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SOIL SCIENCE

Effect of Integration of Seed Bio-priming and Inorganic Fertilization on Soil Physico-chemical Properties of Sunflower (*Helianthus annuus* L.)

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ABSTRACT

Conventional agricultural practices rely on the immense application of agrochemicals that show detrimental effects on soil biota. Negative impacts on soil microbiota and enzymes that involve in nutrient cycles hinder nutrient availability. Application of plant growth-promoting microbes such as *Trichoderma* spp. can evade these hurdles by improving soil biodiversity and performance of the crop. A pot culture experiment was conducted during two consecutive years (2018 and 2019) to figure out the effect of different doses of fertilizers and seed bio-priming with *Trichoderma asperellum* BHUT8 in sunflower (PAC-334) on soil chemical properties. Results showed that the initial soil reaction was slightly alkaline that tend towards neutral with seed bio-priming. The electrical conductivity of soil in both seasons varied randomly among imposed treatments. Soil cation exchange capacity, which permits the soil to hold all essential nutrients, was enhanced 4% with the supply of 70% recommended dose of fertilizer and seed bio-priming. Soil available nitrogen, phosphorus, and potassium were found maximum in the treatments supplied with *Trichoderma* through seed bio-priming. Inoculation of *Trichoderma* showed positive effects on soil chemical properties and reduced the requirement of inorganic fertilizers.

Highlights

- The obtained results of biopriming-inorganic fertilization on the growth parameters and yield of sunflower recommend their use as an alternative tool to reduce chemical fertilizers.
- The use of biopriming inorganic fertilization minimizes negative impacts on natural resources and caused an obvious increase in microbial activity, which is manifested by increased NPK content in the soil.

Keywords: Soil reaction, inoculum, recommended dose of fertilizer (RDF), available nutrients.

In the wake of the green revolution, concerned with crop production, the use of agrochemicals has been increased, which further magnified the impacts. Agro-chemicals contaminate soil, water, vegetation, toxic to beneficial insects, and non-target plants that decline populations of beneficial microorganisms in the soil, also affect humans by causing cancer and other chronic health effects as a majority of foods have detectable levels of chemical residues. Modern agricultural practices that use huge quantities of inputs have deleterious effects on human health, environment, and soil quality as it badly affects soil physical, chemical, and biological properties (Arriaga *et al.* 2017). The physical and chemical properties of soil are also affected due to human activities such as improper irrigation practices, disproportionate application of chemical fertilizers,

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and carbon losses (Aulakh and Sidhu 2015). In the soil system, dissolved ions are accumulated because of severe evaporation losses and fertilizer use that leads to nutritional imbalance, decrease in soil permeability, and other indirect losses (Visconti and Paz 2016). Redundant use of chemical fertilizers causes instability of soil ecosystem and other complications such as groundwater contamination and environmental effects. In developing countries, adverse health effects are noticed in farmers due to exposure to agro-chemicals (Zhang *et al.* 2018). Disproportionate use of agrochemicals can be avoided by adopting sustainable agricultural practices such as the application of microorganisms, which is eco-friendly and economical too. Microbial application through seed needs only a small quantity of inoculum, also improves plant growth and yield (Rocha *et al.* 2019). Among all methods of microbial application, the appropriate technique is through seed bio-priming, which is the combination of two processes, i.e., seed hydration and microbial inoculation by beneficial microorganism (Rakshit *et al.* 2015; Singh *et al.* 2018; Sarkar *et al.* 2018; Rakshit 2019; Sarkar and Rakshit 2021). This study is conducted with an objective to find out the effect of seed bio-priming on soil chemical properties with test crop sunflower, and the microbe selected for study is *Trichoderma asperellum*.

MATERIALS AND METHODS

Seed bio-priming

Seed bio-priming was carried out with micro organism *Trichoderma asperellum* that was cultured on PDA procured from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University. Spore count in suspension was maintained at 10^8 CFU $ml^{-1,}$ and 3-4 drops of tween-20 were added. Sunflower seeds were hydrated for 6 hr followed by surface sterilization with 1% sodium hypochlorite (NaOCl) solution that was placed in moisture chamber and then incubated for 48hr after drenching with spore suspension (Fig. 1).

Experimental details

Pot culture experiment was conducted for two seasons with sunflower (variety: PAC 334) at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (25°20′ N latitude and 83°01′ E longitudes). The experimental design was a completely randomized design with four replications. First season and second season experiments were conducted during February second fortnight to may second fortnight in 2018 and 2019, respectively. Treatments imposed were T_1: control, T_2: 70% RDF, T_3: 80% RDF, T_4: 90% RDF, T_5: 100% RDF (90-50-50), T_6: 70% RDF + Sees bio-priming, T_7: 80% RDF + Sees bio-priming, T_8: 90% RDF + Sees bio-priming. Initial properties of soil are given in table 1.

Soil reaction (pH)

Soil reaction was determined by a combined glass

(a)

(b)

Fig. 1: (a) Culture of *Trichoderma asperellum* **(b)** Bio-primed sunflower seed

Table 1: Initial properties of experimental soil

Soil property	Value	Soil property	Value
Bulk Density (Mg m^{-3})	1.41	CEC (C mol (p^+)/kg)	30.91
Particle density (Mg m^{-3})	2.58	Organic carbon (g kg^{-1})	4.1
Water holding capacity (%)	39.4	Available N (kg/ha)	217
Soil Texture	Sandy loam	Available P (kg/ha)	16.67
pHw (1:2.5)	7.3	Available K (kg/ha)	234
EC (dS/m)	0.38		

electrode in 1:2.5 soil water suspension (Jackson, 1973).

Electrical conductivity (EC)

EC of soil suspension was determined in the same sample used for soil reaction. Reading was taken by using an electrical conductivity bridge in supernatant solution after allowing the suspension to settle down (Jackson, 1973).

Cation Exchange Capacity (CEC)

Soil samples were saturated with 1 N sodium acetate (pH 8.3). The excess sodium acetate was leached out by washing out with 95% ethanol. Then the adsorbed sodium was displaced with neutral normal ammonium acetate. The concentration of sodium in the leachate was estimated flame photometrically (Bower *et al*. 1952).

Soil available nitrogen

Available nitrogen content in soil samples was determined by the alkaline potassium permanganate method (Subbiah and Asija 1956). To the known weight of soil, $KMnO_4$ (oxidizing agent) was added. Nascent oxygen released in the presence of NaOH oxidizes organic matter, and the released ammonia absorbed in a boric acid of known concentration. The excess is known by the titration method by using a mixed indicator.

Available phosphorus

Available phosphorus in the soil samples was extracted with 0.5 M $NaHCO_3$ of pH 8.5, and the phosphorus in the extract was estimated colorimetrically by an ascorbic acid method using a spectrophotometer at 660 nm (Watanabe and Olsen 1954).

Available potassium

Available potassium in soil was extracted using neutral normal ammonium acetate, and potassium in the extract was determined to flame photometrically (Muhr *et al.* 1965).

Statistical analysis

Data analysis was done by using Statistical Package for Social Science (SPSS) software (Version 20). R studio (version 4.0.4) was used for the computation of the correlation matrix.

RESULTS AND DISCUSSION

Soil reaction

Pooled data showed that the soil reaction ranged from 7.2 to 7.5 (Fig. 1). During the first season, the highest soil pH was noticed in control and 100% RDF (7.4), which was slightly alkaline in reaction. Reduction in soil pH towards neutral was noticed in seed bio-priming treatments (7.1 to 7.3). Second season data showed that the highest pH was noticed in control, 70% RDF and RDF (7.5), while the lowest was in treatment supplied with 80% RDF along with bio-priming (7.2). Pooled revealed that the highest pH value was in control (7.5) while the minimum was noticed in T_7 (7.2). Soil physicochemical properties such as soil reaction, electrical conductivity, and cation exchange capacity play a vital role in the chemistry and fertility of the soil (Neina 2019). Soil reaction extensively influences soil physical, chemical, and biological properties that determine crop growth and yield (Minasny *et al.* 2012; Neina 2019). Similar results were found with *Trichoderma* inoculation in Lolium (Song *et al.* 2015), in *Miscanthus*, *Salix*, *Phalaris* and *Panicum* (Kacprzak *et al.* 2014), which might be due to the ability of *Trichoderma* to produce organic acids such as gluconic acid, fumaric acid and citric acid (Wang and Chen 2009; Song *et al.* 2015). In pearl millet and wheat crop, inoculation of cyanobacterial bio-fertilizer resulted in a decrease in soil pH (Nisha *et al.* 2007).

Electrical conductivity

In the current experiment, pooled data showed that the range is between 0.34 dSm^{-1} and 0.43 dSm^{-1} (Fig. 2). There is no specific trend in EC values with imposed treatments, however, in pooled data, maximum conductivity was observed in treatment supplied with 70% RDF (0.43 dSm^{-1}) while the minimum was noticed in treatment supplied with 70% RDF along with bio-priming (0.34 dSm^{-1}). Soil cation exchange capacity is the ability of soil to hold and exchange mineral elements. Soil chemical properties can be assessed by cation exchange capacity (Saidi 2012).

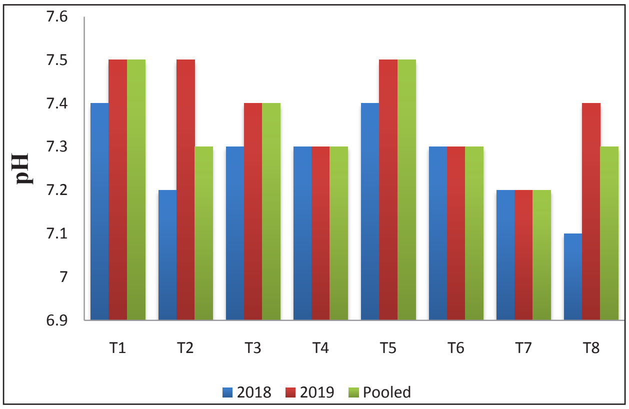

Fig. 2: Effect of different treatments on soil reaction

Cation Exchange Capacity

Cation exchange capacity during the first season experiment ranged between 22.73 and 24.11 cmol (p+) kg^{-1}. Minimum value was noticed in control, wherethe CEC of treatments supplied with 70% RDF + bio-priming and 80% RDF + seed bio-priming were 5.71 and 6.07% higher, respectively. In second season experiment treatments supplied with 80% RDF + bio-priming, 80% RDF + bio-priming and 90% RDF + seed bio-priming were 3.7, 1 and 3.02% higher values were noticed (Fig. 3). An increase in soil CEC with Azotobacter was noticed in *Solanum* (Demir, 2020) whereas in pearl millet and wheat crop, inoculation of cyanobacterial bio-fertilizer showed a slight increase in soil CEC (Nisha *et al.* 2007). According to a study on *Miscanthus*, *Salix*, *Phalaris*, and *Panicum*, there is no effect of *Trichoderma* on soil CEC (Kacprzak *et al.* 2014).

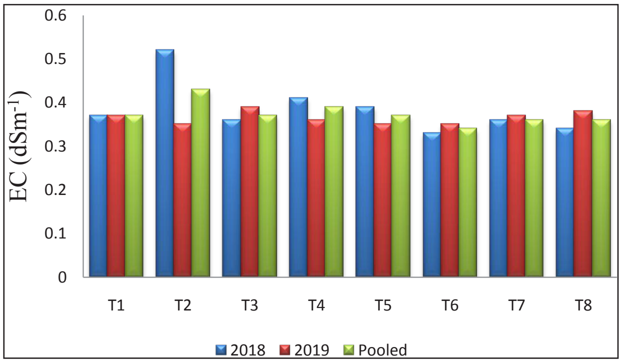

Fig. 3: Effect of seed bio-priming on soil EC (dSm^{-1})

Soil available nitrogen

Soil available nitrogen content varied significantly with different imposed treatments in both the seasons (Fig. 4) and ranged from 205 to 244 kg ha^{-1} on pooled bases. The highest value was recorded in treatment supplied with 80% RDF + bio-priming with *Trichoderma asperellum,* which was significantly superior to control (205 kg ha^{-1}), 70% RDF (225 kg ha^{-1}), and significantly superior to the rest of the treatments.

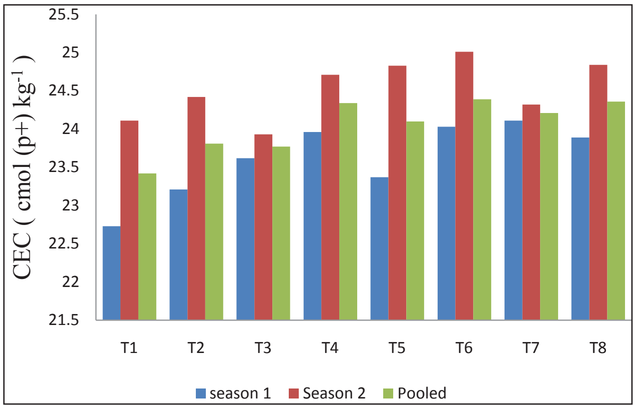

Fig. 4: Effect of different imposed treatments on soil CEC (cmol (p+) kg^{-1})

The highest available soil nitrogen in both the seasons (2018 and 2019) was recorded in the treatment T_7 (80% RDF + bio-priming with *Trichoderma asperellum*), i.e., 240 and 249 kg ha^{-1} respectively that was at par with $T_3 - T_6$ and T_8 treatments. Enhance in soil available nutrients was due to the ability of *Trichoderma* to solubilize soil nutrients (Yadav *et al.* 2009; Kapri and Tewari 2010). Inoculation of *Trichoderma* spp. enhanced soil available nitrogen in *Pinus* (Halifu *et al.* 2019), in pepper (Liu *et al.* 2020), soil available phosphorus in maize (Mercl *et al.* 2020), sugarcane (Yadav *et al.* 2009), in *Lolium perenne* (Song *et al.* 2014), in alfalfa (Zhang *et al.*, 2019), cassava (Hridya and Byju 2014), sugarcane (Thakur *et al.* 2010).

Soil available phosphorus

Available soil phosphorus content was ranged between 15.5 and 24.07 kg ha^{-1} in pooled analysis (Fig. 5). Pots received 80%, 90% RDF along with bio-priming showed maximum available phosphorus content (23.15, 23.32 kg ha^{-1}) that was comparable to 100% RDF treatment (22.58 kg ha^{-1}) and significantly superior to the rest of the treatments in the first season.

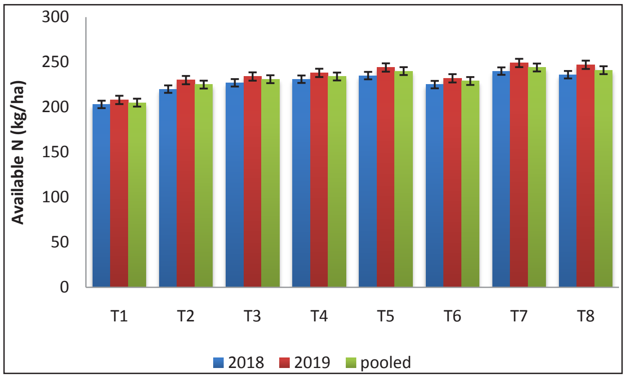

Fig. 5: Treatments impact on soil available nitrogen (kg ha^{-1})

The highest available P at harvest of sunflower in the second season experiment was noticed in T_8 (90% RDF + bio-priming with *Trichoderma asperellum*) (24.82 kg ha^{-1}) that was found at par with the treatments T_3 to T_7 (21.33 to 24.14 kg ha^{-1}) and significantly superior to control (16.62 kg ha^{-1}) and T_2 (20.45 kg ha^{-1}). Phosphorus solubilization of a substrate (calcium phosphate) by *Trichoderma* spp. was ranged between 51.7 % and 90.3 % after 10 days incubation (Bononi *et al.* 2020). Inoculation of *Trichoderma* spp. enhanced soil available phosphorus in *Pinus* (Halifu *et al.* 2019), in pepper (Liu *et al.* 2020), soil available phosphorus in maize (Mercl *et al.* 2020), sugarcane (Yadav *et al.* 2009), in *Lolium perenne* (Song *et al.* 2014), in alfalfa (Zhang *et al.* 2019), cassava (Hridya and Byju 2014), sugarcane (Thakur *et al.* 2010).

Soil available potassium

Available soil potassium content at harvest was ranged from 212 to 247 kg ha^{-1} according to pooled data (Fig. 6) in which the highest was found in the treatment supplied with 90% RDF + bio-priming with *Trichoderma asperellum* (247 kg ha^{-1}) comparable to the treatments T_2 to T_7 and all fertilized treatments except T_2 were significantly superior to control. The highest available potassium content in both first and second season crop was found in T_8 (246 and 248 kg ha^{-1} respectively) that was comparable to the treatments T_2 to T_7 (239 to 247 kg ha^{-1} and 233 to 246 kg ha^{-1} respectively) and significantly superior to control. Enhance in soil available nutrients was due to the ability of *Trichoderma* to solubilize soil nutrients (Yadav *et al.*, 2009, Kapri and Tewari, 2010). Available soil potassium with *Trichoderma* inoculation was recorded in alfalfa (Zhang *et al.*, 2019), cassava (Hridya and Byju, 2014), sugarcane (Thakur *et al.* 2010).

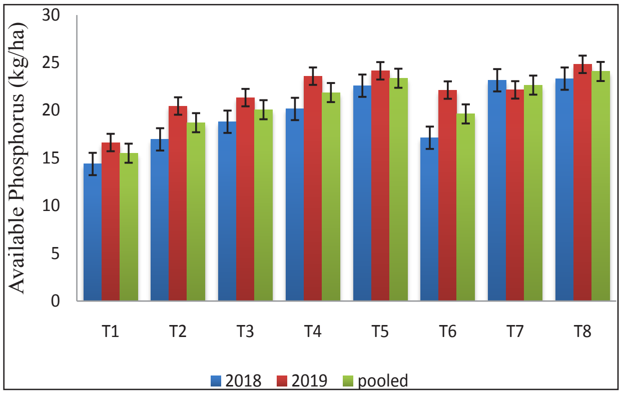

Fig. 6: Effect of treatment combinations on soil available phosphorus (kg ha^{-1})

Correlation

Correlation among soil pH, EC, CEC, available N, P, and K was presented in Fig. 7.

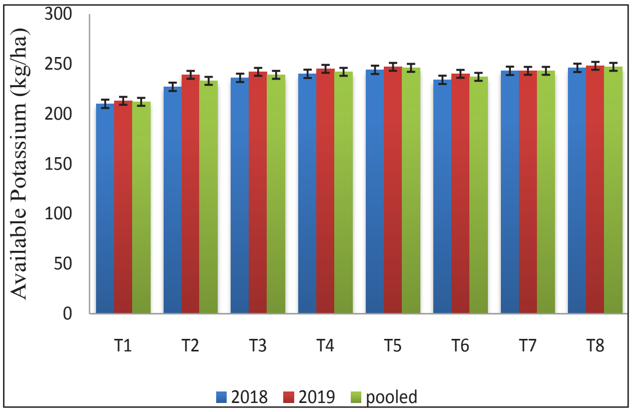

Fig. 7: Influence of seed bio-priming on soil available potassium content (kg ha^{-1})

Normal curve of each parameter is depicted. The correlation coefficient ranged from -0.611 to 0.971. Soil reaction and electrical conductivity showed a negative correlation with all studied parameters *viz.*, CEC, available nitrogen, phosphorus, and potassium ranged from -0.611 to -0.025 with pH and -0.359 to -0.148 with EC. Cation Exchange Capacity was positively correlated with Soil available nitrogen (0.779), phosphorus (0.777) and potassium (0.803). Soil nitrogen strongly correlated with soil phosphorus (0.959) and potassium (0.971), while phosphorus and potassium were strongly correlated with each other (0.949).

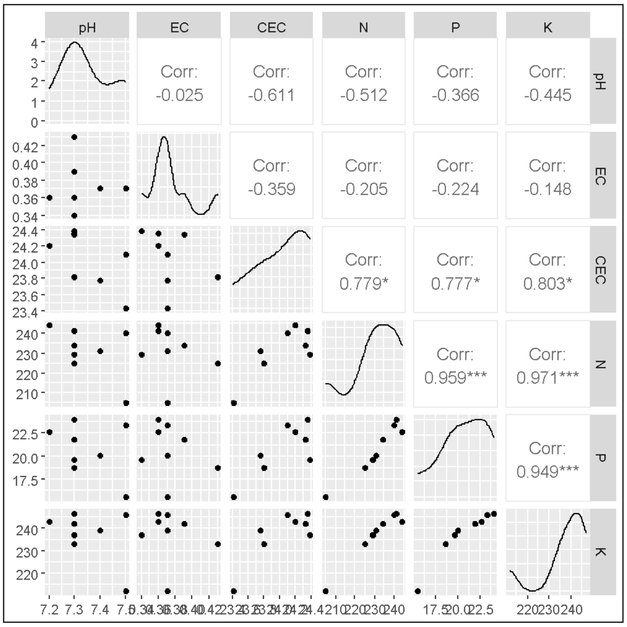

Fig. 8: Correlation among soil chemical parameters. Abbreviations: pH- Soil reaction, EC- Electrical Conductivity, CEC- Cation Exchange Capacity, N- Soil available nitrogen, P- Soil available phosphorus, K- Soil available potassium.

CONCLUSION

The current experiment revealed the gradual improvement in soil chemical properties with bio-priming intervention. All the treatments which received *Trichoderma asperellum* through seed bio-priming gave positive results with respect to soil reaction, electrical conductivity, cation exchange capacity, soil available N, P, and K. Integration of seed bio-priming with chemical fertilization was performed well as compared to a recommended dose of fertilization. Bio-priming treatments are effective for improving soil chemical properties that reduced the crop requirement of agrochemicals as well as minimize the negative impacts of intensive agricultural practices. Practicing seed bio-priming techniques is feasible, eco-friendly, economical, and helpful for plant growth.

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