

## EFFECT OF COMBINED USE OF VERMICOMPOST AND ORGANIC MANURE FORTIFIED WITH RHIZOBACTERIA CONSORTIA ON SOIL NUTRITION

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### ABSTRACT

The soil in Maharashtra state of India is majorly black, red and loamy soil. In the application part, improvement of such soil's nutrition is always demanded for better crop productivity. In the recent study, these soils were treated with vermicompost and additionally organic manure fortified with known and identified rhizobacteria consortium with an aim to ascertain the real change in nutrition value with the respective supplement addition. The observed results highlight that presence of organic manure along with vermicompost imparted many beneficial nutritional values to the soil irrespective of texture or type of the soil. Further, upon fortification of soil with bacterial consortium did not convey any beneficial value to the soil and hence in conclusion it is not recommended to use in combination with organic manure and vermicompost in the soil.

**Keywords:** Vermicompost, Organic manure, Bacterial consortium, Soil nutrition

### Introduction

Through ages, fertile soils have proved to an essential and efficient resource for humanity. Conversely, research still lags behind in defining the mechanisms of formation and refurbishing the fertility of soils (Furey and Tilman., 2021). Scientists are faced with a great challenge to have global crop productivity to feed the ever-increasing population. Amidst several management strategies, fertilization stands out as a conventional and promising strategy to increase crop yields (Cai et al., 2019). All agricultural practices for crop production utilizing synthetic fertilizer which are costly and hazardous in many senses. To maintain better soil fertility agriculturists, demand better nutrient balancing material especially of biological origin (Basak et al., 2020). To serve better nutrition supply- agriculture based learning focusing toward soil configuration, available nutrient type and what type of ecosystem required for growing particular plant has been reported (Babu et al., 2020). Different fertilizer types have varied effect on different crop yields defined by their respective mechanisms. Thus, high crop productivity is influenced by the requirement and understanding the impact of different fertilizer inputs as well as contents of soil on crop yields (Cai et al., 2019).

In a typical application- use of biologically origin farmyard manure, vermicompost in

different combinations preferred to get improved yield of plants (Babu et al., 2020). With an increasing food demand, soil has been treated with enormous quantities of chemical fertilizers that in return impart many ill effects to the ecosystem as well as to the human. Exogenous fertilization enhances soil fertility, via soil carbon, nutrients, and pH (Tian et al., 2016). Soil organic carbon (SOC) acquisition can be augmented by fertilization including addition of crop residues or the direct application of manure, indicative of high carbon additives (Cai et al., 2016). In a remedy part use of organic substitute has been demanded in great deal (Willer and Kilcher., 2011). The soil richness is now better improved by implementing organic farming that assures its better quality and fetches more price in market for crops (Yadav et al., 2013, Das et al., 2017). To replace organic fertilizers, the theme of organic farming is getting popularized with better safety, superior quality, and environment friendly produce (Das et al., 2017, Kumar et al., 2018). In a real sense organic farming imparts many positive impacts on environment quality and really is in demand with growing population concern (Boone et al., 2019, Abdelrahman et al., 2020)

Vermicomposts (VC) has been known to improve structure of the soil structure and soil microbial niche. These may add higher levels of activity and biological metabolites, such as plant growth regulators. Not much research has been diverted on the effects and/or

interactions of soil type and VC application (Zucco et al., 2015). Various reports show the nutrient profile in vermicompost exceeds the traditional compost. It is also reported to boost soil fertility physically, chemically and biologically (Lim et al., 2015).

The better use of organic farming also imparting several positive impacts towards human and animals and further improves soil structures, soil organic carbon, ecosystems services and nature (Seufert et al., 2012, Patel et al., 2015, Reganold et al., 2016). In a negative side of organic use, agronomists noted the 20-40% reduction in crop yield once only organic farming concept applied instead of chemical-based farming (Kremen and Miles, 2012). Vermicompost-treated soil depicts improved aeration, porosity, bulk density and water retention ability while pH, electrical conductivity and organic matter content are also enhanced, expected to contribute for better crop yield (Missellbrok et al., 2012). However, it's not yet scientifically correlated with respect to the nutrient content improvement of the soil and plant growth, thus shedding light on other influencing factors for soil enrichment (Lim et al., 2015).

In the present study combined use of organic manure with vermicompost has been put forward once soil treated with it. Further the soil also fortified with rhizobacteria consortium in the presence of vermicompost and organic manure to ascertain the real effect of treatment on change in nutritional level of black, red and loamy soil of Maharashtra, India.

## Materials and Methods

### Soil collection

In the country of India, the state of Maharashtra represents different soil textures such as Black (B), Red (R) and Loamy (L). Red soil was acquired from Mahabaleshwar, Loamy from Bhandara and Black from Nagpur district. These soil samples were collected during the winter period and tested in the laboratory as per the protocol.

### Isolation of Rhizobium from the soil

The prominent presence of Rhizobacteria confirmed in Red, Black and Loamy soil of Maharashtra. In a process, by using Rhizobium agar and by Azotobacter agar (Hi-Media) a number of prominently growing Rhizobium

species and Azotobacter species sampled successfully. The isolates are then targeted for 16S rRNA gene sequence using universal primer mentioned in protocol (Rai et al., 2013).

### 16S rRNA gene sequencing

The presumptive identified Rhizobium and Azotobacter species processed for genomic DNA isolation, 16S rRNA primer-based PCR amplification and finally amplicon successfully sequenced for both strands up to 1500 base pairs using Sanger Sequencing. The 1500 base pair sequence is then pairwise aligned with a 16S rRNA specific database using the BLASTN program. The BLASTN based identity is used to identify genus and species level information. The best five homologs obtained from BLASTN then aligned by multiple sequence alignment program (CLUSTALW) to generate phylogram. In the last phase obtained 16S rRNA gene sequence submitted to NCBI database for the accession number (Rai et al., 2013).

### Preparation of Vermicompost plus organic manure soil with/without consortium of Rhizobacteria

In the present study the real effect of combined use of vermicompost plus organic manure with 4% and 8% consortium of soil isolated rhizobacteria tested under laboratory conditions has been put forward.

In a set of protocols- tested with red, black and loamy soil once inoculated with 1:1 ratio of sharing 34% or 25% of soil as vermicompost plus organic manure used as control. While in the second set, similar preparation was additionally fortified with 4% or 8% consortium prepared by the four isolates previously identified upto genus and species level once added in equal percentage.

### Incubation Period of treated soil

In the study red, black and loamy soil once treated with organic manure, vermicompost and with consortium, all soil sets were intermittently kept humid and tested upon 10 days of incubation for N, P, K content change.

### Soil Testing

The comparative change in soil upon exposure to the vermicompost, organic manure and with/without rhizobacteria consortium tested using soil testing kit (Hi-media K054). The

parameters tested in the study are pH, phosphate (kg per hectare), organic carbon (% oxidizable organic carbon), potassium (kg/ha), nitrogen (kg per hectare).

**Result**

**Soil collection**

The soils of Maharashtra available as red, black and loamy represented (figure-1) and studied further.

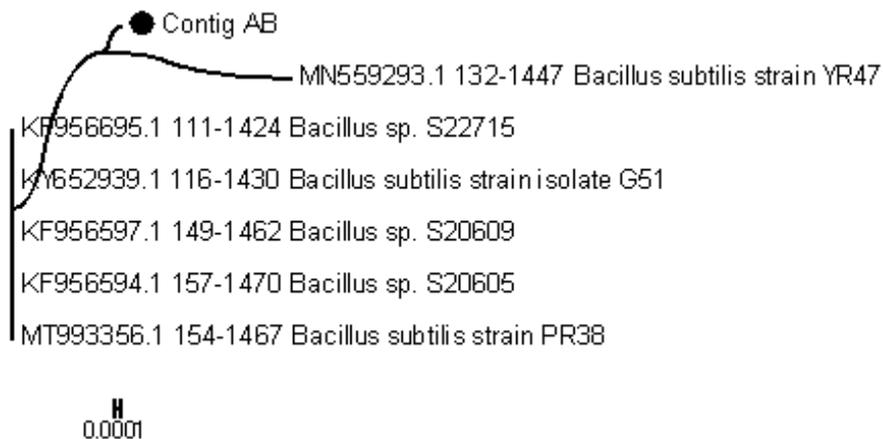


**Fig. 1.** Soil colour and texture as red, Black and Loamy sample from Maharashtra state

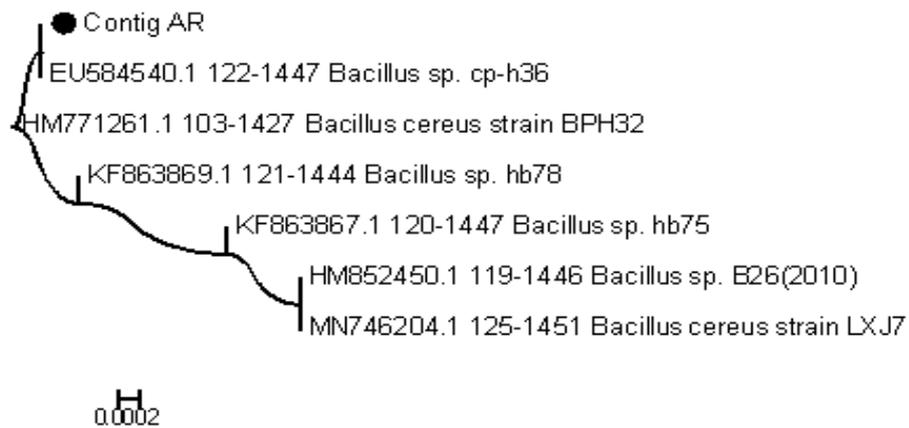
**16S rRNA gene sequencing**

The four bacterial species confirmed as rhizobacteria isolated from red and black soil identified as, *Bacillus subtilis* strain AB with

1317 nucleotide sequence of 16S rRNA having assigned accession number MW548665.1 (figure-2).

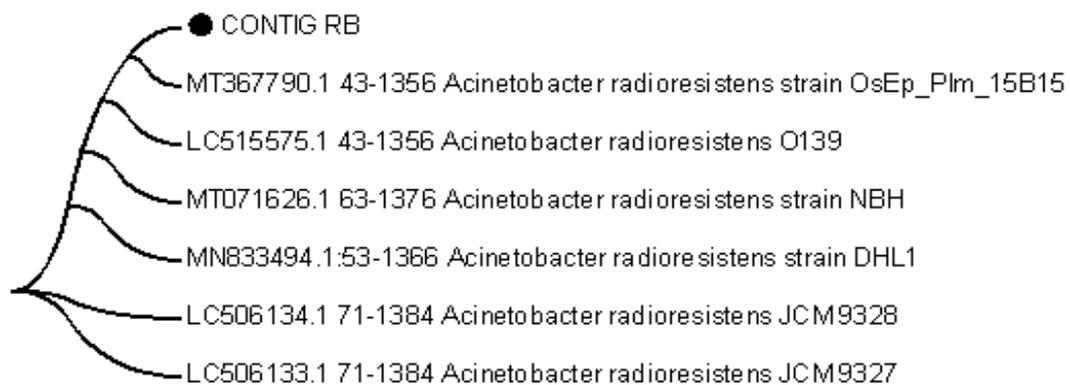


**Fig. 2.** Isolate AB identified as *Bacillus subtilis* as per 16S rRNA gene sequence alignment. Isolate *Bacillus cereus* identified with 1327 nucleotide 16S rRNA gene sequence with assigned accession number HM771261.1 (figure-3).



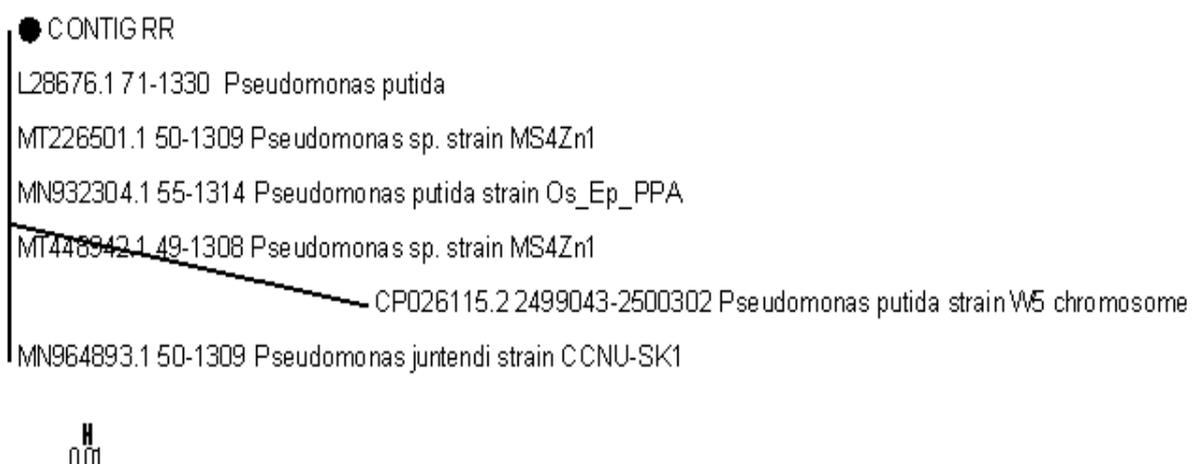
**Fig. 3.** Isolate AR identified as *Bacillus cereus* as per 16S rRNA gene sequence alignment.

Isolate *Acinetobacter radioresistens* strain RB identified successfully with 1314 nucleotides of 16S rRNA gene with assigned accession number MW548666.1 (figure-4).



**Fig. 4.** Isolate RB identified as *Acinetobacter radioresistens* as per 16S rRNA gene sequence alignment.

Isolate *Pseudomonas putida* identified with 1260 nucleotides of 16S rRNA gene sequence with assigned accession number L28676.1 (figure-5).



**Fig. 5.** Isolate RR identified as *Pseudomonas putida* as per 16S rRNA gene sequence alignment

**Comparative nutrient level change in vermicompost and nutrient manure enriched soil fortified with rhizobacteria consortium and in absence of rhizobacteria consortium  
pH of the soil**

As per the pH analysis test, red soil normal without any fortification noted 8.40 for pH. Soil becomes acidic with addition of 25% and 34% vermicompost plus organic manure. With the further addition of 4% and 8% consortium pH shifted once again towards control soil values ranging between 7.90-8.15 pH.

In case of black soil, control soil pH (without any fortification) remains at 7.55 pH. Addition of vermicompost plus organic manure made soil pH acidic (6.99-7.16) and further addition of 4% and 8% consortium pH shifted towards alkalinity ranging from 8.01-8.96 pH.

In case of loamy soil, the original soil pH noted at neutrality at 7.45. Treatment of soil with 25% and 34% organic manure plus vermicompost, pH shifted towards alkalinity (8.09-8.22). Addition of 4% and 8% consortium further shifted the pH towards the higher alkaline side (8.99-9.48) are listed in table-1.

**Table 1.** pH of the red, black and loamy soil

Samples	pH chart reading	pH meter reading	Samples	pH chart reading	pH meter reading	Samples	pH chart reading	pH meter reading
Red soil Control	9.5	8.40	Black Soil Control	7.5	7.55	Loamy soil Control	7.0	7.45
RII A	9.0	7.8	BII A	8.5	6.99	LII A	9.0	8.22
RII B	9.0	7.91	BII B	8.5	7.16	LII B	9.0	8.07
RII Ai	8.5	7.90	BII Ai	9.0	8.32	LII Ai	9.5-9.0	8.99
RII Aii	8.5	8.12	BII Aii	9.0	8.96	LII Aii	9.5-9.0	9.12
RII Bi	8.5	8.15	BII Bi	9.0	8.01	LII Bi	9.5-9.0	9.24
RII Bii	8.5	7.23	BII Bii	9.0	8.45	LII Bii	9.5-9.0	9.48

A: 12.5% vermicompost plus 12.5% organic manure ; B: 17% vermicompost plus 17 % organic manure; Ai: 12.5% vermicompost plus 12.5% organic manure plus 4% consortium; Aii: same like Ai but fortified with 8% consortium ; Bi and Bii are similar to Ai and Aii

**Organic carbon**

In an original red soil, % oxidizable organic carbon noted as 0.505-0.750. It has not improved with addition of vermicompost plus organic manure (25% or 34%) even with an addition of consortium (4% or 8%).

In case of black soil % oxidizable organic carbon noted high 1.00-1.50% but with the addition of 25% of vermicompost plus manure

and fortification with consortium it reduced down organic carbon.

In a loamy soil, original % oxidizable organic carbon noted as 0.750-1.00% as medium high. The boost has been received when 25% and 34% vermicompost plus organic manure given as an inoculum with 1.00-1.50% value. Thereafter addition of % consortium (4% or 8%) brought back organic carbon level to normalcy indicating organisms utilized % oxidizable organic carbon as shown in table-2.

**Table 2.** Available organic carbon in red, black and loamy soil

Samples	Range (% oxidizable organic carbon)	Samples	Range (% oxidizable organic carbon)	Samples	Range (% oxidizable organic carbon)
Red soil Control	0.505-0.750	Black Soil Control	1.00-1.50	Loamy soil Control	0.750-1.00
RII A	0.505-0.750	BII A	0.505-0.750	LII A	1.00-1.50
RII B	0.750-1.00	BII B	1.00-1.50	LII B	1.00-1.50
RII Ai	0.505-0.750	BII Ai	0.300-0.500	LII Ai	0.300-0.500
RII Aii	0.505-0.750	BII Aii	0.300-0.500	LII Aii	0.300-0.500
RII Bi	0.505-0.750	BII Bi	0.300-0.500	LII Bi	0.300-0.500
RII Bii	0.505-0.750	BII Bii	0.300-0.500	LII Bii	0.300-0.500

### Phosphate

In a normal Red soil phosphate noted to be 22 to 56 kg per hectare which has not improved in any of the treatment groups and remained constant throughout treatment.

In case of normal Black soil phosphate level remained 22 to 56 kg per hectare. In fortified

groups no significant change was recorded and so the treatment showed neutral effect.

Loamy soil in normal state for phosphate level recorded 22 to 56 kg per hectare and even after treatment no significant increase was recorded in table-3.

**Table 3.** Available phosphate in red, black and loamy soil

Samples	Range (Kg. per hectare)	Samples	Range (Kg. per hectare)	Samples	Range (Kg. per hectare)
Red soil Control	22 to 56	Black Soil Control	22 to 56	Loamy soil Control	22 to 56
RII A	22 to 56	BII A	22 to 56	LII A	22 to 56
RII B	22 to 56	BII B	22 to 56	LII B	22 to 56
RII Ai	22 to 56	BII Ai	22 to 56	LII Ai	22 to 56
RII Aii	22 to 56	BII Aii	22 to 56	LII Aii	22 to 56
RII Bi	22 to 56	BII Bi	22 to 56	LII Bi	22 to 56
RII Bii	22 to 56	BII Bii	22 to 56	LII Bii	22 to 56

### Potassium

Red normal soil noted with 112 kg/hectare potassium level which has not improved upon treatment with vermicompost plus organic manure and even after the fortification of consortium.

In case of black normal soil, level of potassium recorded to be around 392 kg/hectare which was dramatically reduced to 112 kg/ha in case of addition of only vermicompost plus organic

manure but once fortified with 4% and 8% consortium an improvement has been noted which shifted the potassium level to 112 to 280 kg/hectare.

The potassium level in normal loamy soil is noted to be medium (112 to 280 kg/ha) which remains unchanged even after fortification by vermicompost plus organic and with consortium addition also shown in table-4.

**Table 4.** Available potassium in red, black and loamy soil

Samples	Range (kg/ha)	Samples	Range (kg/ha)	Samples	Range (kg/ha)
Red soil Control	Below 112	Black Soil Control	Above 392	Loamy soil Control	112 to 280
RII A	Below 112	BII A	Below 112	LII A	112 to 280
RII B	Below 112	BII B	Below 112	LII B	112 to 280
RII Ai	Below 112	BII Ai	112 to 280	LII Ai	112 to 280
RII Aii	Below 112	BII Aii	Below 112	LII Aii	112 to 280
RII Bi	Below 112	BII Bi	112 to 280	LII Bi	112 to 280
RII Bii	Below 112	BII Bii	112 to 280	LII Bii	112 to 280

### Ammoniacal nitrogen

Level of ammoniacal nitrogen in red soil noted to be on the lower side 15 kg per hectare which has not improved further by fortification of vermicompost plus organic manure and also by addition of consortium.

In a black soil low level ammoniacal nitrogen noted with 15 kg/hectare which did not improve upon addition of fortified material.

Loamy soil showed slightly high ammoniacal nitrogen levels to 73 kg per hectare which did not improve upon addition of vermicompost plus organic manure and even once supplemented with bacterial consortium showcased in table-5.

**Table 5.** Available Ammoniacal Nitrogen in red, black and loamy soil

Samples	Range (Kg. per hectare)	Samples	Range (Kg. per hectare)	Samples	Range (Kg. per hectare)
Red soil Control	About 15	Black Soil Control	About 15	Loamy soil Control	About 73
RII A	About 15	BII A	About 15	LII A	About 73
RII B	About 15	BII B	About 15	LII B	About 73
RII Ai	About 15	BII Ai	About 15	LII Ai	About 73
RII Aii	About 15	BII Aii	About 15	LII Aii	About 73
RII Bi	About 15	BII Bi	About 15	LII Bi	About 73
RII Bii	About 15	BII Bii	About 15	LII Bii	About 73

### Nitrate Nitrogen

In a red soil level of nitrate nitrogen noted on the lower side which has not improved upon addition of vermicompost plus organic manure and even after addition of bacterial consortium. The black soil also reported a low level of nitrate nitrogen i.e., 15 kg/hectare. The situation remained unchanged even upon addition of vermicompost plus organic manure

and even when fortified with bacterial consortium except once when 25% of vermicompost plus organic manure was added. Lastly, Loamy soil noted with slightly higher nitrate nitrogen 20 kg/hectare which remains unaffected upon addition of vermicompost plus organic manure and even when fortified with bacterial consortium listed in table-6.

**Table 6.** Available Nitrate nitrogen in red, black and loamy soil

Samples	Range (kg/ha)	Samples	Range (kg/ha)	Samples	Range (kg/ha)
Red soil Control	About 04	Black Soil Control	About 04	Loamy soil Control	About 20
RII A	About 04	BII A	About 20	LII A	About 20
RII B	About 04	BII B	About 10	LII B	About 20
RII Ai	About 04	BII Ai	About 20	LII Ai	About 20
RII Aii	About 04	BII Aii	About 20	LII Aii	About 20
RII Bi	About 04	BII Bi	About 20	LII Bi	About 20
RII Bii	About 04	BII Bii	About 20	LII Bii	About 20

### Discussion

Vermicomposts (VC) are finely divided, peat-like materials resulting from a non-thermophilic biodegradation of organic materials via interactions of earthworms and microorganisms. These are with high porosity, aeration, drainage, water-holding capacity, and microbial activity (Edwards 2004; Pandya et al. 2014). Vermicompost are known to enhance nutrient mineralization, and rapidly convert the wastes into a humus-like substance that has a finer structure than ordinary composts while possessing greater and more diverse microbial populations. (Zucco et al., 2015)

Addition of vermicompost plus organic manure shifts the soil pH towards acidity while in case of addition of bacterial consortium along with vermicompost plus organic manure transfers pH towards alkalinity. In present study, addition of 25% vermicompost plus organic

manure improved organic carbon, in red soil. Further addition of 4% or 8% consortium did not improve any nutritional level parameter. In case of black soil, better improvement in nitrate nitrogen recorded with the addition of 25% vermicompost plus organic manure, addition of 4% or even 8% bacterial consortium failed to impart any nutritional value.

Previous findings suggest organic carbon improves soil quality by enhancing the input use-efficiency consequences in favorable and sustainable crop yield and environmental safety (Hammad et al., 2020). Two important nutrients, Nitrogen (N) and phosphorus (P) are vital for plant growth as well as key factor to improve crop yield (Liu et al., 2020). Nitrogen aids in photosynthetic efficiency of the leaves and flower bud differentiation, quality formation of crops, improve fruit setting rate, and increase in yield (Xu et al., 2020).

Potassium (K) is important as it assures optimal plant growth and also is an activator of various important enzymes, like those involved in protein synthesis, sugar transport, Carbon metabolism, and photosynthesis. Furthermore it's also involved in yield and quality improvement (Marschner, 2012; Oosterhuis et al., 2014).

In case of loamy soil, improvement in organic carbon recorded once loaded with 25% and 34% organic manure plus vermicompost. Here once again addition of bacterial consortium along with vermicompost plus organic manure failed to showcase any impact. Hence it could be suggested for the present study that one should not fortify soil with organic manure, vermicompost and rhizobacteria consortium rather only use of vermicompost plus organic manure is advisable.

In similar reports, soil organic dynamics are noted to improve once plants are grown in rotation, once fortified with vermicompost along with organic fertilizers (Sarma et al., 2018). In a plant green gram defined improvement in carbon, nitrogen, phosphate and potassium recorded once soil fortified with 75% and 50% concentration of vermicompost (Rajkhowa et al., 2003). One of the reasons for better activity reported with vermicompost noted with its better water holding capacity,

increased organic content, and cation exchange capacity tested on different soil conditions (Parthasarathi et al., 2008). The role of organic manure has already been mentioned earlier in being able to improve potassium uptake once investigated with yellow poplar<sup>17</sup>, maize<sup>18</sup>, and lentil plants (Han et al., 2016, Kandil et al., 2020, Zeidan., 2007). Ultimately, similar to the present study, combined use of organic manure, green manure along with fertilizer noted to be catalysts for N, P and K level in pearl millet (Tolanur and Badanur, 2003).

In the summary, it has been put forward that fortification of soil with rhizobacteria consortia once soil already added with vermicompost plus organic manure do not improve its soil nutrition level further. Hence farmers should not add all three in-together in any soil.

### Conclusion

Study put forward an improvement of soil nutrition quality once added with vermicompost and organic manure but noted that further fortification of enriched soil with rhizobacteria consortia did not improve nutrition level of soil. Hence addition of rhizobacteria consortia along with vermicompost and organic manure not advised in the soils of Maharashtra studied as red, black and loamy.

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