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Improving the physio-chemical properties of soil by application of *Pseudomonas fluorescens*

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Abstract
The present work was aimed to evaluate the efficiency of *Pseudomonas fluorescens, Trichoderma viridae* and *Trichoderma harzianum* as plant growth promoting and biocontrol agents to enhance the physical and chemical properties of the sandy clay loam soil under the transplanted rice field. *Pseudomonas fluorescens, Trichoderma viridae* and *Trichoderma harzianum* enhanced growth through direct and indirect mechanisms by production of phytohormones, siderophores, and HCN, Nitrogen fixation and Phosphate solubilisation. The vermicompost based formulation was used for soil and seedling treatments.The application of vermi-based formulation of *Pseudomonas fluorescens in combination with Trichoderma viridae* and *Trichoderma harzianum* at the two different dosages (50 and 500 kg per ha) showed properties like pH, OC, NPK Sulphur and micronutrients content were significantly influenced compared to control. Thus *Pseudomonas fluorescens* has great potential to improve the soil fertility due to higher accumulation of nutrients and besides contributing to promotion of plants growth.

Keywords: *Pseudomonas fluorescens, Trichoderma viridae, Trichoderma harzianum*, rice crop, soil physio-chemical parameters

Introduction
The plant growth promoting rhizobacteria (PGPR) which can be used as biofertilizers for enhancing nutrient use efficiency. Moreover, these rhizobacteria play a vital role in change the organic matter decomposition dynamics and provided the available form of nutrients such as nitrogen (N), phosphorus (P), potassium (K) and other nutrients to the plants. (Duarah *et al*., 2011) [3]. The application biocontrol agent such as *P. fluorescens* separately or combination with organic manure and chemical fertilizer to significantly improved the macronutrients and micronutrients of soil (Ahamd *et al*., 2015) [2]. Zinc (Zn) is one of the essential micronutrients required for optimum plant growth. Goteti *et al*., (2013) [4] reported that zinc solubilizing bacteria has great potential enhanced total dry mass and uptake of N, P, K, Mn, and Zn into plants. Mohammadi Khosro, 2012) [7] described the soil bacterial communities, both ectorhizospheric strains of *Pseudomonas* and *Bacilli*, and endo-symbiotic rhizobia genera *Pseudomonas*, *Bacillus* and *Rhizobium* are have been ability to solubilizing phosphates. *Pseudomonas fluorescence* and *P. putida* are able to enhance P availability, by production of secondary metabolites viz. organic acids, phosphatase enzymes and siderophores. Similar studied showed that PGPR can also increase Fe solubility and hence uptake by plant (Jalili *et al*. 2009) [6]. Phosphate solubilizing microorganisms such as *fluorescent*, *Pseudomonas* enhance plant growth under poor phosphorus soil condition. (Pratibha Vyas and Arvind Gulati., 2009) [8].

Arbuscular mycorrhizal fungi (AFM) and *Pseudomonas fluorescens* has act as source of nutrients which is improve the biological, chemical and physical properties (macro- and micro-nutrients) of sandy soils of elements for better growth of plants. It is also inhibit pathogenic microorganisms and removal of heavy metals uptake by cowpea plants (Radwan S.M.A., 2004) [9].

The application direct or indirect of biofertilizer which composed by (namely, *Azotobacter*, *Azospirillum*, *Rhizobium* and *Pseudomonas*), farmyard manure (FYM) and mineral fertilizer (NPK) on wheat crop. A result showed improving plant growth, yield, anatomical structure and physiological activity of soil. (Agamy, R.A., *et al*, 2012) [1]

Materials and Methods
The experiment was carried out at rice field in sandy clay loam under the transplanted condition. Before sown of crop to well-prepared of land by using ploughing followed by puddling. Further, application of various vermi compost based formulations (*Pseudomonas fluorescens* and *Trichoderma harzianum*, *Trichoderma viride*) in soil and there used

control along with treated plants for comparison study. Rice seedlings were transplanted in each plot under randomized block design with three replications. The soil samples were collected from all the experimental plots than analysis of physical and chemical properties. The physical and chemical properties of soil analysed at Biotech Lab Training and Demonstration Centre, Collectorate campus Ambikapur.
Soil treated with *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Trichoderma viride* test physical and chemical properties of the soil.

Procedure of pH

- Take 100 ml beaker.
- Take 20 gm. of soil sample in the beaker.
- Add 50 ml of distil water (soil: water: 1: 2.5).
- Stir with a glass rod occasionally for about 30 minutes. Or, continuously shake the soil water suspension with a reciprocating mechanical shaker for 5 minutes.
- Connect the pH meter with electricity source. Allow it to warm up as per operating instructions of the model (usually about half an hour).
- Take a known standard buffer solution (say, pH 7.0) in a 50 ml beaker. Immerse the combine glass electrode (or both the electrode if provided), into the buffer solution. By rotating the knobe, place in the indicator noddle of the pH meter add the same pH reading as that of the buffer solution (i. e. pH 7.0).
- Remove the beaker containing buffer solution.
- Wash the electrode with a strong stream of distilled water from wash water.
- To check, repeat step no 6 taking another known standard buffer solution (say, pH 4.0) instead of the previous buffer solution (i.e. pH 7.0).
- Immerse the glass electrode of the pH meter in the soil-water suspension in the beaker. Take reading.
- Remove the beaker.
- Wash the glass electrode with a strong steam of distilled water from wash water.

Procedure of electrical conductivity

- Take a 100 ml beaker.
- Take 20 gm. of the soil sample in the beaker.
- Add 40 ml of distil water (soil: water ratio is 1:2).
- Stir with a glass rod occasionally for about 30 minutes. Or, continuously shake the suspension with a reciprocating mechanical shaker for 5 minutes.
- Let the suspension stand till the soil particles settle down.
- Use the supernatant liquid in the conductivity meter and cell. Take reading.

Procedure of organic carbon

- Take a 500 ml conical flask.
- Weigh out accurately 1 gm. of soil sample and place it in the flask.
- Add accurately 10 ml of $k_2Cr_2O_7$ solution with a 10 ml bulb type pipette. Gently rotate the flask to mix them (soil and $k_2Cr_2O_7$) thoroughly.
- Add about 20 ml of concentrated H_2SO_4 with a 25 ml measuring cylinder don't measure the acid with pipette. Gently rotate the flask for about 1 minute to mix. The entire soil particles must come in contact with $k_2Cr_2O_7$ and H_2SO_4.
- Let the flask stand for about 30 minutes. Carry on the same procedure without soil sample (blank titration) to find out the correct strength of ferrous iron solution at that movement.
- Add about 200 ml of distilled water with a measuring cylinder (500 ml).
- Add 10 ml of H_3PO_4 with a 10 ml measuring cylinder.
- Rotate the flask to mix them.
- Fill the burette with 0.5 N and ferrous iron solution upto 50 ml mark.
- Add 1.5 ml of diphenylamine and mix. Dull green colour will appear.
- Immediately after addition of diphenylamine start titration by addition of ferrous iron solution from burette with $k_2Cr_2O_7$ solution left over after oxidation of carbon in soil in the conical flask. First turbid blue colour appears. Then add ferrous iron solution drop by drop till a brilliant green colour appears indicating the end point of the titration.
- Carry on the same procedure without soil sample to find out the correct strength of ferrous iron solution at that movement.

Procedure of nitrogen

- Take sample weight as mentioned above and dilute to 250 ml by using distilled water in a standard flask.
- Take 25 ml of 0.1 N H_2SO_4 with indicator (methyl red) in the receiver end.
- Take 25 ml from that 250 ml diluted solution by using 25 ml pipette.
- Then add 2.5 gm. NaOH palates and fit the tube in the unit without mixing.
- Pit the processing (Receiver solution 150 ml).
- Titrate the distilled water solution against 0.1 N NaOH, end point is yellow.

Testing of P, K, Ca, Mg, Mn, Fe, Cu, Zn, and Mo

Procedure for Triacid Digestion

- Prepare a mixture a mixture of 750 ml conc. HNo3 Nitric acid + 300 ml of 60% HclO3 per chloric acid + 150 ml H_2SO_4 sulphuric acid.
- Take 10 ml of the acid mixture and add 0.2 gm. of sample. Place the solutions in low temperature initially and when frothing subsides increase the temperature to 150 C max to digest the sample.

Procedure for Diacid Digestion

Prepare a mixture of 100 ml of 60% HclO3 Perchloric acid + 500 ml conc. H_2SO_4 sulphuric acid. Take 10 ml of acid mixture and add 0.2 gm. of sample.
Place the solution in low temperature (100 C) for half an hour and when frothing subsides increase the temperature to 200 C Max to digest the sample.
Add 20-50 ml of distilled water and filter the solution through Whitman's filter paper no 40 into a 250 ml volumetric colourless solution.
Initially during digestion the solution will turn from clear to yellow colour and after digestion for nearly one and half hours will turn it to transparent clear solution. Now take the digested solution and add distilled water to make up the volume to 100 ml in that take 10 ml for distillation.

Result and Discussion

Data presented in table: 1and figure i.e. 1, 2 and 3 indicate that the *Pseudomonas fluorescens* applied used as soil treatment significantly influenced the physical and chemical properties (pH, EC, OC, NPK, S and micro nutrient) of soil.

Table 1: Effect of *Pseudomonas fluorescens* on physical and chemical properties of soil

Treatment details	pH	EC (ds/m)	OC (%)	N (kg ha^{1})	P (kg ha^{-1})	K (kg ha^{-1})	S (ppm)	B (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
T_1 Farmer practice NPK @ 80:60:40 kg ha^{-1}	6.45	0.66	0.22	205.12	13.23	260.54	12.21	0.56	2.61	0.84	15.85	3.11
T_2*P. fluorescens* seedling dip	6.05	0.26	0.61	235.21	11.32	259.52	18.34	0.30	1.25	0.42	19.54	2.65
T_3*P. fluorescens* soil application @50 kg ha^{-1}	6.26	0.26	0.81	284.54	12.52	313.51	19.62	0.35	2.17	0.44	20.92	2.47
T_4 *T. harzianum* + *P. fluorescens* soil application @50 kg ha^{-1}	6.52	0.32	0.54	242.45	15.54	259.34	14.75	0.35	2.24	0.42	14.85	2.87
T_5 *T. viride* + *P. fluorescens* soil application @50 kg ha^{-1}	6.39	0.34	0.68	246.43	14.54	221.55	13.32	0.35	2.33	0.71	20.45	3.12
T_6 *T. harzianum* + *P. fluorescens* soil application @500 kg ha^{-1}	6.27	0.56	0.35	219.67	14.51	228.34	20.12	0.38	1.72	0.52	17.52	3.45
T_7 *T. viride* + *P. fluorescens* soil application @500 kg ha^{-1}	6.51	0.41	0.73	265.32	13.32	292.52	17.32	0.37	1.95	0.43	17.21	2.22
T_8 Control	5.66	0.21	0.19	171.32	8.51	193.55	10.55	0.21	1.21	0.31	11.86	1.73
SEm(±)	0.12	0.03	0.08	16.90	1.08	22.55	1.99	0.04	0.17	0.03	1.57	0.32
CD 5%	0.38	0.09	0.24	51.28	3.30	68.40	6.05	0.13	0.51	0.11	4.77	0.99

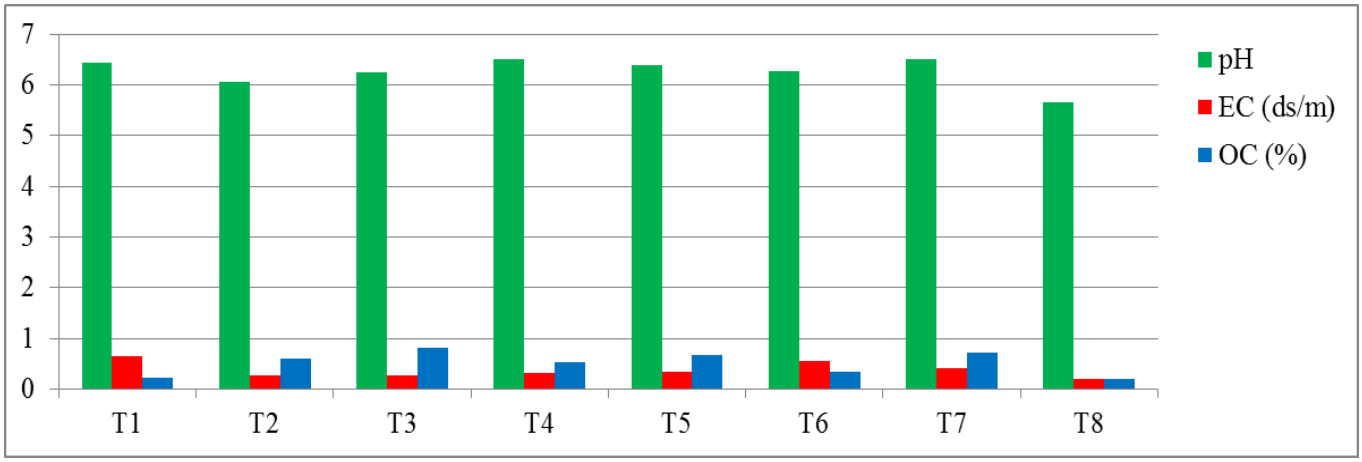

Fig 1: Effect of *Pseudomonas fluorescens* on physical properties of treated soil

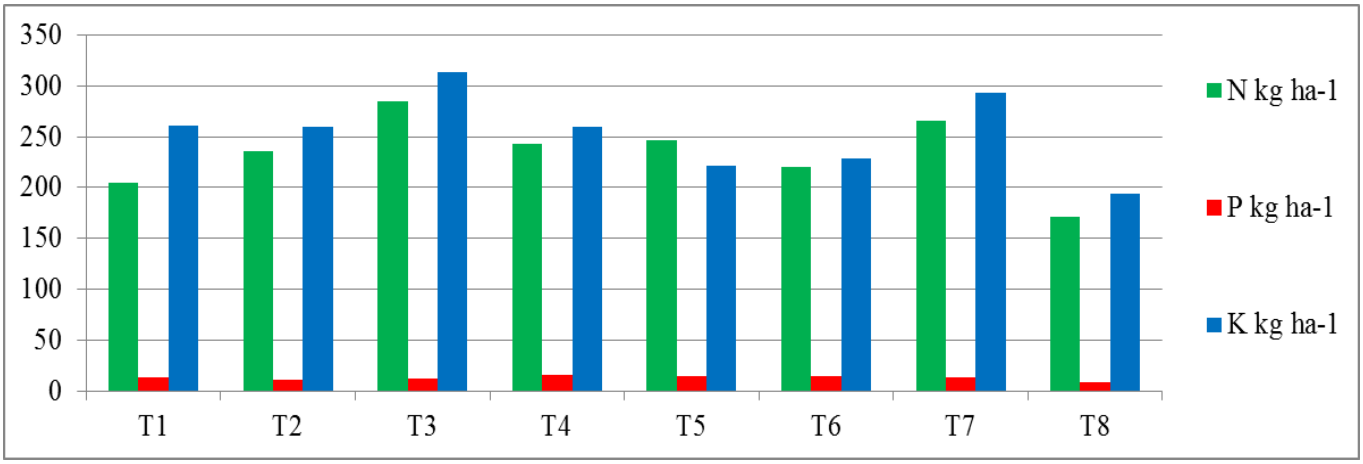

Fig 2: Effect of *Pseudomonas fluorescens* on chemical properties of soil

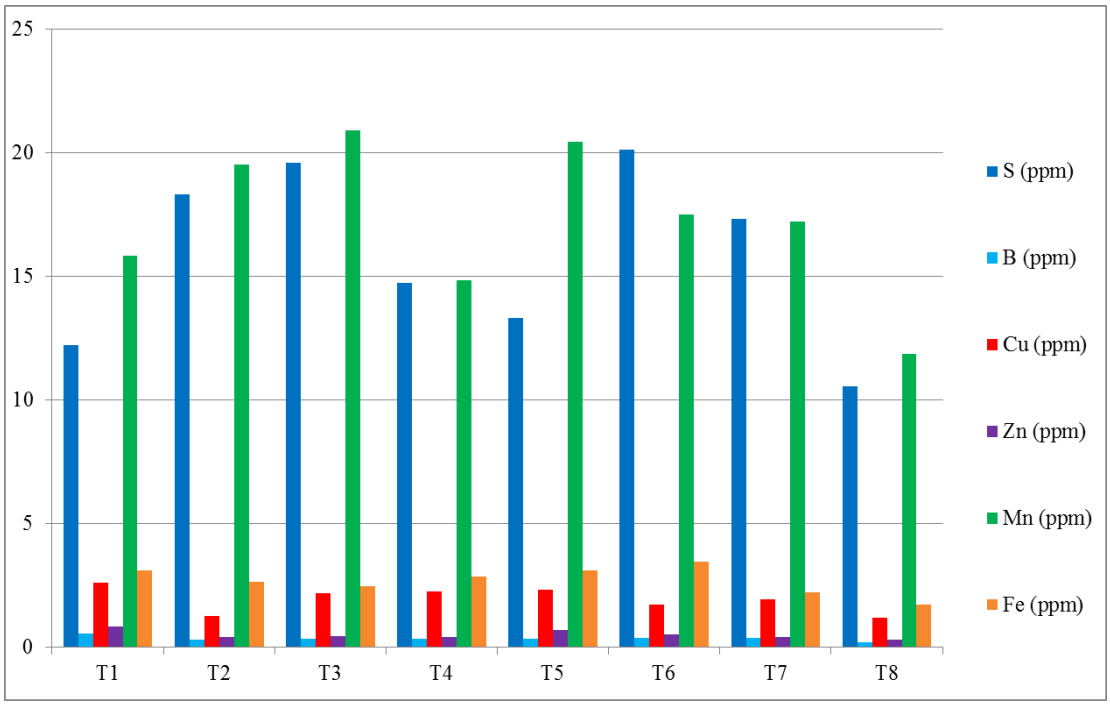

Fig 3: Effect of *Pseudomonas fluorescens* on chemical properties (micronutrients) of soil

Soil pH: The soil pH was ranged from 5.75 to 6.75. The highest pH (6.75) was recorded in T7 (pure vermi @ 40 kg ha^{-1}) followed by T2 (control 80:60:40@ kg ha^{-1}) and T10 (*Trichoderma viride* + *Pseudomonas fluorescens*, vermi @ 60 kg ha^{-1}), Showing acidic of soil pH, whereas, lowest pH (5.75) was recorded in control.

Electrical Conductivity: The electrical conductivity was ranged from 0.13 to 0.62 dSm^{-1}, the highest electrical conductivity (0.62dSm^{-1}) was recorded in T_7 (pure vermi @ 40 kg ha^{-1}) followed by T_6 (pure vermi @ 60 kg ha^{-1}) and T_4 (control 40:40:20 @ kg ha^{-1}), 0.56 and 0.55 dSm^{-1} electrical conductivity respectively, whereas, lowest electrical conductivity (0.13dSm^{-1}) was recorded in control.

Organic Carbon: The organic carbon was ranged from 0.24 to 0.78 %. The highest organic carbon (0.78%) was recorded in T_4 (control 40:40:20 @ kg ha^{-1}) followed by T_5 (pure vermi @ 80 kg ha^{-1}) and T_7 (pure vermi @40 kg ha^{-1}), 0.77 % and 0.72 % organic carbon respectively. The lowest organic carbon (0.24%) was recorded in control.

Nitrogen: The nitrogen content was ranged from 141.50 to 318.00 kg ha^{-1}. The highest nitrogen content (318.00 kgha^{-1}) was recorded in T_{11} (*T. harzianum* + *P. fluorescens*, @40 kg ha^{-1}) followed by T_8 (*P. fluorescens* vermi @ 60 kg ha^{-1}) and T_2 (Control 80:60:40 @ kg ha^{-1}), 302.50 kg ha^{-1}and 284.50 ha^{-1} kg nitrogen respectively, whereas lowest nitrogen (141.50 kg ha^{-1}) was recorded in control.

Phosphorus: The phosphorus content was ranged from 11.05 to 21.00 kg ha^{-1}. The maximum phosphorus content (21.00 kgha^{-1}) was recorded in T_6 (pure vermi @ 60 kg ha^{-1}) followed by T_{11} (*T. harzianum* + *P. fluorescens*, @40 kg ha^{-1}) and T_{10} (*T. viride* + *P. fluorescens*, vermi @ 60 kg ha^{-1}), 20.60 kg ha-1and 16.40 kg ha^{-1}phosphorus respectively, whereas minimum phosphorus content (11.05 kgha^{-1}) was recorded in control.

Potassium: The potassium content was ranged from 188.00 to 383.50kgha^{-1}. The highest potassium content (383.50 kgha^{-1}) was recorded in T_5 (pure vermi @ 80 kg ha^{-1}) followed by T_{12} (*T. viride* + *P. fluorescens*, vermi @ 40 kg ha^{-1}) and T_3 (Control 60:50:30 @ kg ha^{-1}), 326.00 kg ha^{-1}and 302.50 kg ha^{-1} potassium respectively, whereas lowest content of potassium (270.45 kg ha^{-1}) was recorded in control.

Sulphur: The sulphur content was ranged from 15.50 to 32.35 ppm. The highest sulphurcontent (32.35 ppm) was recorded in T_2 (Control 80:60:40 @ kg ha^{-1}) followed by T_6 (pure vermi @ 60 kg ha^{-1}) and T_7 (pure vermi @40 kg ha^{-1}), 32.20 and 23.25 ppm sulphur respectively, whereas lowest content of sulphur (15.50 ppm) was recorded in control.

Boron: The boron content was ranged from 0.18 to 0.45 ppm. The highest boron content (0.45ppm) was recorded in T_{12} (*T. viride*+*P. fluorescens*, vermi @ 40 kg ha^{-1}) followed by T_5 (pure vermi @ 80 kg ha^{-1}) and T_2 (Control 80:60:40 @ kg ha^{-1}), 0.41 and 0.39 ppm boron respectively, whereas lowest content of boron (0.18 ppm) was recorded in control.

Copper: The copper content was ranged from 1.61 to 2.71 ppm. The highest cupper content (2.71ppm) was recorded in T_7 (pure vermi @ 40 kg ha^{-1}) followed by T_8 (*P. fluorescens*, vermi @ 60 kg ha^{-1}) and T_5 (pure vermi @ 80 kg ha^{-1}), 2.50 and 2.39 ppm copper respectively, whereas lowest content of copper (1.61 ppm) was recorded in control.

Zinc: The zinc content was ranged from 0.17 to 0.73ppm. The maximum zinc content (0.73 ppm) was recorded from T_5 (pure vermi @ 80 kg ha^{-1}) followed by T_{10} (*T. viride*+*P. fluorescens*, vermi @ 60 kg ha^{-1}) and T_{11} (*T. harzianum* + *P. fluorescens* @ 40 kg ha^{-1}), 0.59 and 0.52 ppm zinc respectively, whereas lowest content of zinc (0.17ppm) was recorded in control.

Manganese: The manganese content was ranged from 11.90 to 19.80 ppm. The highest manganese content (19.80 ppm) was recorded from T_8 (*P. fluorescens*, vermi @ 60 kg ha^{-1})

followed by T_7 (pure vermi @ 40 kg ha^{-1}) and T_{10} (*T. viride* + *P. fluorescens*, vermi @ 60 kg ha^{-1}), 19.75 and 18.95 ppm manganese respectively, whereas lowest content of manganese (11.90 ppm) was recorded in control.

Iron: The iron content was ranged from 1.95 to 4.12 ppm. The highest iron content (4.12 ppm) was recorded fromT_3 (Control 60:50:30 @ kg ha^{-1}) followed by T_2 (Control 80:60:40@ kg ha^{-1}) and T_6 (pure vermi @ 60 kg ha^{-1}), 3.78 and 3.50 ppm iron respectively, whereas lowest content of iron (1.95 ppm) was recorded in control.

Present studied indicates that the soil amendment of vermicompost based formulation of *P. fluorescens* not only maintains neutral pH of soil but also influence the physical and chemical properties of soil and Its showed higher available content of NPK and micronutrients also increases the nitrogen fixation and phosphorus solubilizing by rhizo bacteria *P. fluorescens.*

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