.

Figure 4c demonstrates the variances in soluble potassium of soil upon treatment with inorganic fertilizer, organic manures and microbes. Application of biozote max with poultry manure resulted in higher soil potassium of 5.78 mgkg⁻¹ which was significantly greater than all other treatments as well as control.

Organic matter contents of soil were significantly affected by the addition of organic manure and synthetic fertilizer with microbes as amendment (Fig. 4d). Organic matter was increased as the organic amendments FYM, poultry manures were added. The microbial inoculants also enhanced the organic matter in soil. Highest amount of organic matter (0.67%) was found in biozote max + farmyard manure treated soils; it was statistically similar to farmyard manure treated soil (0.65%) but were significantly higher than all other treated as well as non-treated plots while least (0.52%) was found in untreated control.

Soil enzyme activities: The variation in acid and alkaline phosphatases are exhibited in figure 5(a, b). The application of mineral fertilizer NPK along with biozote max yielded 2.68 acid phosphatase that is significantly greater than all other treatments and non-treated soils. While poultry manure + biozote max application resulted in 0.72 alkaline phosphatase that was statistically at par with farmyard manure + biozote max and NPK + biozote max treated soils but was suggestively greater than all other treatments as well as control.

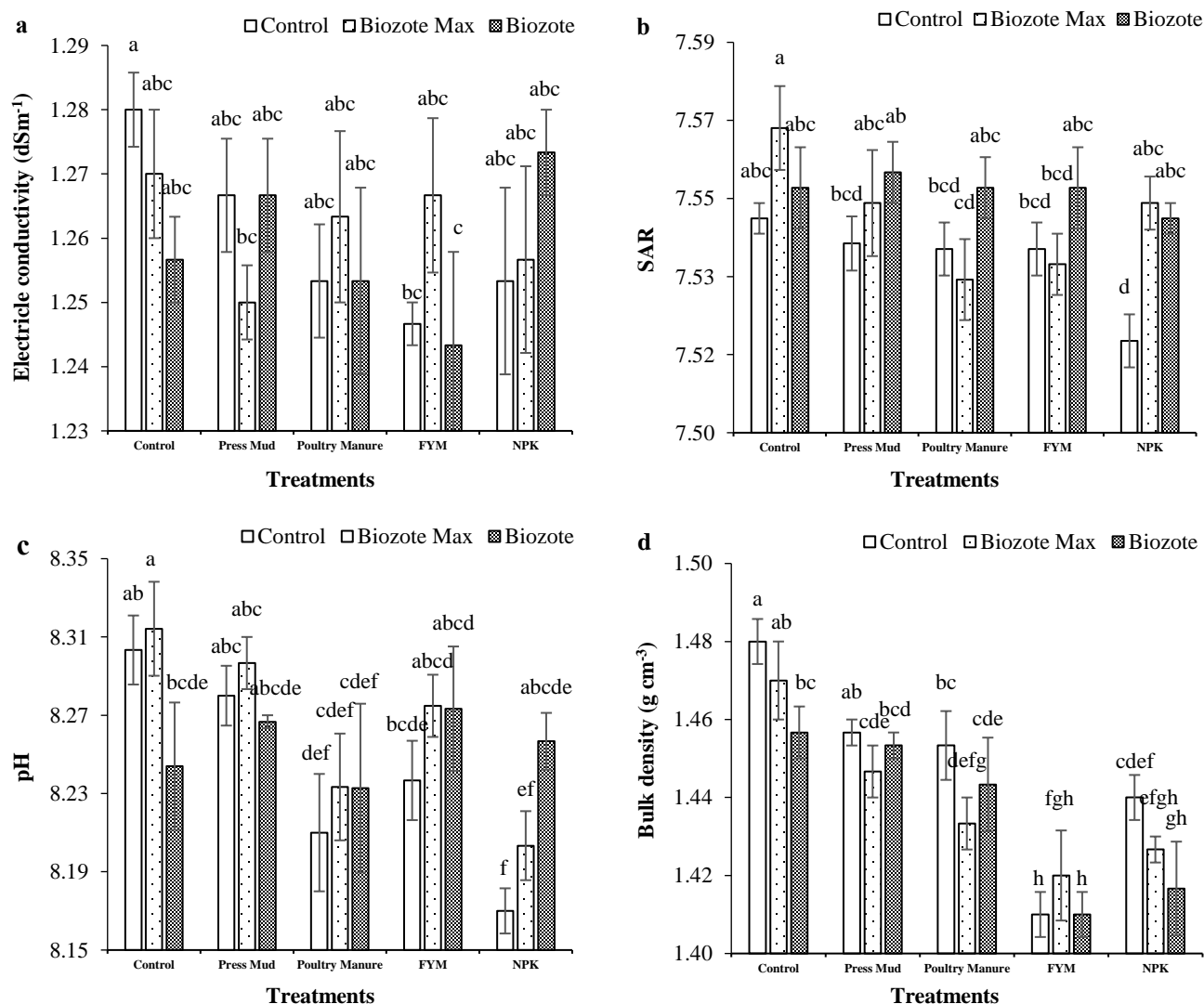


Fig. 3. Effect of Organic manures and inorganic fertilizers, inoculated with microbes on soil physicochemical parameters a) Electrical conductivity b) Soil pH c) SAR and d) Bulk Density.

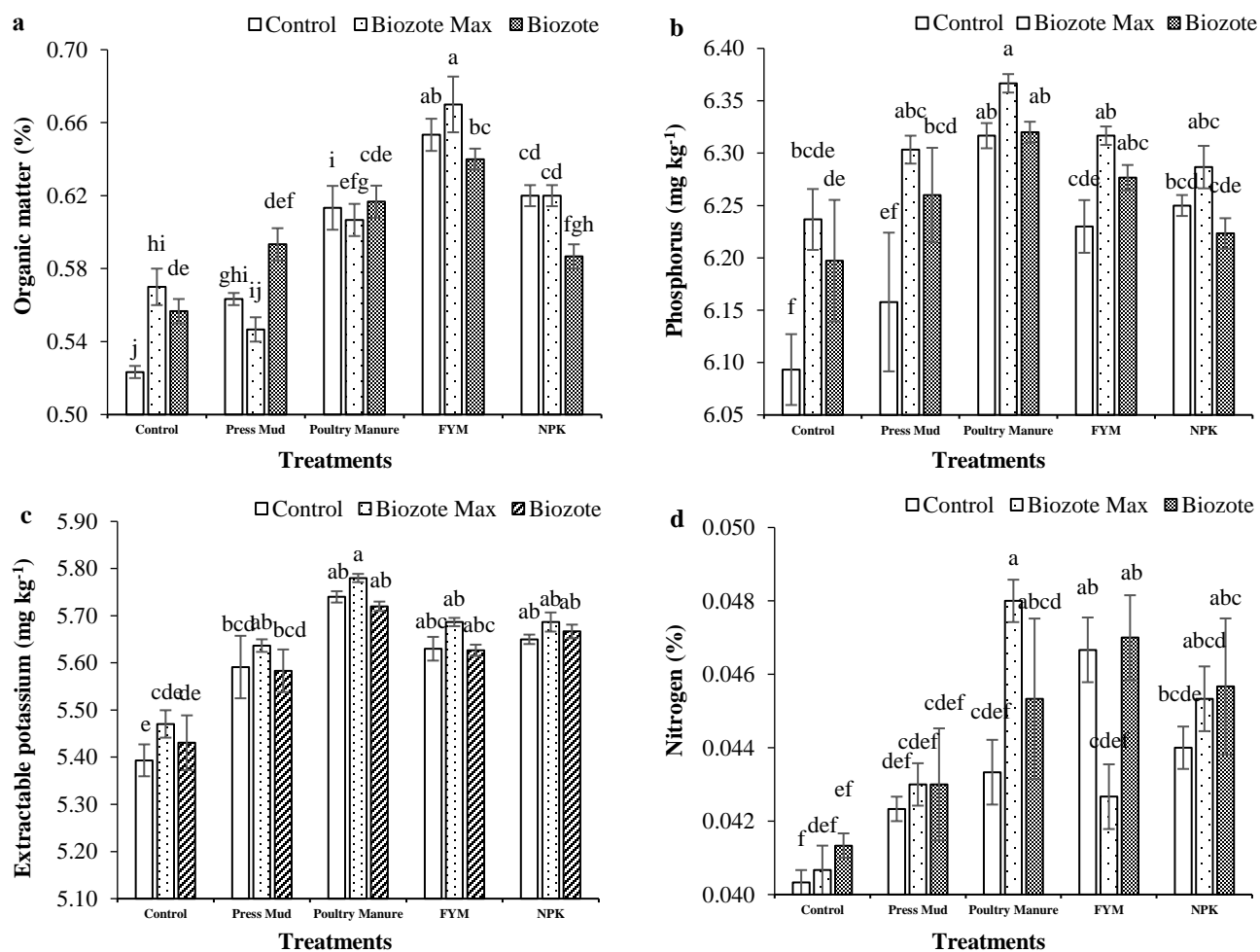


Fig. 4. Effect of Organic manures and inorganic fertilizers, inoculated with microbes on soil physicochemical parameters a) Total Nitrogen b) Extractable Phosphorus c) Extractable Potassium and d) Organic matter.

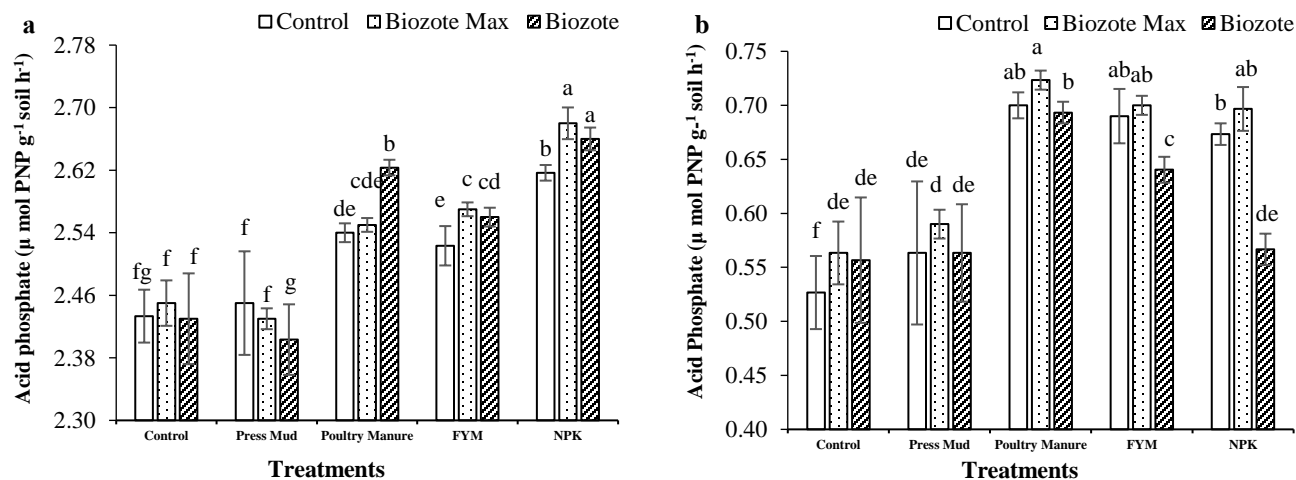


Fig. 5. Effect of Organic manures and inorganic fertilizers, inoculated with microbes on Enzyme activity a) Acid Phosphatase b) Alkaline Phosphatase.

Discussion

In this study, the implications of a synergistic interaction of bacteria and manures on agronomic and biochemical parameters of wheat, soil physicochemical characteristics, soil nutrients phosphorus and phosphatase enzymes activities were studied. Even though the influence

of interactions between P fertilizer amendment and microbial inoculation was explored. In general, the findings revealed that bacteria have the potential to significantly alter soil processes when organic inputs are made.

The wheat plants in the trial that received manure and microbe modification produced much drier biomass than the wheat plants in the experiment that received no

manure modification. It is unknown how much of the plant's reaction is influenced by bacteria and manures, but they both have a significant impact on how the roots develop. Ma *et al.*, 2020 reported similar results by inoculation of microbes and organic fertilizers which has enhanced the plant biomass and phosphorus levels. The root exudates during the microbial processes in the rhizosphere by soil microbes play important role in nutrient cycling and absorption (Sokol *et al.*, 2019). When no phosphorus is lost through exudation, the organic phosphorus is metabolised and incorporated into the microbial biomass, which is thought to be the source of microbial growth in the rhizosphere (Malik *et al.*, 2013). We can conclude that the microbial loop on P mineralization is to blame for most of the direct nutrient effects given that most studies documented the direct nutrient effects brought on by micro-faunal grazing on the rhizosphere bacteria, or the "microbial loop on P mineralization," and consequently the increased growth and P transfer to the plants (Irshad *et al.*, 2011). We discovered that the changes in the plant biomass were remarkably equivalent to the interactions between microbial inoculants and the addition of manure (Fig. 1d, e). Organic phosphate is the largest soil pool, phosphorus is immobilized by the microbes (Fasusi *et al.*, 2021), preventing it from losses and making it available for plant uptake. In the current study it was found that the manure application had a sizable influence, with the solo bacterial treatment having the greater impact. The overall substrate effect was mostly attributed to the improvements in soil C pools and soil pH that were brought about by the addition of manure because all the microbial treatments, even without the addition of manure, resulted in a significant drop in plant P.

There is a possibility that due to buffering capacity of the soil the organic inputs had lower influence on the soil pH. Lim *et al.*, (2015) revealed that the pH of the soil was decreased because of the presence of organic acids in the soil. The pH of the soil may have dropped because of the microbial degradation of manures, which produced NH_4 , CO_2 and organic acids (Song *et al.*, 2020). The pH of the soil is brought down when manure is added to it, which is a beneficial impact. The results obtained lend credence to the conclusion reached by (McCauley *et al.*, 2009), which stated that the pH of soil was decreased when organic matter was added to it. Following the incorporation of organic materials into the soil, researchers observed a drop in the pH of the soil (Niwa *et al.*, 2007; Luo *et al.*, 2011).

Even though various treatments caused the soil's EC to decline, the actual readings remained above the 4.0 dSm^{-1} threshold limit. Similar studies showed that the electrical conductivity (EC) of acidic and alkaline soils was improved when organic materials of various types and microbes were added to the soil (Wu *et al.*, 2013; Kim *et al.*, 2016; Ouni *et al.*, 2013).

The increase in soil organic carbon by the application of organic amendments have been depicted in Figure 2d. The overall amount of organic carbon in the soil increased significantly relative to its beginning point as a direct result of the addition of organic amendments. The organic content of the soils that had received organic amendments

was higher than that of the control soils. The results of our study showed that the increase in organic matter was proportionate to the number of manures that was applied, in accordance with the findings of other studies (Tian *et al.*, 2017; Lin *et al.*, 2019; Su *et al.*, 2021).

The results showed that different manure treatments had a significant effect on the total amount of nitrogen that was present in the soil. The treated soils with poultry dung had a higher total nitrogen content compared to untreated soils. It's possible that the plots to which manure was applied had a higher level of lingering nitrogen in the soil than the control plots. There are also other cases of increased amounts of nitrogen in soil because of the application of manure and inoculation with microbes (Singh, 2018). A tremendous quantity of nitrogen is made available to plants in environments that have organic matter, a pH that is acidic, and appropriate soil moisture (Liu *et al.*, 2020).

Phosphorus levels in the soil can be altered using organic amendments and microbes, which influence the overall fertility of the soil (Fig. 3b). The concentration of mobile phosphorus in the control soil was the lowest of any of the soils that were tested. The capacity of the soil to have phosphorus taken from it was greatly improved by the introduction of organic manures and microorganisms. When compared to the control plots, the soils treated with microbes and manure had a significant more amount P. Utilizing these adjustments may allow for the recovery of the contents, which are nutritional in nature. This indicated that the constant infusions of P into the soil were probably coming from manures with a slow-release rate, and that the release of P was largely produced by the activities of soil microorganisms (Bargaz *et al.*, 2018). Following the addition of organic amendments Marinari *et al.*, (2000) found that there were comparable increases in the amount of phosphorus found in the soil. More mineralization occurred because of increased phosphatase activity and the breakdown of physical substance (Sherene, 2017). Asghar *et al.*, (2021) reported that use of poultry manure enhanced the phosphatase activity in soil.

The addition of manures and microorganisms, which may be attributed to the manures with high K contents, resulted in an increase in the amount of potassium that could be extracted using DTPA (Fig. 3c). When compared to the levels found in the control group, the levels of available potassium that were achieved after the addition of dung and microbes were much higher (Fig. 3c). By adding organic matter to a soil in the form of mulch, compost, or microbes, one can enhance the number of components that are biologically available to plants (Khosro *et al.*, 2011). As the amount of organic matter in the soil was increased and microbial action got enhanced, the amount of potassium that was fixed in the soil also reduced, which led to an increase in the available potassium (Biswas & Kole, 2017). The use of mineral fertilizers, manure, compost, and other ameliorants over an extended period increases the amount of potassium that is present in the soil (Adeniyi *et al.*, 2011). A boost in soil potassium levels is brought about by the high K content of organic additions, which in turn raises CEC. These findings lend credence to the conclusions of subsequent studies (Chen *et al.*, 2021).

Conclusion

In the current study sole and integrated use of organic manures with inorganic fertilizer in combination with microbial inoculants have been investigated. The result depicted significant influence of the manure viz. FYM and poultry manure along with biozot max and biozot agronomic parameters. FYM as an amendment with biozote showed significantly higher value for No. of tillers per plant, 1000 grain weight, grain yield, straw yield and Harvest index. The physico – chemical characteristics of soil including soil pH, EC, SAR and bulk density were significantly improved by the application of amendments. The NPK measured in soil along had been enhanced by the application of poultry manure along with biozote inoculant, while organic matter was increased in the FYM treated plots. The enzyme activities were found higher in the plots receiving sole NPK fertilizers and alkaline phosphatase was maximum in the organic amendments' plots. It may be concluded that combine application of organic amendments with microbial inoculants may improve soil health, physico – chemical properties and agronomic characteristics.

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(Received for publication 16 November 2022)