

Optimizing soil health: comparative effects of humic acid, PGPR, and RDF on soil properties and fertility

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Abstract: Soil fertility and productivity can increase with the addition of organic fertilizer. The continuous cropping, long-term use of inorganic fertilizer, soil erosion, and overgrazing may lead to a decline in soil organic matter. Soil fertility can be enhanced by using organic substances such as humic acid (HA) and root-associated bacteria like plant growth-promoting rhizobacteria (PGPR). The study aimed to compare the impact of organic (HA and PGPR) and inorganic (RDF- Recommended dose of fertilizer) fertilizer on the soil's physicochemical parameters during the winter seasons at G.B. Pant University of Agriculture and Technology in Pant Nagar in the year of 2020-21 and 2021-22. This study used a randomized block design (RBD) with ten treatments and three replicates. The contents of the treatments used are as follows: T₁; Control (Only RDF), T₂; HA- 2.5 kg/ha (soil application), T₃; Seeds treated with HA -20 g/kg of soil), T₄; seeds treated with PGPR - 20 g/kg of seed, T₅; RDF (NPK mixture), T₆; RDF + HA (soil application), T₇; RDF +HA (seed treatment), T₈; RDF +PGPR (Seed treatment), T₉; HA (soil application) +PGPR (seed treatment), T₁₀; RDF + HA (soil application) + PGPR (seed treatment). The results showed that T₁₀ (RDF+ HA-soil application + PGPR) had the most significant impact on the soil's particle density (PD), water retention, organic carbon content (OC), and accessible nitrogen (N), phosphorus (P), and potassium (K) content in the soil during both years of experiment. The detailed research concluded that applying humic acid, PGPR, and RDF positively improved soil properties.

Keywords: Humic acid, soil physicochemical properties, rhizobacteria, organic matter

1. Introduction

The detrimental effects of soil degradation on soil productivity have been widely recognized in the last three decades. This issue has been exacerbated in the present agrosystem by the widespread use of continuous tillage and the lack of attention paid to sustainable soil management. Because of the inadequate chemical features of these soils, agricultural practices have led to a steady depletion of organic matter in the A horizon, drastically lowering the soil's initial productivity. Organic matter is an essential indicator of soil quality (Bouajila, K., and Sanaa, M. 2011). Organic resources, including plant residues and animal and green manures, can be applied to soils to improve fertility, soil physical characteristics, and microbial activity and to decrease metal toxicity via complexation (Johnston, 1986). When organic matter decomposes in the soil, macronutrients like nitrogen, phosphorous, and sulfur are released, along with a trace amount of certain other micronutrients (Escobar *et al.*, 2003).

Humus is made up of organic residue that is resistant to further breakdown. The primary source of HA is the Leonardite layer. These are sedimentary layers found under the soil and formed after an extended period, although coal and peat are the other sources. (Man- Hong *et al.*, 2020 & Bhatt and Singh, 2022). There are three components of humus: fulvic acid, humic acid, and humin. All three have different characteristics and impacts on soil health. The application of humic acid in agriculture is quite popular nowadays due to its wide properties. Humic acid (HA) can be found in organic humus and can greatly influence soil quality and plant growth. Also, it aids in enhancing soil structure and water retention (Fahramand *et al.*, 2014).

The prolonged use of synthetic fertilizer may somehow degrade soil fertility. People are looking for an organic alternative to inorganic fertilizers or pesticides. Plant Growth promoting rhizobacteria is a bio-fertilizer, a root-associated bacteria used in large-scale crop production. It has a direct and indirect role in soil and plant systems. Seeds treated with PGPR encourage a robust root system with many more root hairs and a wide surface. Several growth-regulating phytohormones, including IAA, GA₃, zeatin, ethylene, and abscisic acid, are secreted by PGPR and are responsible for increased agronomic production.

Regarding plant and soil nutrition, rhizobia and phosphorus (P) solubilizing bacteria play crucial roles (Singh, 2013). PGPR produces chemicals protecting plants from soil-borne diseases, including antibiotics and siderophores (Kyei-Boahen *et al.*, 2002). PGPR and humic acid both benefit plant growth. Plants benefit from their presence; in the long run, they can aid in maintaining a healthy ecosystem and crop yield (Dasgupta *et al.*, 2015) (DÖNDER and

TOĀAY, 2021). Most humic acid studies have been conducted in greenhouses and laboratories; however, the field conditions may vary, and various factors can affect crop and soil response performance (Ranjan et al., 2021). Therefore, a field experiment was conducted to examine the effect of humic acid on soil properties under field conditions and to compare the effect of humic acid, PGPR, and RDF on soil physical and chemical properties. Chickpea is a most critical pulse crop and occupies the most extensive area among pulses in India.

2. Material and Methods

Research field

The investigation was conducted at the D₆ block in GBPUAT's Norman E. Borlaug Crop Research Center at Pantnagar-263145, US Nagar, Uttarakhand (India). The research place is located at 29 °N latitude, 79.5 °E longitude at an altitude of 243.83 m above mean sea level in the foothills of the Himalayas. The climate is humid subtropical, with cold winters and hot summers that are quite dry. The mean weekly maximum and minimum temperature ranged from 26.3 to 10.6 °C and 25.2 to 11.4 °C during 2020-21 and 2021-22, respectively. The total precipitation received in the first and second year of the experiment during the crop period was 26.0 and 158 mm, respectively. The experiment site comes under an irrigated area. Humic acid and PGPR both help to neutralize the pH of soil by adding organic acid. Thus, the acidic soil can be more acidic by using HA and PGPR. Also, the dry land area is unsuitable because optimum moisture is needed to activate soil microorganisms.

Soil study

The order and texture of the soil at the experimental site were mollisols and silty clay loam, respectively. The detailed parameters, methods employed, and results obtained from soil investigation before the trials of both years are mentioned in Table 2.

Soil sampling and observation

Five soil samples were taken from each plot at a depth of 0-15 cm at the time of sowing and after harvesting of the crop for analysis of the soil's physical properties (bulk density, particle density, porosity, and water holding capacity) while for the analysis of chemical properties (organic carbon content, available nitrogen, phosphorus, and potassium) samples were collected at 45, 75 days after sowing, and harvesting.

Details of the experiment

The study was designed as a randomized block design (RBD), with ten treatments and three replications. There were 30 plots, and the individual plot size was 5 × 3.5 meters. Humic acid was procured from Shahi Krishi Seva Kendra, Udham Singh Nagar. The powder form of HA was used in the experiment. The PGPR (*Bacillus cereus* NE-10, NCBI Acc No. KR868766) was obtained from AICRP on Pulses, Department of Soil Science, GBPUAT, Pant Nagar. HA was applied as soil application and seed treatment, while PGPR was applied only as a seed treatment. HA (2.5 kg/ha) was mixed with soil to increase the volume and then applied to respective plots. Seeds were treated with HA (20 g/kg seeds) and PGPR (20 g/kg seeds) before sowing. For seed treatment with PGPR, seeds were mixed in jaggery solution one hour before sowing, and then, the seeds were left to dry for 15 to 20 minutes. RDF was applied through an NPK mixture with a grade of 12:32:16. Chickpea variety PG-186, released from Pantnagar, was used during both years of study. The seed rate was applied at the rate of 60 kg/ha. The treatment details are the following:

Table 1: Details of treatment provided to a series of specimens.

S. No	Treatments
1	Control (only RDF)
2	HA-2.5 kg/ha (soil application)
3	HA- 20 g/kg seed (Seed treatment)
4	PGPR -20 g/kg seed (seed treatment)
5	RDF (through NPK mixture, 150 kg/ha)
6	RDF+ HA-2.5 kg/ha (soil application)
7	RDF+ HA- 20 g/kg seed (Seed treatment)
8	RDF+ PGPR -20 g/kg seed (seed treatment)
9	HA-2.5 kg/ha (soil application) + PGPR -20 g/kg seed (seed treatment)
10	HA-2.5 kg/ha (soil application) + PGPR -20 g/kg seed (seed treatment) + RDF

Table 2: Preliminary soil properties of the trial site

Parameters	Initial status		Method employed and References
	2021	2022	
A) Soil physical properties			
1. Bulk density (g/cm ³)	1.39	1.40	Hand Auger (Baver, 1956)
2. Particle density (g/cm ³)	2.50	2.62	Baver (1956)
3. Porosity (%)	44.40	46.56	Baver (1956)
4. Water holding capacity (%)	24.13	27.21	Hilgard apparatus (Piper, 1950)
B) Soil chemical properties			
1. Soil pH	6.29	6.30	Glass electrode pH meter (Jackson, 1973)
2. Electrical conductivity (ds/m)	0.22	0.23	Conductivity meter (Bover and Wilcox, 1965)
3. Organic carbon (%)	0.65	0.69	Modified Walkley and Black method (Walkley and Black, 1934)
4. Available nitrogen (kg/ha)	175.5	206.3	Alkaline KMnO ₄ method (Subbiah and Asija, 1956)
5. Available phosphorus (kg/ha)	14.2	16.0	Olsen's method (Olsen <i>et al.</i> , 1954)
6. Available potassium (kg/ha)	133.2	142.4	Flame emission spectrometry method (Jackson, 1973)

3. Statistical Analysis

The experimental data received from the observations on growth, yield, and soil parameters were analyzed by using the standard procedure for Randomized Block Design (RBD) with the help of a computer analysis program OPSTAT developed by O.P. Sheoran Programmer, Computer department, CCS HAU, Hisar. One-way ANOVA (F-test) was used, and the data was interpreted as per the CD (critical differences) values at a 5% significance level (Sheoran, 2010).

4. Results and Discussion

From Table 3, it can be inferred that adding humic acid, PGPR, and RDF improved the soil's physical properties. Treatments significantly enhanced soil particle density and water-holding capacity during both years, while bulk density and porosity of soil were not significant in the second year of the experiment. In the case of chemical properties, treatments significantly showed a positive effect. T₁₀ (RDF+HA-soil application+ PGPR) was found superior to all the treatments, while T₆ (RDF+ HA-soil application) and T₈ (RDF+ PGPR) were found at par in most of the parameters. HA are organic molecules that improve soil quality, plant growth, and other agronomic variables. HA-based products have been integrated into crop production to ensure the long-term sustainability of agricultural output in recent years. The findings showed that HA may improve the soil's physiochemical characteristics. Soil characteristics consist of things like particle aggregation and relative proportion, water-holding capability (WHC), cation exchange capacity (CEC), pH, carbon content (OC), enzyme activity, macronutrient cycling, and availability (Ampong *et al.*, 2022; Brannon and Sommers, 1985; Li *et al.*, 2019).

Bulk Density

Table 3 shows a statistically significant effect of treatments on soil bulk density in the first year of the research, but no such effect was detected in the second year. Earlier, the experimental site was occupied by an exhaustive crop (cereal), and humic acid was introduced at that site, which showed some effect on soil bulk density as this property of soil takes time to change so it may be due to this reason, the results were found non-significant. In the first year, T₁ (Control) had a bulk density that was much greater than that of the other treatments, including T₂ (HA-soil application) and T₃ (HA-seed treatment). In both years, the bulk density (1.39, 1.37, and 1.35 g/cm³) of the humic acid treatments (T₂, T₆, and T₁₀) was lower than that of the other treatments.

There is a negative correlation between pore size and density. Humic acid is very efficient at holding soil particles together. This enhances the soil's structure, leading to greater porosity and decreased bulk density (Fahramand *et al.*, 2014). The bulk density of the control group was found to be much higher than that of the PGPR treatment group T₈ (RDF+ PGPR), which showed a significant reduction. Because of the increased availability of nutrients around the rhizosphere, an increased number of microorganisms were drawn to the area, resulting in a greater breakdown rate. Moreover, some organic acid was produced, which bound the soil particles and improved the pore space in the soil (Bhattacharya and Chandra, 2013).

Table 3: Soil physical properties as influenced by the addition of HA, PGPR, and RDF alone or in combination after harvesting of the crop during both the years of experiment

Treatment	BD (g/cm ³)		PD (g/cm ³)		Porosity (%)		WHC (%)	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
T ₁	1.41	1.44	2.35	2.37	39.99	39.20	25.26	24.22
T ₂	1.39	1.37	2.43	2.46	42.76	44.18	27.45	30.46
T ₃	1.41	1.39	2.36	2.38	40.32	41.54	26.27	26.58
T ₄	1.40	1.38	2.44	2.45	42.61	43.55	26.53	27.51
T ₅	1.39	1.39	2.50	2.52	44.50	44.61	27.51	26.55
T ₆	1.37	1.36	2.50	2.53	45.34	46.38	30.36	33.45
T ₇	1.38	1.36	2.45	2.47	43.66	44.75	27.53	26.55
T ₈	1.37	1.35	2.50	2.51	45.35	46.23	28.10	28.15
T ₉	1.40	1.39	2.45	2.47	43.00	43.79	27.51	29.32
T ₁₀	1.35	1.33	2.55	2.56	47.03	47.87	30.28	35.46
C.D.(P=0.05)**	0.03	NS	0.10	0.10	2.23	NS	2.67	2.33

**Critical difference at 5% level of significance, Treatment details: T₁- Control (only RDF), T₂ – HA-soil application, T₃ -HA -seed treatment, T₄ - PGPR -seed treatment, T₅- RDF, T₆ - RDF + HA -soil application, T₇ -RDF +HA -seed treatment, T₈- RDF +PGPR -Seed treatment, T₉- HA -soil application +PGPR -seed treatment, T₁₀- RDF + HA -soil application + PGPR - seed treatment.

Particle Density

In all treatments, particle density rose throughout the second year of the trial. In both years, the particle density values for T₂ (HA-seed treatment) and T₄ (PGPR treatment) were substantially higher than those of T₁ (Control) and T₃ (HA-seed treatment).

The maximum value of particle density was reported in both years when all three treatments (HA, PGPR, and RDF) were combined into a single treatment. The synergistic action of HA, PGPR, and RDF may be considered for this. HA has a carbon content between

51 and 56 %, which is the primary source of nutrition for soil organisms. By decomposing the plant waste, they contribute organic matter to the soil, which is light in weight and might lead to a drop in the particle density of the soil. Adding carbon to soil increases the number of soil microbes (Khatana *et al.*, 2021). On the other hand, PGPR is responsible for adding organic acid to the soil through decomposition, which helps to make the soil lighter in weight due to the presence of humus (Walley *et al.*, 2014), leads to lower particle density, while RDF is responsible for an increase in the availability of nutrients in the soil.

Porosity

When compared to T₁ (control) with T₂ (HA- soil application) and T₄ (PGPR), each reported 2.8, 2.6, and 4.9, 4.4% greater porosity in the first and second years, respectively. In the first year of the study, T₁₀ (RDF+HA-soil application +PGPR) performed at the same level as T₆ (RDF+HA-soil application), whereas T₈ (RDF+PGPR) determined a value of porosity that was much higher than that of any of the other treatments.

The combined application of HA, PGPR, and RDF increased porosity across both years. This might be because humic acid comes from an organic source; after all, it is a substance that works to bind the particles of soil together. It is also a component of the clay complex, which tends to increase aggregate stability, leading to improved soil structure and improving porosity (Regelink *et al.*, 2015). Since HA is a byproduct of humus, which is composed of organic matter, the addition of humic acid to soil led to an increase in the population of microorganisms, which in turn led to an increase in the porosity of the soil (Sellamuthu and Govindaswamy, 2003). On the other side, PGPR increases the population of soil microorganisms by providing a favorable environment, which leads to increased aeration and porosity in the soil.

Water Holding Capacity

When compared to the previous year, the water storage capacity had a 4.15% rise in the second year. In comparison to T₁ (control), the water holding capacity of T₂ (HA- soil application) and T₄ (PGPR) rose by 2.2, 1.3, and 6.2, 3.3% in the first and second years, respectively.

T₁₀ (RDF+HA-soil application +PGPR) reported values of water holding capacity of soil that were 5.0, 2.8, and 11.2, 8.9% higher than those recorded by T₁ (control) and T₅ (RDF), respectively, in the first and second years. Throughout both years of the research, the humic acid treatments (T₂ and T₆) had a more significant percentage of WHC than the PGPR-seeded treatments. This may be because of how humic acid is structured on a molecular level. Humic acid is composed of various substances, such as macromolecules, hydrophobic, hydrophilic,

and functional groups. Because of its hydrophilic character, humic acid contains many sites that may attach hydrogen ions, improving soil capacity to store water (Fahramand *et al.*, 2014).

Table 4: Organic carbon content in soil enhanced by different treatments at 45, 75 days after sowing (DAS) and after harvesting the crop in both years of investigation

Treatment	Organic carbon contents (%)					
	45 DAS		75 DAS		After harvest	
	2020-21	2021-22	2020-21	2021-22	2020-21	2021-22
T ₁	0.50	0.46	0.52	0.50	0.46	0.46
T ₂	0.67	0.68	0.76	0.70	0.70	0.69
T ₃	0.49	0.49	0.55	0.53	0.51	0.51
T ₄	0.68	0.68	0.72	0.70	0.67	0.68
T ₅	0.63	0.73	0.76	0.75	0.68	0.72
T ₆	0.79	0.88	0.88	0.85	0.82	0.74
T ₇	0.64	0.79	0.85	0.81	0.77	0.83
T ₈	0.78	0.82	0.87	0.82	0.80	0.82
T ₉	0.62	0.78	0.71	0.72	0.66	0.67
T ₁₀	0.83	0.88	0.90	0.90	0.86	0.88
C.D.(P=0.05)**	0.10	0.09	0.10	0.12	0.12	0.12

** Critical difference at 5% level of significance, Treatment details: T₁- Control (only RDF), T₂ – HA-soil application, T₃-HA -seed treatment, T₄ - PGPR -seed treatment, T₅- RDF, T₆-RDF + HA -soil application, T₇ - RDF +HA -seed treatment, T₈- RDF +PGPR -seed treatment, T₉- HA -soil application +PGPR -seed treatment, T₁₀- RDF + HA -soil application + PGPR -seed treatment.

Organic Carbon

Table 4 indicates that T10 recorded a much larger organic carbon content at each observation stage over the years than the other treatments, except for T₆ (RDF+ HA-soil application) and T₈ (RDF+ PGPR). This was the case regardless of which treatment was being compared. The organic carbon content in the soil was lower in the beginning stage (45 DAS); after that, it grew to 75 DAS but then again decreased at the harvesting stage over both years (Table 4). Chickpeas experience nodule production between the ages of 35 and 75 DAS, after which the nodules

degenerate. As a result, the level of microbial activity is at its highest during this period and declines while harvesting comes (Kumar and Kumar, 2023). Humic acid has a stronger resistance toward organic carbon degradation and a slower release of nutrients. The humic acid treatment resulted in more organic matter (Verma *et al.*, 2017). The synergistic action of HA and PGPR may also be responsible since humic acid is a rich carbon source. It contains 51 to 56% carbon. Adding humic acid led to an immediate increase in the organic carbon content of the soil (Gumus and Seker, 2015).

Available Nitrogen content

After analyzing the results, it was noted that the treatments considerably impacted the amount of readily available soil nitrogen. T₁₀ (RDF+HA-soil application + PGPR) reported 79.2, 27.1, and 85.8, 38.3% greater contents of available nitrogen in the soil than T₁ (control) and T₅ (RDF) at 75 DAS in the first and second year, respectively. During the initial stage of the crop, the soil had a low nitrogen content; later, it was found that the nitrogen level had started to increase up to 75 DAS but again decreased by harvest time. Mostly, pulses have an innate quality of nodule development, which takes place five to six weeks after sowing. Nodules help with nitrogen fixation in plants. However, the nodules start to disintegrate after 105 DAS.

Consequently, by the time the plants reached maturity, they had once again used nitrogen from the soil, which resulted in a low nitrogen content in the soil at the time of harvest (Pastapure *et al.*, 2021). The combined action of HA, PGPR, and RDF may be the reason for this. Indirectly improving soil qualities, such as mineralization and solubilization of available minerals in the soil and nutrient uptake by plants, are all outcomes of humic acid's role as a soil enhancer (Bhatti *et al.*, 2011). On the other hand, the inoculation of PGPR accelerated the nitrogen fixation process by increasing nodulation, resulting in a high N content in the soil. Being a primary nutrient, nitrogen plays a crucial role in the biosynthesis of chlorophyll, amino acids, proteins, and nucleotides. However, all are important for crop growth and development (Marschner, 2011).

Available Phosphorus content

Soil-accessible phosphorus levels in T₁₀ (RDF+HA-soil application +PGPR) were 68.6%, 28.51%, and 68.9%, 28.1% higher than in T₁ (control) and T₅ (RDF) at 75 DAS in the first and second years, respectively (Table 5).

At each point of observation, the PGPR-seeded treatment (T₈) had the highest level of accessible phosphorus. PGPR (*Bacillus cereus*) had the potential for P solubilization, which

helped in the solubilization of inorganic phosphorus through the excretion of organic compounds and allowed the release of phosphorus into the soil solution. It was reported by Billah *et al.*, 2020 that bio-fertilizers have properties of P-solubilization into the soil as phosphorus is highly reactive in the soil, thus making chemical compounds with Iron, aluminum, and calcium in soil solution and become insoluble and non-available to plants. On the other hand, HA acts as a chelating agent, which helps break down the connection between phosphate and organic molecules, releasing P into the soil and increasing the amount of phosphorus available (Turgay *et al.*, 2011). Phosphorus is considered an “energy currency” because it is important for the energy storage and transfer required for plants' physiological and metabolic functions (Bhardwaj *et al.*, 2023).

Available Potassium content

Every treatment showed considerably higher levels of accessible potassium than T₁ (control). At 75 DAS, T₁₀ reported a 35.0, 7.1, and 40.5, 11.9% greater available potassium content in soil than T₁ (control) and T₅ (RDF), respectively, in both years (Table 5). The application of PGPR (T₄) recorded a higher potassium content available in soil than T₂ (HA-soil application). Potassium is important for plant enzyme activation, osmoregulation, and stomatal movement. It is highly reactive with water and oxygen; thus, free form is unavailable in the soil. (Marschner, 2011). Generally, potassium is present in soil in three primary forms: exchangeable form, absorbed on clay complex, and fixed in the lattice. The inoculation of PGPR may help transform the lattice's fixed or inaccessible potassium into an available form (Maurya *et al.*, 2014). On the other hand, HA enhanced the soil's structure, which led to a mineralization process. This process was responsible for the increased availability of nutrients in the soil and the regulation of nutrient cycling (Ampong *et al.*, 2022).

Table 5: Influence of HA, PGPR, and RDF alone or in combination with the contents of available N, P, and K in the soil at different stages during both the years of the experiment

Treatment	Available N content (Kg/ha)						Available P contents (Kg/ha)						Available K contents (Kg/ha)					
	45 DAS		75 DAS		After harvest		45 DAS		75 DAS		After harvest		45 DAS		75 DAS		After harvest	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
T₁	150.9	147.6	153.0	155.5	149.3	148.9	12.9	13.4	14.6	15.1	13.1	13.2	121.4	121.5	125.0	126.8	120.0	122.2
T₂	189.4	185.7	198.2	195.7	188.2	184.0	16.0	15.6	17.1	17.5	16.7	14.4	132.9	147.8	144.2	151.8	153.6	146.7
T₃	158.1	153.9	169.3	161.4	164.3	155.1	15.3	15.4	16.2	15.9	14.6	13.8	134.0	145.2	139.5	148.6	135.6	144.3
T₄	172.7	172.7	173.9	178.5	180.6	171.0	17.4	17.1	20.9	18.8	15.7	15.7	140.8	147.2	153.7	150.3	151.9	145.0
T₅	201.5	196.1	215.8	208.6	189.4	199.0	17.3	18.0	19.2	19.9	17.0	16.4	135.2	157.1	157.5	159.2	154.8	154.8
T₆	252.6	268.4	265.9	280.1	253.4	276.0	19.6	20.6	21.2	23.0	20.3	19.3	156.0	166.5	166.1	174.8	164.6	167.5
T₇	233.7	236.7	244.2	245.4	213.3	237.5	19.0	19.3	16.7	21.8	13.9	18.1	154.0	161.4	162.6	164.3	149.4	159.9
T₈	249.6	251.3	272.6	266.8	239.2	253.0	20.6	21.4	22.1	24.0	21.4	20.2	158.0	164.0	164.7	170.4	163.1	164.5
T₉	215.8	207.4	227.9	214.9	201.5	207.8	18.9	20.1	19.3	20.9	18.7	17.1	146.9	159.5	160.5	164.6	158.2	149.5
T₁₀	261.3	278.9	274.3	288.5	273.5	282.7	21.3	22.2	24.7	25.5	20.8	21.2	160.3	170.2	168.7	178.2	165.1	172.2
C.D.(P=0.05)**	14.3	25.1	20.3	27.2	18.1	34.2	1.5	1.5	2.2	4.6	2.1	3.1	12.5	13.3	12.4	12.7	11.0	13.5

** Critical difference at 5% level of significance, Treatment details: T₁- Control (only RDF), T₂– HA-soil application, T₃-HA -seed treatment, T₄ -PGPR -seed treatment, T₅- RDF, T₆-RDF + HA -soil application, T₇-RDF +HA -seed treatment, T₈- RDF +PGPR -Seed treatment, T₉- HA -soil application +PGPR -seed treatment, T₁₀- RDF + HA -soil application + PGPR -seed treatment.

5. Conclusion

Humic acid and PGPR enhance the soil's physical and chemical properties, which in turn helps improve soil's overall health. The findings of the current experiment determined that the combined application of HA, PGPR, and RDF (T₁₀-RDF+ HA-soil application +PGPR) was more successful than the application of each of these substances separately. Soil application of HA was found to be more effective in all the soil properties than seed treatment; also, it was found to be significantly superior to control (only RDF) in all the soil parameters. In the present scenario, fertilizers have become an essential part of agriculture to meet the demands of a growing population, which in turn leads to a constant reduction in soil fertility. The incorporation of organic materials in the soil gradually improved the quality of the soil over time. Both HA and PGPR are naturally formed; applying either to soil can lower the amount of fertilizer required, which can be a better approach to promote sustainability in agriculture. As an organic substance, humic acid has not been reported to negatively impact soil health and plant growth, according to the literature, but the overdose of humic acid can change the soil structure in the long run. A detailed future study is required to estimate the optimum dose and an appropriate application method for humic acid in different crops.

Conflict of Interest

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References

Among, K., Thilakaranthsa, M. S. and Gorim, L. Y. 2022. Understanding the role of humic acids on crop performance and soil health.

Baver, L. D. 1956. Soil physics. 3rd Ed. John Wiley and Sons, Inc., New York. 489 pp.

Bhardwaj, A., Kumar, S. and Singh, D. 2023. Tannery effluent treatment and its environmental impact: a review of current practices and emerging technologies. *Water Quality Research Journal*, 58(2), pp.128-152.

Bhattacharya, S. and Chandra, R. 2013. Effect of inoculation methods of *Mesorhizobium ciceri* and PGPR in chickpea (*Cicer arietinum* L.) on symbiotic traits, yields, nutrient uptake and soil properties. *Legum. Res. Int. J.*, 36(4): 331-337.

Bhatti, M.B., Rajpar, I., Hassan, Z.U., Shah, A.N. and Tunio, S.D. 2011. Humic acid improves growth, yield and oil content of *Brassica Compestris* L., *Pak. J. Agri.*, 27 (2): 125-133.

Billah, M., Khan, M., Bano, A., Nisa, S., Hussain, A., Dawar, K. M., and Khan, N. 2020. Rock phosphate-enriched compost in combination with Rhizobacteria; A cost-effective source for better soil health and wheat (*Triticum aestivum*) productivity. *Agronomy*, 10(9): 1390.

Bouajila, K., & Sanaa, M. 2011. Effects of organic amendments on soil physico-chemical and biological properties. *J. Mater. Environ. Sci*, 2(1), 485-490.

Bover, C.A. and Wilcox, L.A. 1965. Chemical and Microbiological properties *Agronomy Monograph 9*. ASA and SSSA, Madison, Wisconsin, USA. 433-451 p.

Brannon, C. A., & Sommers, L. E. 1985. Preparation and characterization of model humic polymers containing organic phosphorus. *Soil Biology and Biochemistry*, 17(2), 213-219.

Dasgupta, D., Ghati, A., Sarkar, A., Sengupta, C., & Paul, G. 2015. Application of plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of *Sesbania bispinosa* on the growth of chickpea (*Cicer arietinum* L.). *Int J Curr Microbiol App Sci*, 4(5), 1033-1042.

DÖNDER, E., & TOĞAY, Y. 2021. The effect of humic acid and potassium applications on the yield and yield components in chickpea (*Cicer arietinum* L.). *ISPEC Journal of Agricultural Sciences*, 5(3), 568-574.

Escobar, M. O., & Hue, N. V. 2008. Temporal changes of selected chemical properties in three manure–Amended soils of Hawaii. *Bioresource technology*, 99(18), 8649-8654.

Fahramand M., Moradi, H., Noori, M., Sobhkhizi, A., Adibian, M., Abdollahi, S., and Rigi, K. 2014. The influence of humic acid on increased yield of plants and soil properties.

Gumus, I. and Seker, C. 2015. Influence of humic acid applications on soil physicochemical properties. *Solid Earth*, 7 (3):2481–500.

Jackson, M.L. 1973. *Soil Chemical Analysis*. 1st edition. New Delhi, India. Prentice Hall of India Private Limited, 521p.

Johnston, A. E. 1986. Soil organic matter, effects on soils and crops. *Soil use and management*, 2(3), 97-105.

Khatana, R. N. S., Thomas, T., Barthwal, A. and Kumar, T. 2021. Effect of NPK levels and Rhizobium on soil physico-chemical properties, growth, yield and economics of summer black gram (*Vigna munga L.*) var. Shekhar-2. *Pharma Innov. J.*, 10(8): 1555-1561

Kumar, S. and Kumar, D. 2023. Biofiltration of Volatile organic compounds Using Chir Pine Cone Nuts Inoculated with *Pseudomonas putida*. *Ecological Questions*. 35, 2 (Oct. 2023), 23–32.

Kyei-Boahen, S., Slinkard, A.E. and Walley, F.L. 2002. Evaluation of Rhizobial Inoculation Methods for Chickpea. *Agron. J.*, 94: 851-859.

Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G., ...and Tan, D. 2019. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. *Scientific reports*, 9(1), 12014.

Marschner, H. (Ed.). 2011. *Marschners mineral nutrition of higher plants*. Academic press.

Maurya, B.R., Meena, V.S. and Meena, O.P. 2014. Influence of inceptisol and alfisol's Potassium Solubilizing Bacteria (KSB) isolates on release of K from waste mica. *Vegetos*, 27(1): 181-187.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. 1954. Estimation of Available phosphorus in soil by extraction with sodium bicarbonate U.S., Washington; D. C. Circular 9: 39-49.

Pastapure, V., Singh, D. and Kumar, S.2021. Effects of open dumping of municipal solid waste on surrounding soil characteristics: a review. In *Indian Geotechnical and Geoenvironmental Engineering Conference* (pp. 47-54). Singapore: Springer Nature Singapore.

Piper, C.S. 1950. Soil and Plant Analysis. The University of Adelaide Press, Adelaide, Australia, 368 pp.

Ranjan, S., Singh, D. and Kumar, S., 2021, November. Analysis of landfill leachate and contaminated groundwater: a review. In Indian Geotechnical and Geoenvironmental Engineering Conference (pp. 55-62).

Regelink, I.C., Stoof, C.R., Rousseva, S., Weng, L., Lair, G.J., Kram, P., Nikolaidis, N.P., Kercheva, M., Banwart, S. and Comans, R.N.J. 2015. Linkages between aggregate formation, porosity and soil chemical properties. *Geoderma*, 247: 24–37.

Sellamuthu, K.M., and Govindaswamy, M. 2003. Effect of fertilizer and humic acid on rhizosphere microorganisms and soil enzymes at an early stage of sugarcane growth. *Sugar Tech.*, 5(4): 273-277.

Sheoran, O.P. 2010. Online statistical analysis (OPSTAT) software developed by Chaudhary Charan Singh Haryana Agricultural University, Hisar, India.

Singh, J. S. 2013. Plant growth promoting rhizobacteria: potential microbes for sustainable agriculture. *Resonance*, 18(3): 275-281.

Subbiah, B.V. and Asija, G.L. 1956. A Rapid procedure for estimation of available nitrogen in rice soils. *Curr. Sci.* 25: 259-260.

Turgay O.C., Karaca A., Unver S. and Tamer N. 2011. Effects of coal- derived humic substance on some soil properties and bread wheat yield. *Communications in Soil Science and Plant Analysis* 42: 1050–70.

Verma, R., Maurya, B. R., Meena, V. S., Dotaniya, M. L., Deewan, P., and Jajoria, M. 2017. Enhancing production potential of cabbage and improves soil fertility status of Indo-Gangetic Plain through application of bio-organics and mineral fertilizer. *Int. J. Curr. Microbiol. App. Sci.*, 6(3): 301-309.

Walkley, A. and Black, C.A. 1934. An examination of Degtjareff method for determining soil organic and a proved modification of chromic acid titration method. *Soil Sci.*, 37: 29-38

Walley, F. L., Gillespie, A. W., Adetona, A. B., Germida, J. J., Farrell and R. E. 2014. Manipulation of rhizosphere organisms to enhance glomalin production and C sequestration: pitfalls and promises. *Canadian Journal of Plant Science*, 94(6), 1025–1032.

Y. Man-hong, Z. Lei, X. Sheng-tao, N. B. McLaughlin, and L. Jing-hui. 2020. Effect of water-soluble humic acid applied to potato foliage on plant growth, photosynthesis characteristics and fresh tuber yield under different water deficits, *Sci Rep*, vol. 10, no. 1, p. 7854.