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Effect of seed bio-priming and N doses under varied soil type on nitrogen use efficiency (NUE) of wheat (*Triticum aestivum* L.) under greenhouse conditions

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ABSTRACT

This study was aimed to investigate an impact of bio-priming by *Trichoderma harzianum* BHU51, varied N doses (100%, 75%, 50% and 25% RDN) along with varied soils types (alluvial, red and black) on wheat (*Triticum aestivum* L.) under greenhouse conditions. Results revealed that significantly enhanced plant height, chlorophyll content, root length, effective tillers growth and yield parameters of wheat with different soil type under greenhouse conditions by the application of various treatments. Significantly higher effective tillers, chlorophyll content and root length were recorded with recommended dose of fertilizers (RDF) of NPK @ 120-60-60 kg ha^{-1} followed by seed bio-priming with *T. harzianum* + 3/4th N and RDF of PK. The seed bio-priming also enhanced the plant growth and nitrogen use efficiency (NUE). Agronomic use efficiency (AUE) and physiological use efficiency (PUE) were significantly higher with alluvial soil as compared to black and red soils. The principal component analysis revealed that root length and N uptake were the most important property to improve the NUE. The results indicated suitability of *Trichoderma harzianum* BHU51 as a bio-priming agent in improving NUE and wheat crop productivity.

1. Introduction

World food insecurity is a chronic problem and is likely to worsen with climate change and rapid population growth. It is largely due to poor yields of the cereal, pulse and millets crops caused by many factors which including soil, plant and environmental. The world's population is assumed to increase from 7 billion now to 8.3 billion in 2025. The world will need 70–100% more food by 2050 (Godfray et al., 2010). Nowadays soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which caused a serious threat to human health and environment (Bhardwaj et al., 2014). The exploitation of beneficial microbes as a biofertilizer has become paramount importance in agriculture sector for their potential role in food safety and evergreen crop production (Meena et al., 2013; Youssef and Eissa, 2014). Intensive uses of agricultural inputs, particularly fertilizers are costly to farmers. N fertilizer is one of the main inputs for cereals production systems doubling agricultural food production worldwide over the past four decades is associated with a 20-fold increase in N fertilizer use (Brennan et al., 2014). In most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant and is lost through a combination of leaching, surface run-off, denitrification, volatilization, and microbial consumption (McAllister et al., 2012). It is estimated that a 1% increase in NUE could save ~$1.1 billion annually. Therefore, to minimize the loss of N, reduce environmental pollution, and decrease input cost, it is crucial to develop crop varieties with a higher NUE. In cereals it is estimated to be ~42% and 29% in developed and undeveloped nations, respectively (Raun and Johnson, 1999). The NUE may be affected by crop species, soil type, temperature, N application, soil moisture condition and crop rotation (Meena et al., 2015a).

One possible way to improve the NUE in soil–plant–environment system through eco-friendly approaches mediated *via* application of plant–growth promoting rhizobacteria (PGPR) in soil (Meena et al., 2015d) and seed bio-priming process using microorganisms such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Trichoderma* species. These efficient soil microorganisms have multifunctional impact on soil-plant system which leads to improve NUE, nutrient uptake, plant growth and plant tolerance to multi-stress which in

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turn help to reduce the environmental pollution and increasing the agricultural sustainability (Meena et al., 2014, 2015b).

An efficient use of *Trichoderma*-enriched biofertilizers increased yield reduces the soil borne pathogens and improves soil health (Haque et al., 2012). With the inoculation of *Trichoderma* through seed bio-priming in many cereal and vegetable crops has increased the levels of plant growth hormones and improved seed performance (Haque et al., 2012). Bio-priming helps seeds to evenly germinate even under adverse soil conditions (Babu et al., 2014; Contreras-Cornejo et al., 2014; Meena et al., 2015c). Wheat is second most important cereal crops in India. Its rank first in the world cereal production with the productivity of ~2.98 million t/ha and is a staple food of about one third of the world's population. India is the second largest producer of wheat (~95 million tons) next only to China (125.60 million tons) and cover the largest area under its cultivation (~30 mha), which is about ~14% of the world wheat area 217 mha (FAOSTAT, 2014). It is very important to determine the effect of *Trichoderma* strain along with varied soil types on various parameters of NUE and plant root length, to understand the beneficial effects of varied soils and the relative contribution of each doses of nitrogen fertilization on wheat crop. This will ultimately lead to selection of soil types for efficient utilization of *Trichoderma* strain. Therefore, the present study was undertaken to investigate the comparative effect of three soils with levels of N fertilization on wheat crop which is a major food crop and cultivated worldwide.

2. Materials and methods

2.1. Experimental soils

The soils of three orders; Entisol, Inceptisol and Alfisol were collected. The Alluvial soil sample was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The Red soil was collected from the Semara village in Shahabganj, Mirzapur and Black Soil was collected from Arziline block Sehansapur, Varanasi, initial soil properties present in Table 1.

2.2. Trichoderma strain

Trichoderma strain was isolated from different regions of Uttar Pradesh by Department of Mycology and Plant Pathology, Institute of Agricultural Science, BHU, Varanasi and characterized for their plant growth promotion activity. The most efficient and compatible *Trichoderma* isolates *Trichoderma harzianum* BHU51 (Gene Bank accession no. JN 618343) was used in this study.

Table 1
Initial physicochemical properties of experimental soils.

Physical parameter	Alluvial soil	Red soil	Black soil
Bulk density (Mg m^{-3})	1.4	1.3	1.5
Particle density (Mg m^{-3})	2.6	2.5	2.6
Water holding capacity (%)	39.9	38.9	44.9
Sand (%)	48.8	46.0	11.7
Silt (%)	30.5	32.8	52.7
Clay (%)	20.4	21.5	35.6
Soil texture	Sandy loam	Silty clay loam	Clayey
Electro-chemical and chemical properties			
pHw (1:2.5)	7.2	6.4	7.4
EC (dS/m)	0.4	0.3	0.6
CEC (C mol (p$^+$) kg^{-1})	29.0	18.0	30.9
Organic carbon (g kg^{-1})	3.7	3.1	3.9
Available N (kg ha^{-1})	229.0	173.0	236.0
Available P (kg ha^{-1})	17.0	08.0	13.0
Available K (kg ha^{-1})	230.0	109.0	235.0

2.3. Seed bio-priming

Trichoderma strain BHU-51 formulations (containing approximately 10^8 spores/mL) were used for seed treatment (Fig. 1). The slurry of the formulations was made and then seeds were dipped to slurry, mixed and allowed to soak for 30 min. Control seeds were not treated with formulations. After that seeds of wheat were used for pot trial with wheat cultivar- HUW-234.

2.4. Treatments details

The experiment has been under taken on three different soil types at the Institute of Agricultural Sciences, Banaras Hindu University, during December 2012 to April 2013, under greenhouse conditions, bio-primed seed of wheat cultivar HUW-234 with *Trichoderma harzianum* along with different recommended fertilizer doses were grown (Table 2).

2.5. Root length

Root systems were separated from shoots and the fresh root biomass was weighed immediately. Half of each root sample was fixed in FAA (37% Formaldehyde–Glacial Acetic Acid–95 Ethanol, 9:0.5:0.5, V:V:V) for quantification of AM fungal colonization and vesicular numbers. Roots were carefully separated from soil by washing and flooding over sieves. After cleaning of any foreign material, roots were preserved in 20% ethanol for measurement of root length by line interception method of Tennant (1975), using the formula:

$$Root\ \ length\ \ (RL) = (11/14) \times N \times G$$

where N is total numbers of intercepts of root with vertical and horizontal grid lines; G is grid square dimensions, cm; RL is root length, cm.

2.6. Chlorophyll content

The chlorophyll content was estimated at 60 DAS of wheat crop by following procedure of Arnon (1949). The leaf sample from sample plants were selected and 0.5 g weighed. The leaves were macerated with 80% acetone in a pestle and morter and then it was filtered by Whatman No. 1 filter paper and collected the supernatant. The volume of supernatant raised up to 50 mL and absorbance was recorded at 645 nm using a spectrophotometer and from the absorbance (A) values. Total chlorophyll content was determined as follows.

Total chlorophyll (mg g^{-1} fresh weight)=(20.2 × A 645) +(8.02 × A 663) × V/W × 1/1000.

2.7. Nitrogen use efficiency (NUE) formulas

$$Physiological\ \ use\ \ efficiency\ \ (PUE) = \frac{(Yield\ \ F\ \ kg\text{–}Yield\ \ C\ \ kg)}{Nutrient\ \ uptake\ \ F,\ \ kg\text{–}Nutrient\ \ uptake\ \ C,\ \ kg}$$

$$Agronomic\ \ use\ \ efficiency\ \ (AUE) = \frac{(Yield\ \ F\ \ kg\text{–}Yield\ \ C\ \ kg)}{Quantity\ \ of\ \ nutrient\ \ applied,\ \ kg}$$

$$Apparent\ \ nutrient\ \ recovery\ \ efficiency\ \ (ANR) = \frac{Unit\ \ of\ \ yield\ \ kg}{Unit\ \ of\ \ element\ \ in\ \ tissue,\ \ kg}$$