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IMPACTS OF BIOENHANCER (*TRICHODERMA HARZIANUM*) ON *ZEA MAYS* PLANTS UNDER REDUCED FERTILIZATION

Mohsin Raza[*1], Mohib ullah[2], Humza[3], Hamd ullah[4], Amad iftikhar[5]

[*1,4]*Department of Botany, Government Postgraduate College Kohat 26000, Khyber Pakhtunkhwa, Pakistan*

[2]*Department of Biotechnology, CECOS university of IT and Emerging Sciences, Peshawar25000, Khyber Pakhtunkhwa, Pakistan*

[3]*Department of Botany, Government Post Graduate College Karak 27200, Khyber Pakhtunkhwa, Pakistan*

[5]*Department of Microbiology, Kohat University of Science and Technology Kohat26000, Khyber Pakhtunkhwa, Pakistan*

[*1]mohsinrazakohati77@gmail.com,[2]mohibullah830@gmail.com, [3]hamd01121@gmail.com,[4]humzakhattak69146@gmail.com [5] amadiftikhar4949@gmail.com

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Mohsin Raza

Abstract

There are no definite p fertilizer reduction guidelines for maize growers using beneficial microbes, resulting in economic and environmental problems. It was found that this field study was able to determine the possibility of growth of maize at low phosphorus application level and identify the most appropriate fertilization strategy in the presence of *Trichoderma harzianum* (Biofit) and the ability to maintain the maize growth under reduced application of phosphorus. The experiment was conducted in Randomized Complete Block Design with seven treatments and three replications for hybrid CS220 at Tarnab farm, Peshawar, Pakistan during 2023 kharif season. The treatments were the association of 6.17 g of *Trichoderma harzianum* with phosphorus (25–100% of recommended) and nitrogen (25–100% of recommended). The results revealed that plants responded to nutrients depending on the stage, with T7

(25% P, 100% N) producing the tallest plants (14.41%), T4 (100% P, 25% N) the highest number of plants per plot (14.63%), greatest grain weight and grain number. This divergent response is due to the different nutrient requirements of vegetative growth and establishment/grain filling, respectively, for nitrogen and phosphorus. In particular, the phosphorus treatments of 50–75% performed the same as the 100% phosphorus in most parameters, suggesting that *T. harzianum* is effective in improving the use of phosphorus through the solubilization of native soil phosphorus. Soil analysis after harvest showed neutral soil but very low residual phosphorus, meaning that crop uptake was high. According to overall yield parameters, T4 (100% P, 25% N + *Trichoderma harzianum*) is recommended for maximum grain yield whereas T7 (25% P, 100% N + *Trichoderma harzianum*) can be applied for vegetative growth. The results indicate that *Trichoderma harzianum* provides for moderate reduction in phosphorus (50–75%) without any significant loss in yield, providing a viable option for the farmers to reduce the input cost while maintaining productivity. The use of *Trichoderma harzianum* as a bioenhancer in sustainable agriculture to improve the phosphorus use efficiency in maize production.

Introduction:

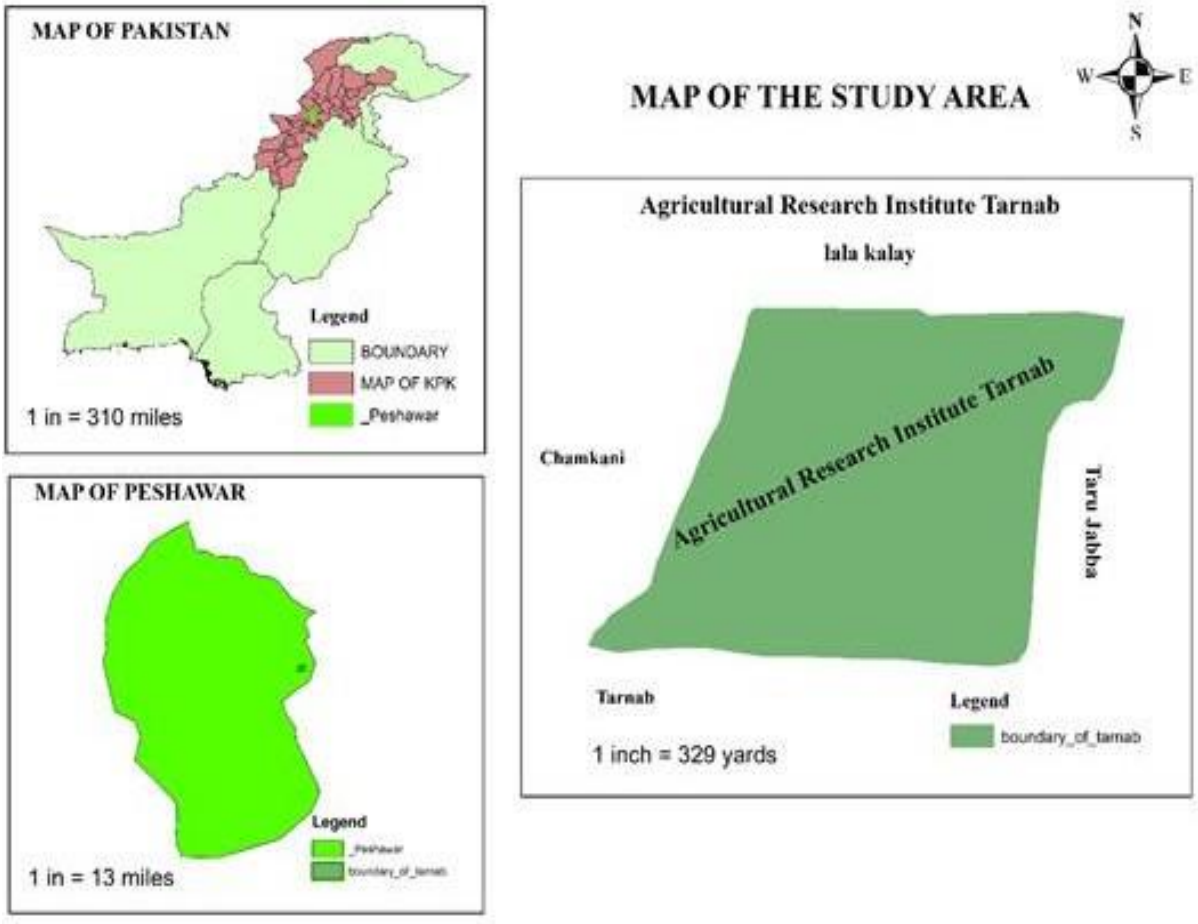
The heavy use of chemical fertilizers and pesticides fully support the global food security but leaves long-term negative effects that harm each and every part that associated with man and its cultivation like soil, microflora, the environment, and human health. Therefore, many of our scientists in today modern era of smart and with advanced approaches are going toward sustainable agriculture, where organic farming, biofertilizers, composting, and biocontrol agents replace harsh chemicals to conserve nature (Baweja, Kumar, & Kumar, 2020).It's true that chemical fertilizers like Nitrogenous, Phosphate, and Potassium enhance the crop productivity but on the other hand completely alter soil physicochemical and biological properties. Whenever we continuous use it in our fields for crops improvement, will be depleted soil organic matter (SOM) and degrades overall agricultural soil quality(Pahalvi, Rafiya, Rashid, Nisar, & Kamili, 2021). In our very latest time many agrochemicals, once seen as a boon, and now known to persist in soil and continuously contaminating our groundwater, harming soil microbial communities and human health too through hormone disruption, reproductive abnormalities, and cancer. With the global population projected to reach ~9.7 billion by 2050, agriculture remains dependent on these chemicals, making it urgent to review their impacts and adopt

ameliorative measures for sustainable soil and ecosystem health (Hossain, Shahrukh, & Hossain, 2022). Chemical fertilizers boost crop yield but its misuse especially nitrate pollution contaminates food and water, with vegetables and maize most at risk. Mixed agrochemical application by farmers further complicates risk assessment, demanding more research (Tayoh, 2020). As far we know, from field and lab experimentations, reached to conclusion that agricultural practices almost have degraded our soil structure and biological equilibrium, driving the need for alternative approaches that are less invasive, cheaper, more efficient, and easier to implement than conventional techniques. Biofertilization has emerged as one such technology(Carvajal-Muñoz & Carmona-Garcia, 2012).Modern agriculture's have so many good points but regarding chemical fertilizers we have noted that heavy reliance on synthetic fertilizers has caused so many problems. For instance, greenhouse effects, soil deterioration, air, and water pollution, creating an urgent need for eco-friendly alternatives. Biofertilizers—microorganisms like bacteria, fungi, and algae offer a natural, economical solution by enriching soil nutrients, promoting plant growth, and suppressing soil borne diseases, thereby reducing chemical fertilizer dependence for sustainable agriculture(Thomas & Singh, 2019). Biofertilizers usually originated from microorganisms and use microorganisms to supply nutrients and support plant growth, requiring crop- and region-specific strains due to varying agro-climatic conditions. In addition, They are eco-friendly, economical tools that can enhance soil fertility, with leguminous crops fixing atmospheric nitrogen for their own and subsequent crops' benefit (Al Abboud, Ghany, & Alawlaqi, 2014).*Trichoderma* on plant growth and development have own important economic implications such as shortening the plant growth period and time, as well as Improving plant stamina to deal with biotic and/or abiotic stress. stresses, resulting in increased plant productivity and yields. Moreover, the reduction in lignification which was The strains of *T. harzianum* are beneficial when cultured in induced fungi. The results indicate the positive effect of the fresh state of *Zea mays* stalks on their quality (Akladious & Abbas, 2012). Therefore, we decided to check the significant role of *Trichioderma harzianum* as a bioenhancer in order to investigate that what is the optimum amount of phosphorus as a fertilizer which is more suitable for the plant growth. So, the main purpose of fertilizers applications while different levels of it. Clearly indicates that, what level is more beneficial for the *Zea mays* growth improvement.

Materials and methods:

During the Kharif program of the year 2023, a field experiment was conducted at the experimental site of the Agricultural Research Institute, Tarnab farm in Peshawar, Khyber Pakhtunkhwa, Pakistan. The experiment aimed to assess the performance of maize CS220 variety in combination with Biofit, a product containing fungus called *Trichoderma harzianum*, which was provided by the Directorate of Soil and Plant Nutrition (DSPN) at the Agriculture Research Institute, Tarnab farm. In addition, various levels of Nitrogen (N), Phosphorus (P), and Potassium (K) were also tested to evaluate their effects on the growth and yield of the maize variety. Design of this experiment was (Randomized Completely Block Design) RCBD. lay out has been design in three replications in which six different treatments applied

along with T1 which was control. However, three times, soil sampling conducted at the experimental site of directorate of soil and plant nutrition.

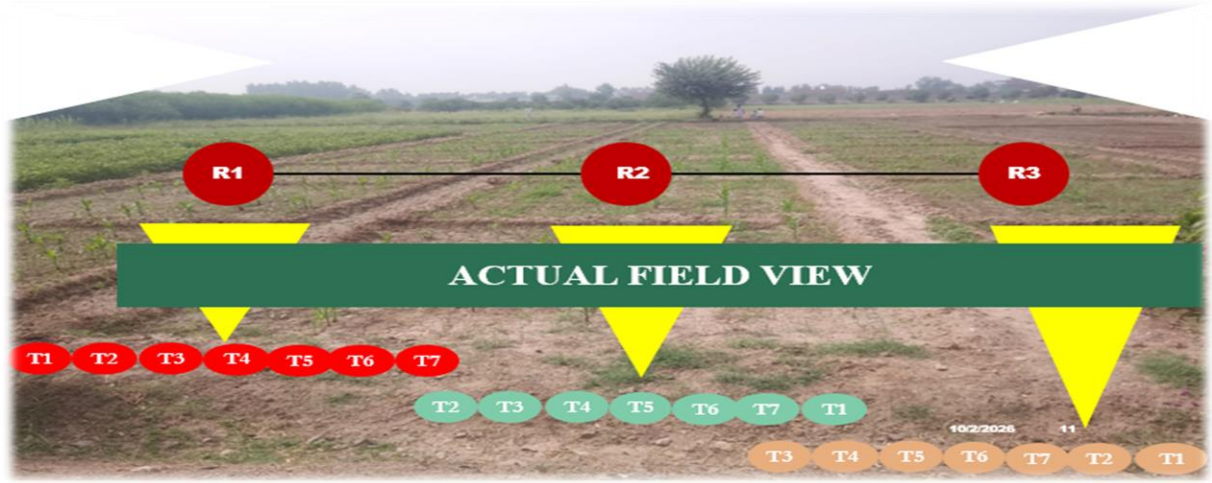


***Trichoderma Hrazianum* mass production**:

In the Soil Microbiology Laboratory, the culturing of *Trichoderma harzianum* followed a specific protocol by applying Potato Dextrose Agar (PDA) as the growth medium, composed of 200 grams of peeled potato, 20 grams of agar, and 20 grams of dextrose. Sterilization was achieved using 70% ethanol for equipment and media. Various equipment was essential for this process, including an analytical balance for precise measurements, an autoclave for high- level sterilization at 121°C for 15-20 minutes at 15 psi, a Laminar Flow Unit (LFU) to maintain a sterile environment during culturing, and a refrigerator for sample storage. An incubator served as a growth chamber for Trichoderma harzianum, maintaining temperatures between 28°C and 30°C for optimal growth, with monitoring after seven days. For sample preservation, an ultra-low freezer at -88°C was employed. Additionally, a shaker facilitated the isolation process by mixing 1 gram of soil with 9 ml of distilled water for 3-5 minutes before pouring onto the growth medium.

Experimental Design: Randomized Complete Block Design (RCBD) shows the organization of treatments from (T1 to T7):

Rep I	Rep II	Rep III
T1	T2	T3
T2	T3	T4
T3	T4	T5
T4	T5	T6
T5	T6	T7
T6	T7	T1
T7	T1	T2



Treatment details:

T1	Control					
T2	Green soil	4.3grams				
T3	Biofit	6.17grams				
T4	DAP 100%	430grams	Nitrogen 25%	500grams	Potassium	150grams
T5	DAP 75%	322grams	Nitrogen 50%	540grams	Potassium	150grams
T6	DAP 50%	215grams	Nitrogen 75%	560grams	Potassium	150grams
T7	DAP 25%	107grams	Nitrogen 100%	600grams	Potassium	150grams

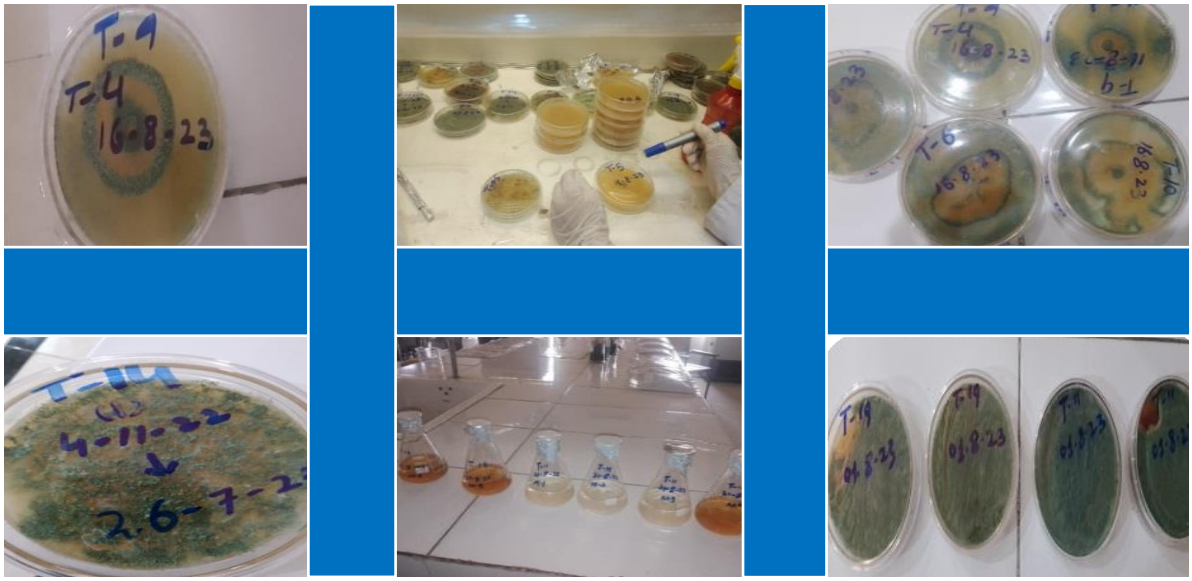
Statistical analysis:

Data were analyzed using analysis of variance (ANOVA) with a significance level of $p < 0.05$. Treatment means were compared using the Least Significant Difference (LSD) test by using computer software statistix 8.1

Results and discussion:

Glimpse of lab. Work in soil microbiology:

We collected our samples from the soil microbiology lab. at Agriculture research institute Tarnab Peshawar, Pakistan and prepared our product and gave a name to it that is Biofit and then, we moved into our next step that's was mixing of it with soil in order to investigate its efficacy that how much is efficient in comparison to chemical fertilizers.



Effects of *Trichoderma harzianum* and different levels of fertilizers on various parameters

1- Height of maize plants (in cm):

The application of *Trichoderma harzianum* in combination with varying fertilizer levels significantly influenced maize plant height **(Figure 1).** Treatment T7, which received 25% phosphorus (107 g), 100% nitrogen (600 g), and 6.17 g of *Trichoderma harzianum*, produced the tallest plants, with a 14.41% increase over the control. This suggests a synergistic interaction between the bioenhancer and high nitrogen availability. In contrast, T1 (control) and T3 (bioenhancer only) showed comparatively lower height gains, indicating that *Trichoderma harzianum* alone was less effective than when combined with balanced nutrient inputs. The positive effect of 100% DAP (phosphorus), 25% nitrogen, and the bioenhancer on plant height was clearly evident across the treated plots. In comparison to another paper its very well explained that *Trichoderma* as a bio fertilizer did not show any effects on its emergence. However, it was great influence on height of maize plants (Ali, Khan, Jan, & Muhmmad, 2025)..According to (de Oliveira et al., 2025) their overall finding like plant growth significantly

affected by *Trichoderma harzianum* it clearly indicate that Trichoderma is the best source to enhance the productivity of crops.

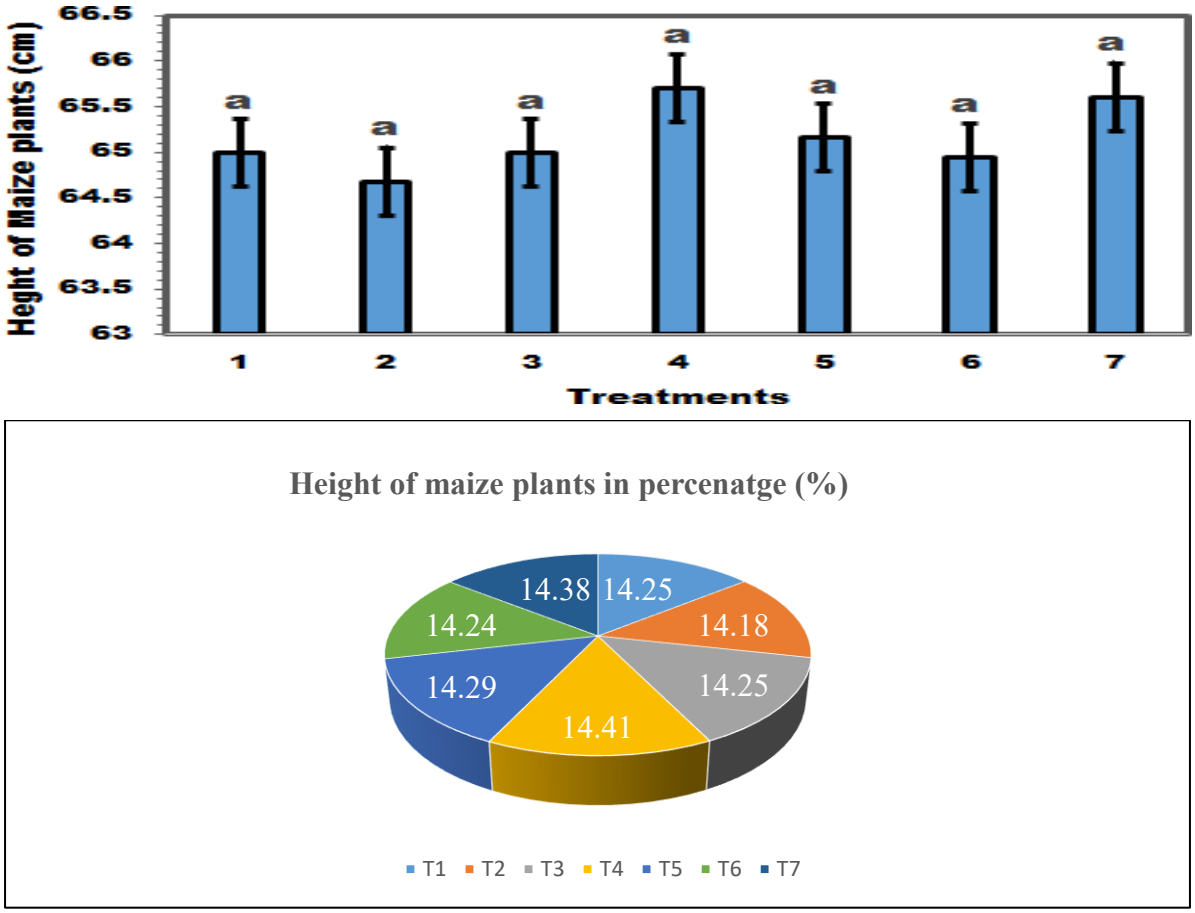


Figure No.1

2- Numbers of plants in each plot:

The influence of 100% phosphorus and the bioenhancer on plant count per plot is presented in **Figure No.2**. Treatment T4, which received 100% phosphorus (430 g), 25% nitrogen (500 g), 150 g potassium, and 6.17 g *Trichoderma harzianum*, recorded the highest number of plants per plot, with a 14.63% increase. This indicates that full phosphorus application combined with the bioenhancer supported better plant establishment and survival. Treatments with reduced phosphorus (T6 and T7) showed comparatively lower plant counts, suggesting that phosphorus availability is critical during early growth stages for maintaining plant density**.** According to an author(Yedidia, Srivastva, Kapulnik, & Chet, 2001)When soil mixed with *Trichoderma harzianum* it showed that emergence in seedling increase by 30% which observed it in total 8th day of experiment.

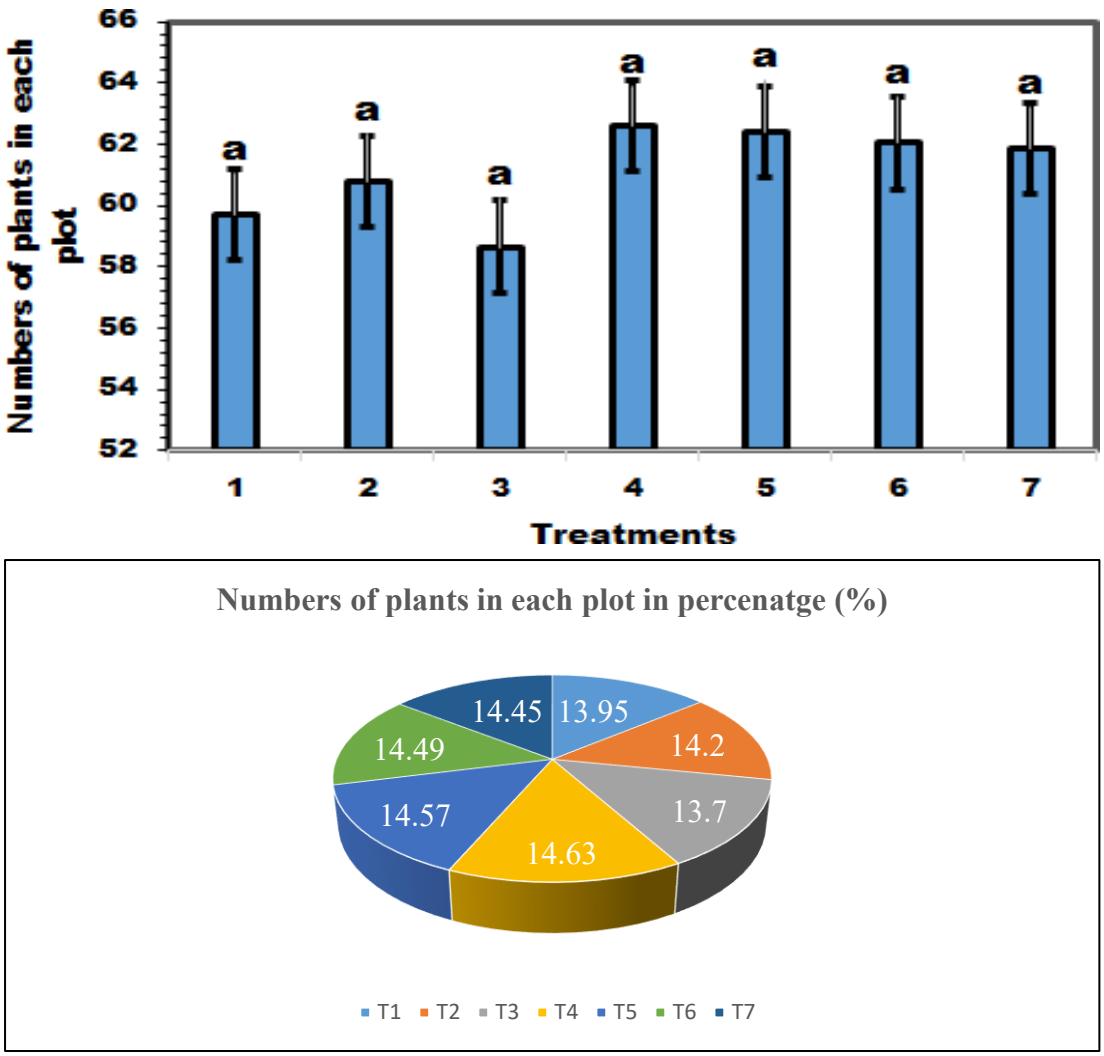


Figure No.2

3- Total grains weight per plot (in g):

The influence of 100% DAP and 25% nitrogen on the weight of grains per plant is shown in **Figure No.3**. Treatments receiving full phosphorus and the bioenhancer produced a higher number of grains per plant compared to the control and bioenhancer-only treatments. This suggests that the combined application of phosphorus and *Trichoderma harzianum* enhanced reproductive development and grain filling. The weight of grains per plant is a key yield component, and its improvement under T4 indicates the potential of integrating bioenhancers with balanced fertilization for higher maize productivity. According to (Sharma, Patel, Saini, & Deep, 2012)The result of grain weight increase in T4 (100 % P, 25 % N + T. harzianum) is similar with the result reported by Sharma et al. (2012) who found an increase in weight of 1000 wheat seeds treated with T. harzianum, Th3. This validates the effect of Trichoderma on increasing grain filling and yield traits via nutrient absorption and root growth**.**

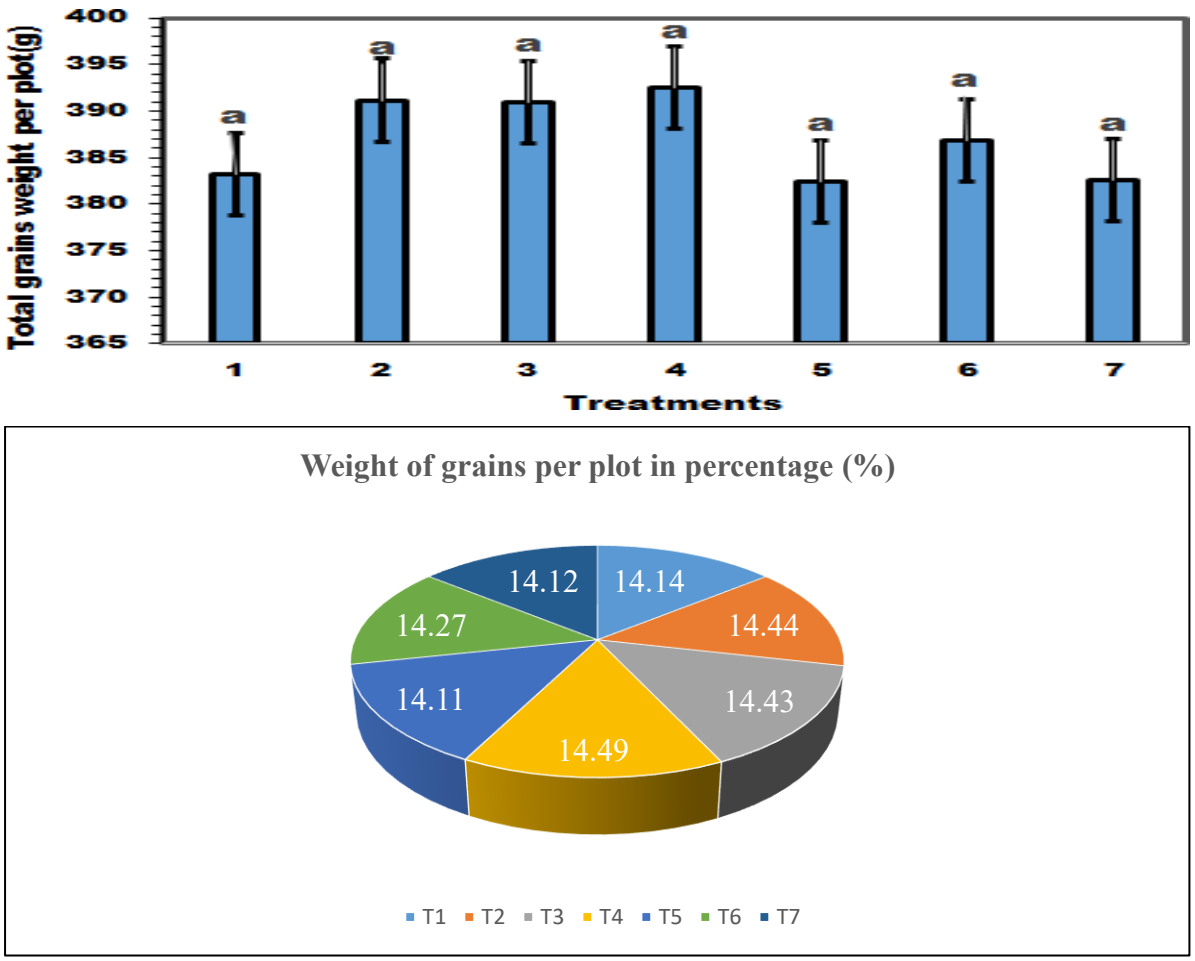
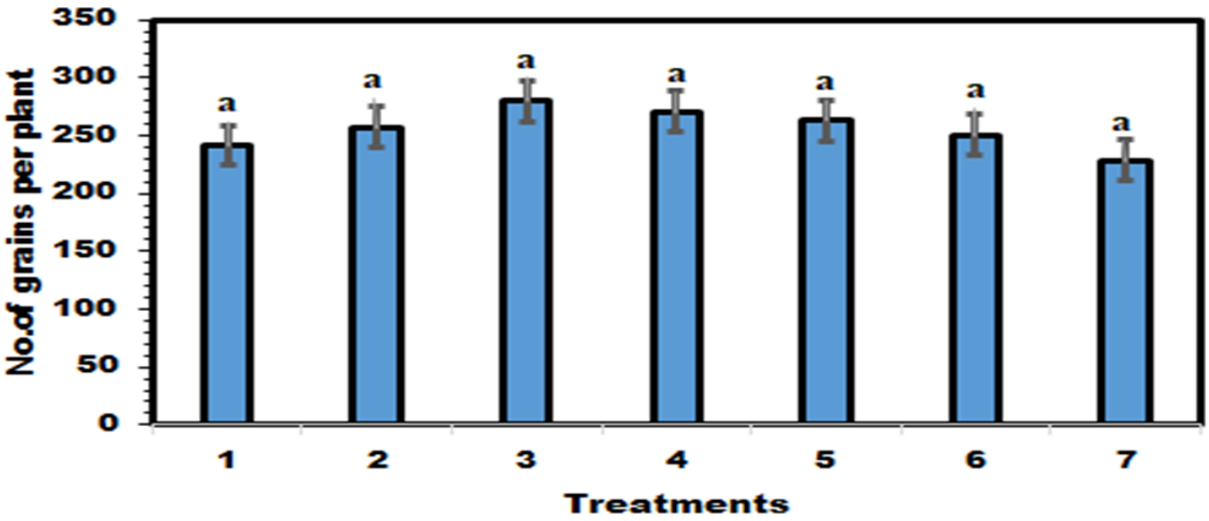


Figure No.3

4- Numbers of grains per plant in each plot:

The influence of *Trichoderma harzianum* (Biofit used as a bioenhancer) only on the number of grains per plant is shown in **Figure 4**. Treatments receiving no phosphorus and only bioenhancer produced a higher number of grains per plant compared to the control and other treatments. This suggests that the bio fertilizer like *Trichoderma harzianum* can enhance reproductive development and grain filling. The number of grains per plant is a key yield component, and its improvement under T3 indicates the potential of bioenhancers. However, if in some amount we apply phosphophorus then, its valuable due to the solubalization abilities of *Trichoderma Harzianum.* According to (Sharma et al., 2012) In the same way, Sharma et al. (2012) found that there was a considerable improvement in the grains per spike in the case of *T. harzianum* treatment, which further confirms that *T. harzianum* is also a bioenhancer agent with a positive effect on the reproductive development and grain filling**.**



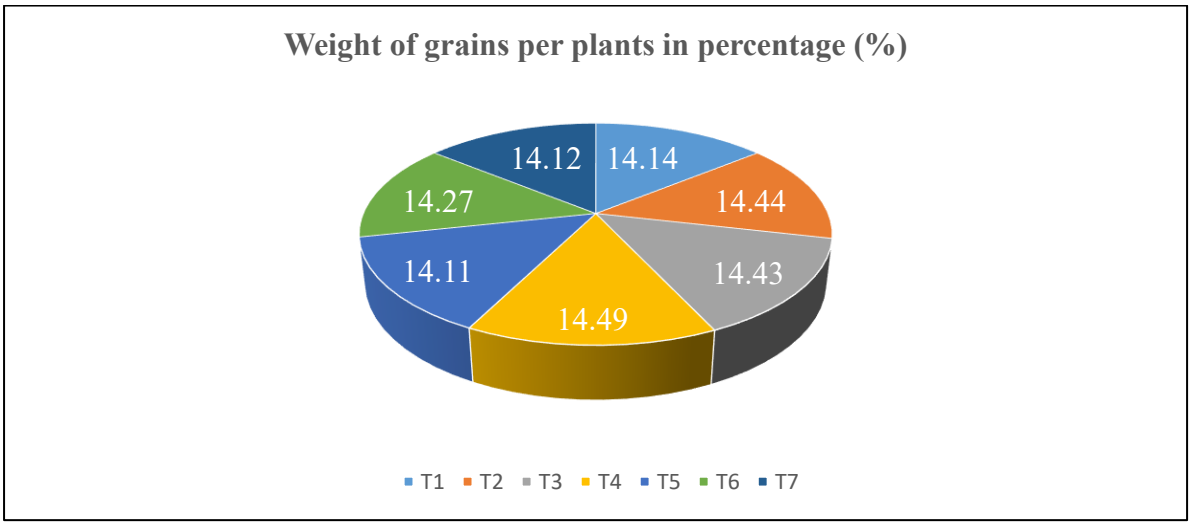


Figure No.4

Soil analysis report:

Soil analysis conducted at the Agriculture Research Institute, Tarnab, Peshawar, revealed significant temporal changes in soil physico-chemical properties **(Table 1).** Before sowing, the soil was silt clay loam with a slightly alkaline pH of 7.73, an EC of 0.43 mS/cm, and moderate organic matter content (2.27%). By October 4, 2023, the soil pH increased to 8.2, while EC dropped sharply to 0.08 mS/cm and organic matter decreased to 1.24%. However, nutrient levels increased dramatically, with nitrogen reaching 7.4%, phosphorus 110%, and potassium 120%. By October 30, 2023, the soil pH shifted to neutral (7.1), EC remained low (0.07 mS/cm), and organic matter declined further to 1.03%. Nutrient levels also decreased, with nitrogen at 5%, phosphorus at 52%, and potassium at 60%. Post-harvest analysis indicated critically low phosphorus levels and elevated potassium, highlighting the dynamic nature of soil composition under different agricultural practices.

Table No.1: Soil analysis:

Sample Date	Clay (%)	Silt (%)	Sand (%)	Texture Class	pH	EC (mS/cm)	TSS (%)	CaCO3 (%)	Organic Matter (%)	N (%)	P (%)	K (%)
Pre-sowing	5.5	70.5	24	Silt Clay Loam	7.73	0.43	0.1088	10	2.27	0.113	0.168	2.27
4/10/023	6	67	27	Silt Loam	8.2	0.08	0.025	10	1.24	7.4	110	120
30/10/2023	5	74	21	Silt Loam	7.1	0.07	0.022	7	1.03	5	52	60

Conclusion and futuristic perspectives:

The results of this study showed that under the different levels of phosphorus fertilization, the bioenhancer *Trichoderma harzianum* (Biofit) had an effect on enhancing growth of maize. The maximum plant height (14.41%) occurred under T7 (25% P, 100% N), but the maximum plant count per plot (14.63%) occurred under T4 (100% P, 25% N), suggesting that there may be stage-specific nutrient requirements with N favoring vegetative growth and P favoring establishment and grain filling. The percentage of phosphorus at 50-75% treatments was similar in most parameters compared to 100% phosphorus, indicating that *Trichoderma harzianum* increases phosphorus use efficiency by solubilizing native soil phosphorus. Post-harvest soil analysis showed neutral pH and very low residual P levels (high crop uptake). T4 (100% P, 25% N + 6.17 g *Trichoderma harzianum)* is recommended for Maximum Yield of grain and T7 (25% P, 100% N + 6.17 g *Trichoderma harzianum*) for vegetative growth based on overall yield performance. The outcomes are in agreement with the use of *T. harzianum* as bioenhancer to lower 50–75% the requirements of phosphorus application without a significant reduction in yield, providing the farmer an economically and environmentally sustainable solution for maize production. Consequently, there is a need for future studies to be conducted in multiple locations and seasons in different agro-climatic zones to confirm repeatability of these results. Molecular analysis of the auxin signaling and phosphate solubilization pathways related to *Trichoderma*–maize interactions are required. Metagenomics analysis of soil microbial communities, economic evaluation of costs and benefits and integration with other beneficial microorganisms like *Azospirillum*, Azotobacter and arbuscular mycorrhizal fungi would further help the practical applicability. Moreover, the study of *Trichoderma harzianum* on various maize varieties and cereal crops, optimization of bioformulation for better shelf life and rhizosphere competence, and its potential in drought and heat stress management in the context of global climate change are areas of promising applications

for sustainable cereal production. In current life after careful reviews of literature main issue is drought tolerance. Whenever, we study it and find its appropriate solution then, in the near future will be solved many problems with it. The strong recommendation from our side is to study the drought tolerance in *Brassica napus*. However, we can combine beneficial microbes with any chemicals and then, will investigate its efficiency in the near future.

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