

EFFECT OF TRICHODERMA BIOFERTILIZERS AND PHOSPHORUS SOURCES ON GROWTH, PHENOLOGY, AND YIELD OF MAIZE

^{1*}Mehran Ali, ²Asad Ali Khan, ³Iftikhar Jan, ⁴Inamullah, ⁵Dost Muhammad

^{1,2,4} Department of Agronomy, The University of Agriculture Peshawar.

³Agriculture Research Institute, Tarnab, Peshawar.

⁵Department of Soil and environmental sciences, The University of Agriculture Peshawar.

*Corresponding Author: Mehranali666@aup.edu.pk

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Abstract

Crop productivity is hampered worldwide by low soil organic matter and plant phosphorus availability, particularly in dry and semi-arid areas. An experiment was conducted during kharif 2022 and 2023 at the research farm of Directorate of Soil and Plant Nutrition (DSPN), Agricultural Research Institute (ARI), Tarnab, Peshawar for two consecutive years. Trichoderma biofertilizers (no biofertilizer, strains TH01, TH05, and green soil) and phosphorus sources (no phosphorus, farm yard manure, poultry manure, DAP, and rock phosphate) were administered at a rate of 100 kg ha⁻¹ and 2 kg ha⁻¹, respectively, in the experiment. The application of DAP as a phosphorus source resulted in a minimum of 54 days for tasseling, 60 days for silking, and 103 days for physiological maturity among the biofertilizer trichoderma strain TH01 at a rate of 2 kg ha⁻¹; a higher number of leaves plant⁻¹ (14), leaf area plant⁻¹ (6165 cm²), leaf area index (4.32), and plant height (205 cm). Regarding the various phosphorus sources, the application of DAP took the shortest amount of time for tasseling (53 days), silking (59 days), physiological maturity (102 days), and leaves plant⁻¹ (14). Therefore, it was determined that applying 2 kg ha⁻¹ of the biofertilizer Trichoderma strain TH01 improved the growth and yield of the maize crop. When compared to alternative phosphorus sources, DAP and poultry dung produced greater growth and yields of maize. In order to improve growth, yield, and yield components for maize production, it is advised that the biofertilizer Trichoderma strain TH01 be applied at a rate of 2 kg ha⁻¹. Poultry manure was a more cost-effective phosphorus supply for optimizing maize growth and phenological characteristics, and it may be suggested for maize's economic productivity.

Keywords:

Maize, Trichoderma strains, Phosphorus sources and Leaf area.

Introduction

After rice and wheat, maize (*Zea mays* L.), a versatile crop in the Poaceae family, is the third most important cereal crop in the world. It has the potential for a high genetic output in comparison to other cereal crops. Maize is one of the most widely cultivated crop in the world, providing food and animal feed as well as a source of biofuel (Yang et al., 2017). By 2020, maize is expected to be the world's major crop (Wang et al., 2016). The countries with the most maize planted areas are the United States, China, and Brazil (Olopade et al., 2019). In 2023–2024, 1.6 million hectares of maize were planted, a 4.5 percent drop from the 1.7 million hectares planted the previous year. Punjab produced 10054.40 thousand tons over a 1243.40-thousand-hectare area, Khyber Pakhtunkhwa produced 923.78 thousand tons over a 468.43-thousand-hectare area, Sindh produced 3.149 thousand tons over a 3.067-thousand-hectare area, and Baluchistan produced 3.18 thousand tons over a 4.52-thousand-hectare area (MNFSR, 2023).

Trichoderma is a bio control agent used to improve the resistance to diseases that may also stimulate plant growth (Oliveira et al., 2016; Roese et al., 2017). Growth promotion mechanisms in plants are associated with microorganisms such as fungi and bacteria, which are capable of solubilizing phosphates through their metabolism, producing phytohormones or fixing nitrogen (Péres-Montañó et al., 2014). Fungi of the *Trichoderma* genus are among the most studied microorganisms, due to their ability to colonize the rhizosphere and different plant organs, promoting beneficial effects on the plant development (Carvalho et al., 2011). These fungi promote growth in plants, since some species have the ability to produce phytohormones (Carvalho et al., 2011) and solubilize phosphorus (Oliveira et al., 2012). The victory of trichoderma in the rhizosphere is due to their high reproductive measurement, capacity to survive under very adverse conditions, regulation in the usage of nutrients, capacity to reshape the rhizosphere and secure impulsiveness against plant pathogenic fungi (Benítez et al., 2004). Some species of trichoderma have the ability to advance plant growth as result of different method such as solubilization of phosphates, micronutrients, and minerals such as iron, magnesium and manganese that have main role in plant growth as well as indirectly with the control of the crucial and petty infesting pathogens in rhizosphere and better nutrient uptake (Hoyos-Carvajal et al., 2009).

The metabolism and growth of plants depend on phosphorus from the very beginning of their existence. It is essential to the development of a plant's root system. This element is also one of the nutrients needed to promote energy transfer among cells, in the photosynthesis process (Magdoff and Van, 2009; Ribas, 2016). However, the Phosphorus resource is very limited word-wide (Gilbert, 2009). However, the transfer of soil Phosphorus is a major cause of Phosphorus-induced eutrophication in surface waters, resulting in a decline in the provision of ecosystem services, and often with serious economic consequences (Syers et al., 2008).

The use of organic amendments such as farm yard manure and poultry manure is an alternative to these harmful effects of inorganic fertilizers due to its wide-spread availability, its additional value for soil organic carbon and its ability for storing and releasing nutrients over a longer time period (Diacono et al., 2010). Application of organic fertilizer could increase the yield through enhancing soil fertility and nutrient use efficiency (Santhi and Selvakumari, 2000). The addition of poultry manure will provide sufficient amount of micronutrients for crop growth (Dozier et al., 2003). Poultry manure decomposes

faster than other animal manure, such as cattle or pig dung; thus it releases its nutrients for plant uptake and utilization rapidly (Gowariker et al., 2009). Poultry manure also increased uptake and concentration of N, P, K, Mg, Ca, Zn, Fe and Cu of maize besides improving soil nutrient (Ayeni and Adetunji, 2010). (Saravanane et al., 2020) reported a 7.62% increase in seed yield of maize with the application of poultry manure. Phosphorus in the manure also plays a key role in plant P nutrient (Koopmans et al., 2007; Sims, 2007). The application of poultry manure is the most practical technology to maintain soil fertility but the effects on crop yield are slow compared to the use of chemical fertilizer. Because of this farmers are reluctant to use this technology as they need to see quick results (Rutunga et al., 2007).

In this regard, use rock phosphate as substrates in phosphorus solubilization by microorganisms is a promising strategy (Soumare et al., 2020). This strategy aims to reduce phosphorus adsorption and precipitation by promoting phosphorus sources with a low solubility instead of soluble phosphorus sources (Soltangheisi et al., 2019). In fact, insoluble rock phosphate could be more efficient than soluble fertilizers in terms of recovery of phosphate by plants especially in sandy soils subjected to heavy leaching (Chen et al., 2006). Numerous of soil microorganisms can solubilize insoluble rock phosphate to release soluble phosphorus nutrients by various secretory mechanisms such as chelation, acidification, ion exchange reactions and production of low molecular weight organic acids (Chaiarn and Lumyong, 2009; Magallon-Servin et al., 2020; Suleman et al., 2018).

Materials and methods

Experimental location

The research farm of Directorate of Soil and Plant Nutrition (DSPN), Agricultural Research Institute (ARI), Tarnab, Peshawar. The experimental site is situated in Khyber Pakhtunkhwa's capital, Peshawar, approximately 14 kilometers to the east. Peshawar has a continental climate because it is situated roughly 1600 kilometers from the north Indian Ocean. The site is 450 meters above sea level and is situated at 34° N and 71.3° E. The research institutes soil is silty loam, with 25% clay and 50% silt. The pH value of soil is 7.7-8.0, and contained 0.67 % organic matter with N 0.053 %, 5.2 P and K 77.2 (mg kg) respectively (DSPN., 2021). The climate of the area is semiarid.

Treatment plans

The productivity of maize and soil fertility were examined using the biofertilizer trichoderma harzianum at a rate of 2 kg ha⁻¹ (no biofertilizer, strain TH01, strain TH05, green soil) and phosphorus sources at a rate of 100 kg p ha⁻¹ (no phosphorus, farm yard manure, poultry manure, DAP, rock phosphate).

Experimental design

Two factors with three replications were included in the randomized complete block design (RCBD) design. After plowing the field with a standard cultivator, a rotavator was used at the appropriate field capacity level. The field was treated with phosphorus sources, such as rock phosphate, farmyard manure, and chicken manure, fifteen days prior to seeding. Each treatment had a 21 m² plot with a row-to-row and plant-to-plant distance of 70 and 25 cm, respectively. At a seed rate of 20 kg ha⁻¹, hybrid maize CS-220 was planted. During the planting period, soil was spread to the field containing the four distinct

strains of *Trichoderma* enhanced biofertilizer (no biofertilizer, strain TH01, strain TH05, and Green Soil) inoculant (1×10^6 spore/g). Similarly, in natural field conditions, five distinct phosphorus sources no phosphorus, farm yard manure (FYM), poultry manure (PM), DAP, and rock phosphate (RP) were tested in conjunction with biofertilizer. Prior to planting, base dosages of potash and nitrogen were applied at rates of 60 and 150 kg ha⁻¹, respectively. Wheat crops were planted without any treatment between two consecutive seasons, while the recommended fertilizer 120:90:60 kg NPK ha⁻¹ was only applied to fill the space between two consecutive maize crops. During the trial phase, all conventional agriculture methods were used.

Data collection

The number of days from sowing until 80% seed emergence in each plot was recorded as days to emergence, while emergence per square meter was determined by counting plants in one-meter rows at three random locations per plot and converting the values to plants m². Days to tasseling and silking were noted as the number of days from sowing until 70% of the plants produced tassels and silk, respectively, whereas days to physiological maturity were calculated from sowing until 70% of the plants reached maturity. Plant height was measured on five randomly selected plants from ground level to the top and averaged. Leaf area per plant was obtained at the silking stage by measuring the length and width of each leaf on five randomly chosen plants, multiplying the averages by a correction factor, and the leaf area index (LAI) was then determined using the formula: $LAI = (\text{Number of plants m}^2 \times \text{leaf area plant}^{-1} (\text{cm}^2)) / 10,000 \text{ cm}^2$.

Statistical Analysis

The data recorded were analyzed statistically according to the procedure relevant to the randomized complete block design (RCBD). Least significant difference (LSD) test at 5% level of significance was used for mean comparison in case of significant difference (Jan et al., 2009).

Results

Crop phenology

The results regarding maize phenology are presented in Tables 1. Days to emergence were not significantly affected by biofertilizer, phosphorus sources, years, or their interactions. However, days to tasseling were significantly influenced by both phosphorus sources and biofertilizers, while all interactions remained non-significant. A year effect was observed, with tasseling occurring at 54 days in 2022 and 55 days in 2023. Among biofertilizers, *Trichoderma* strains TH01 and TH05 showed earlier tasseling (54 days), while green soil plots showed delayed tasseling (55 days). Regarding phosphorus sources, DAP plots reached tasseling earliest (53 days), followed by poultry manure and farmyard manure (54–55 days), whereas no phosphorus and green soil plots were latest (56 days). Similarly, days to silking were significantly affected by years, phosphorus sources, and biofertilizers, with no significant interactions. Silking occurred earlier in 2022 (60 days) than in 2023 (61 days). Plots treated with *Trichoderma* strain TH01 exhibited fewer days to silking (60 days), followed by TH05 and green soil (61 days), while no biofertilizer plots were delayed (62 days). For phosphorus sources, DAP plots reached silking earliest (59 days), followed by farmyard manure (61 days), whereas poultry manure and

no phosphorus plots showed delayed silking (62 days). Days to physiological maturity were significantly influenced by biofertilizer, phosphorus sources, and years, while only the $Y \times PS$ interaction was significant. The shortest days to physiological maturity were recorded with Trichoderma strain TH01 (103 days), followed by TH05 (104 days) and green soil (105 days), while no biofertilizer plots were latest (106 days). Among phosphorus sources, DAP plots matured earliest (102 days), followed by farmyard manure and poultry manure (104 days), whereas rock phosphate and no phosphorus plots showed delayed maturity (106 days).

Table 1. Days to emergence, tasseling, silking, and physiological maturity of maize as affected by biofertilizers and phosphorus sources during 2022 and 2023.

Treatments	Days to Emergence	Days to Tasseling	Days to Silking	Days to Physiological Maturity
Biofertilizers (2 kg ha⁻¹)				
No bio-fertilizer	6	56 a	62 a	103 a
Strain TH01	6	54 c	60 c	103 d
Strain TH05	6	54 c	61 b	104 c
Green Soil	6	55 b	60 b	105 b
LSD (0.05)	NS	0.45	0.57	0.47
Phosphorus sources (100 kg ha⁻¹)				
No phosphorus	6	56 a	62 a	106 a
Farmyard manure	6	55 b	61 b	104 b
Poultry manure	6	54 c	60 c	104 b
DAP	6	53 d	59 d	102 c
Rock phosphate	6	56 a	62 a	106 a
LSD (0.05)	NS	0.51	0.64	0.53
Year (Y)				
2022	6	54	60	103
2023	6	55	61	106
Interactions				
BF $\times$ PS	NS	NS	NS	NS
Y $\times$ BF	NS	NS	NS	NS
Y $\times$ PS	NS	NS	NS	*
Y $\times$ BF $\times$ PS	NS	NS	NS	NS

Growth attributes

The emergence m² of maize was not significantly affected by biofertilizer, phosphorus sources, or their interactions (Table 2). In contrast, plant height was significantly influenced by both factors, while year-to-year variation was non-significant. On average, taller plants were recorded in 2023 (200 cm) compared with 2022 (197 cm). Among biofertilizers, Trichoderma strain TH01 produced the tallest plants (205 cm), followed by strain TH05 (200 cm), whereas no biofertilizer plots produced the shortest

plants (193 cm). For phosphorus sources, DAP (215 cm) and poultry manure (212 cm) resulted in the tallest plants, followed by farmyard manure (199 cm), while no phosphorus plots produced the shortest plants (171 cm). Leaf area per plant was also significantly affected by biofertilizer and phosphorus sources, with no significant interactions, though year had a significant effect. Larger leaf area was observed in 2023 (6083 cm²) compared with 2022 (6002 cm²). Trichoderma strain TH01 produced the largest leaf area (6165 cm²), followed by strain TH05 (6051 cm²) and green soil (5991 cm²), while the smallest was in plots without biofertilizer (5963 cm²). Among phosphorus sources, DAP (6322 cm²) and poultry manure (6287 cm²) produced the largest leaf area, followed by farmyard manure (6104 cm²), whereas no phosphorus plots produced the lowest (5726 cm²), comparable to rock phosphate (5773 cm²). Similarly, leaf area index was significantly affected by biofertilizer and phosphorus sources, with no significant interactions, while year had a significant effect, with 2023 (4.26) surpassing 2022 (4.20). Trichoderma strain TH01 resulted in the highest leaf area index (4.32), followed by strain TH05 (4.24) and green soil (4.19), while no biofertilizer plots had the lowest (4.15). For phosphorus sources, DAP (4.43) and poultry manure (4.40) recorded the highest leaf area index, followed by farmyard manure (4.27), whereas no phosphorus and rock phosphate plots recorded the lowest (4.01 and 4.04, respectively). Furthermore, the number of leaves per plant was significantly influenced by biofertilizer and phosphorus sources, though all interactions were found to be non-significant.

Table 2. Emergence, plant height, leaf area, and leaf area index of maize as affected by biofertilizers and phosphorus sources during 2022 and 2023.

Treatments	Emergence (m ²)	Plant height (cm)	Leaf area plant ⁻¹ (cm ²)	Leaf area index plant ⁻¹
Biofertilizers (2 kg ha⁻¹)				
No bio-fertilizer	11.9	193 c	5963 c	4.17 c
Strain TH01	11.7	205 a	6165 a	4.32 a
Strain TH05	11.9	200 ab	6051 b	4.24 b
Green Soil	11.9	196 bc	5991 bc	4.19 bc
LSD (0.05)	NS	6	61	0.04
Phosphorus sources (100 kg ha⁻¹)				
No phosphorus	11.8	171 c	5726 c	4.01 c
Farmyard manure	11.9	199 b	6104 b	4.27 b
Poultry manure	11.9	212 a	6287 a	4.40 a
DAP	11.8	215 a	6322 a	4.43 a
Rock phosphate	11.7	195 b	5773 c	4.04 c
LSD (0.05)	NS	7	68	0.05
Year (Y)				
2022	11.8	197	6002	4.20
2023	11.9	200	6083	4.26
Interactions				
BF × PS	NS	NS	NS	NS
Y × BF	NS	NS	NS	NS

Y × PS	NS	NS	NS	NS
Y × BF × PS	NS	NS	NS	NS

Correlation

The correlation matrix illustrates the interrelationships among different maize growth and phenological traits. Days to tasseling (DTT) and days to silking (DTS) showed a very strong positive association ($r = 0.97$), indicating their close synchronization, and both were positively correlated with plant height ($r = 0.80$ and 0.78), LA ($r = 0.79$ and 0.76), LAI ($r = 0.79$ and 0.76), and number of leaves ($r = 0.65$ and 0.66). Similarly, days to maturity (DTM) was strongly correlated with DTT ($r = 0.80$) and DTS ($r = 0.81$) but exhibited negative associations with plant height ($r = -0.51$), LA ($r = -0.50$), LAI ($r = -0.50$), and number of leaves ($r = -0.47$), suggesting that prolonged maturity duration reduced vegetative growth traits. Plant height, LA, LAI, and leaves number were strongly interrelated, with PH showing strong positive correlations with LA and LAI ($r = 0.94$ each) and with number of leaves ($r = 0.78$), reflecting their role as indicators of plant vigor. On the other hand, emergence per m² showed very weak correlations with most traits, while plant population at harvest displayed negative associations with DTE ($r = -0.30$), PH ($r = -0.16$), LA ($r = -0.14$), and LAI ($r = -0.14$), indicating reduced final stand under delayed emergence and vigorous vegetative growth. Overall, the results suggest that vegetative traits such as PH, LA, LAI, and number of leaves are strongly interconnected and positively influenced by early tasseling and silking, whereas extended maturity duration and delayed emergence tend to suppress these growth characteristics.

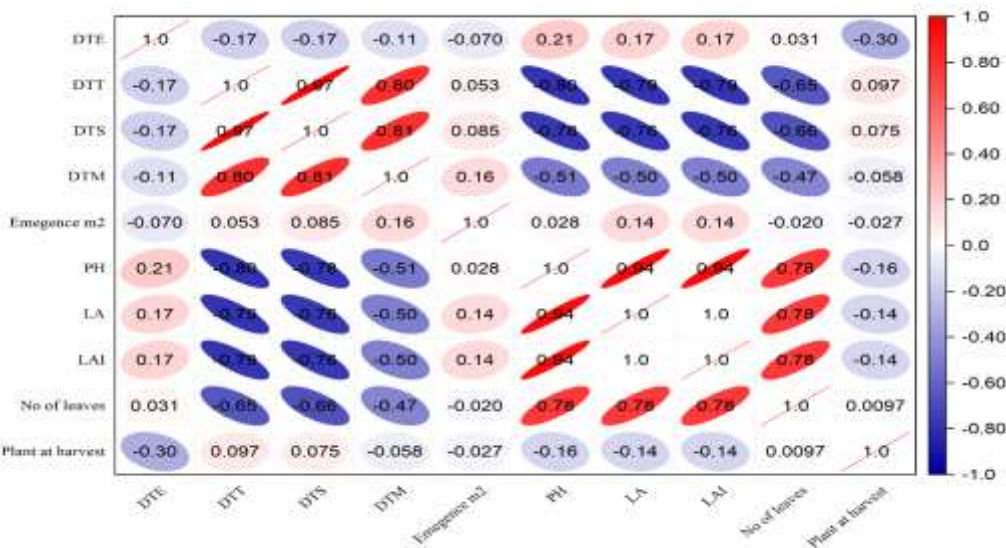


Figure 2. Correlation analysis for evaluated traits.

Discussion

Days till maize emerges and emergence m² as impacted by phosphate and biofertilizer sources. The data showed that the days to emergence and emergence m⁻² of maize were not significantly impacted by any of the parameters investigated, including biofertilizer and phosphorus sources. The lack of effect of externally supplied nutrients may be because at earlier stages of growth, the seedlings obtain their

nutrients from stored food in seeds; additionally, emergence m^2 is dependent on seed rate, and uniform seed rate did not affect emergence m^2 . This could explain the negligible effect of biofertilizer and various P sources on days to emergence and emergence m^2 . Similar results were reported by (Ali et al., 2019) who reported that there was no effect of externally supplied nutrients on emergence m^2 of the maize. The non-significant results for both emergence and emergence m^2 would probably due to the presence of sufficient soil moisture during crop sowing time (Ali et al., 2004). Our results are also in line with (Azarmi et al., 2016; Alam et al., 2003) that stated the innate food stored in the seed endosperm in the form of carbohydrates are sufficient for seed to emerged regardless of the need of external soil nutrients.

The impact of phosphorus sources and biofertilizer on maize phenology (days to silking, tasseling, and maturity). Data showed that the phenology (days to silking, tasseling, and maturity) of maize was significantly influenced by the sources of phosphorus and biofertilizer. When using biofertilizer, trichoderma strain TH01 and strain TH05 showed less days to silking, tasseling, and maturity of maize, whereas plots without biofertilizer showed more days to silking, tasseling, and maturity. This is in agreement with the findings of (Behzad, 2010) who stated that the impacts of trichoderma on growth and development of plants have important agronomical and economic implications such as shortening the plant growth period and time in nursery, as well as improving plant vigor to overcome biotic and/or abiotic stresses, resulting in increased plant productivity and yields. These results also similar with (Mahato and Neupane, 2017) that observed early phenological observation of maize treated with trichoderma. Among the various phosphorus sources, plots fertilized with DAP and chicken manure showed less days to silking, tasseling, and maturity of maize, whereas plots without phosphorus showed longer days to silking, tasseling, and maturity of maize. Our results are also similar with (Essilfie et al., 2024) that observed early tasseling, silking and maturity with the application of organic phosphorus source and inorganic phosphorus fertilizer. These finding are also in line with (Bekele et al., 2018) who observed significantly early tasseling and silking of maize with the integrated use of animal manures and phosphorus application. The reason for the early phenology with the application of higher P levels might be due to better root development which facilitated the plants to obtain more P and other nutrients from the poultry manure resulting in rapid plant growth and development (Amanullah and Khalid, 2015).

The impact of several biofertilizers and phosphorus sources on maize plant height was noteworthy. Trichoderma strains TH01 and TH05 were shown to produce taller plants, while plots lacking biofertilizer showed the smallest plant heights of maize. The combination of Trichoderma species' long-term residual effects may have contributed to the maize plant's continuous nutrition delivery over the growing season. Soesanto et al. (2022) reported similar outcomes that application of Trichoderma harzianum increased plant height. According to (Egamberdieva et al., 2017) who noted that microbial symbiosis for growth promotion and nutrient absorption is related to phytohormones biosynthesis. Plots with DAP and chicken manure were the phosphorus sources that generated taller plants, followed by farmyard manure application; plots without phosphorus produced dwarf plants. According to Ali et al. (2019) who revealed that the probable reason for the increase in plant height due to phosphorus might be that it improved the root development and nutrient absorption which had a great effect on overall plant growth performance which resulted in taller plants. Similar results were reported by (Liu et al., 2022) who reported that application of organic and inorganic P increased the plant height.

The most significant acceptor of photosynthesis is leaf area plant⁻¹, which leads to increased absorption of photosynthetic products. The best indicator of crop productivity and a benchmark for crop development is the functional leaf area of the crop canopy that is on the ground. According to our findings, leaf area plant⁻¹ and leaf area index were strongly impacted by biofertilizer and phosphate sources. Data analysis showed that phosphate and biofertilizer sources had a substantial impact on leaf area and leaf area index plant⁻¹. When it came to biofertilizer, strain TH01 produced larger leaf area plant⁻¹ and leaf area index, but plots without biofertilizer showed lower leaf area plant⁻¹ and leaf area index plant⁻¹. Our results are also similar with the findings of (Kaur and Kumar, 2020) that leaf area and leaf area index were increased with application of trichoderma over control. Plots fertilized with DAP, which was statistically comparable to poultry manure, showed higher leaf area plant⁻¹ and leaf area index plant⁻¹ among the various phosphorus sources, but plots without phosphorus showed lower leaf area plant⁻¹ and leaf area index plant⁻¹. These results are in accordance with (Amanullah et al., 2021) reported that application of P significantly increased the leaf area in maize. The growth parameters were also significantly improved with application of poultry manure as compared to sheep and cattle manures (Yazdani et al., 2009) suggested that poultry manure enhanced the LAI in maize. This information is consistent with a host of other studies that have shown that phosphorus fertilizers improve the growth and development of sweet corn crops, generally by increasing leaf area during the growth period, often leading to a shorter period of maturation and earlier yield (Fahrurrozi et al., 2018). The increase in leaf area per plant and leaf area index at the DAP and poultry manure might be due to the increase in the P availability for the maize plants with positive impacts on plant growth which resulted in an increase in the number of leaves and leaf area per plant. (Varatharajan, et al., 2019) found that a higher leaf area index enhances a crop's ability to intercept, absorb, and utilize radiant energy, leading to a faster rate of photosynthesis and greater accumulation of dry matter in the crops. In essence, increased nutrient availability allows for the production of larger leaf area which ultimately improves crop yield and plant health. (Chaturvedi, 2006) reported that inorganic P positively increased root growth resulting in the more nutrients and water uptake by the plant cells, leading to maximum LA and productivity of the crop.

Conclusion

The performance of maize in terms of growth was enhanced by biofertilizers and phosphorus sources. Trichoderma strain (TH01) biofertilizers increased maize crop output and growth. The growth and yield of maize were higher when phosphorus was obtained from DAP and poultry manure than from other sources. When compared to other phosphorus sources, the application of poultry manure with the biofertilizer Trichoderma strain (TH01) was found to be more effective and cost-effective.

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