IMPACT OF VERMICOMPOST, ORGANIC MANURE, AND *RHIZOBACTERIA* FORTIFICATION ON NUTRIENT QUALITY OF SOILS FROM MAHARASHTRA, INDIA

R.S. HARKAL[#], A.V. MANWAR

Department of Microbiology, DSM College, Parbhani Dist. Parbhani M.H., India,
[#]e-mail: ramprasadharkal.85@gmail.com

Abstract. Soils in Maharashtra presents variety for their color, texture, and nutrient balance. Hence it is important to learn about region cultivated land configuration since it assures the nutrient supply prerequisite for quality crop production. Maharashtra soils represent the red, black, and loamy texture, and resultant nutrition level change was investigated when those fortified with *rhizobacteria*, organic manure, and vermicompost. As per the findings, all soils exhibited different responses for change in nutrient level once fortified with said treatment and majorly positive impact noted with the organic manure and vermicompost compared to *Rhizobacteria* inoculation once prepared from *Bacillus subtilis, Bacillus cereus, Acinetobacter radioresistens,* and *Pseudomonas putida.* In a summary, it could be advisable to inoculate organic manure and vermicompost in Maharashtra red, black, and loamy soils to improve its nutrient content *via* an organic farming approach.

Key words: organic farming, *Rhizobacteria*, vermicompost, soil type.

INTRODUCTION

In the present scenario, heavy use of costly synthetic fertilizers put forward many hazardous effects on soil nutrient balance and demanding an alternative nutrient formulation mainly of biological sources [3]. In recent times, soil-based studies put forward the need to understand land configuration, available ecosystem, type of nutrient supply given, and kind of plantation done so that the best crop yield could be achieved [2]. The common use of farmyard manure, vermicompost in a combination preferred to use in different ratios that assure improved plants yield (e.g., corn) [2]. Across the world, public awareness has increased about the irrational use of synthetic agrochemicals that can bring about many ill effects in humans, and resultant demand has increased for their organic substitutes which will match the growing food demand across the globe and remain safe [18]. The soil rich in organic nutrient are widely accepted by the farmers and fetch a better price in the market for

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the derived crops [5, 19]. Soil nurtured with organic farming assures produce with superior quality, safe to consume, and environmentally friendly than any other synthetic chemical-based farming [5, 9]. Organic farming assures many positive impacts towards environmental safety [1, 3]; human and animals' development [16]; and resultant able to improve soil texture [15]; soil organic carbon [11], ecosystems, and nature [13]. However, it has also been reported that only the use of organic farming reduces yield by 20–40 % as compared to chemical-based farming and hence demands the proper balance [8]. As per the detailed study, the magnitude of yield reduction with organic farming also depends on the ecosystem, crop cultivated, soil conditions, and management practices [16].

Considering all these factors present study investigated the effects of organic manure, vermicompost, and *Rhizobacteria* in combinations as well as a sole effector to improve soil nutrient content when investigated with red, black, and loamy textured soil of Maharashtra, India.

MATERIALS AND METHODS

SOIL COLLECTION

In the study, the country India having the central state Maharashtra considered for the soil sampling available for red, black, and loamy type. Red soil was collected from the Mahabaleshwar city, black from Nagpur city, and loamy soil from Gondia city. The soils were collected from a one-foot depth from the surface level and 1–2 kg of those were collected from the sampling sites. Soils were brought to the laboratory and tested further. The soil was allowed to dry for five days, and ground to a fine powder; it was then sieved through muslin cloth and packed in a glass bottle till further use.

ISOLATION OF *RHIZOBACTERIA* FROM THE SOIL

The soil samples selected for the isolation of *Rhizobium* and *Azotobacter* species were red, black, and loamy soils. For isolation of these species, general-purpose media (NA) and special-purpose media (nitrogen-free mannitol agar) were used. Each soil sample was then processed and diluted serially. For the preparation of serial dilution, six test tubes were loaded with 10 mL of sterile distilled water and lid closed with cotton plugs. First tube was inoculated with one gram of soil and mixed well. One mL of supernatant from this soil suspension was then loaded in second dilution tube. Thereafter, similar procedure of serial dilution was carried out till fifth test tube. All the dilutions were immediately used for inoculation.

To inoculate on media, 100 µL of each sample was placed on the plate. Plates were then incubated at 28 °C for five days to record the typical growth of the isolates. These isolates were then sub-cultured on nutrient agar and the obtained pure cultures were identified by 16S rRNA gene sequencing.

16S rRNA GENE SEQUENCING

The bacterial species preliminary identified as *Rhizobium* species and *Azotobacter* species were processed for genomic DNA and targeted by the 16S rRNA universal primers in polymerase chain reaction. The bacterial 16S rRNA gene amplicon obtained via the polymerase chain reaction (PCR) methodology was then successfully sequenced. Using Basic Local Alignment Search Tool Nucleotide (BLASTN) software pair-wise alignment was carried. Simultaneously, phylogenetic relationship was also studied. The obtained information of bacterial species was submitted to National Center for Biotechnology Information (NCBI) via a sequin program to obtain an accession number.

PREPARATION OF SOIL MIXTURE WITH VERMICOMPOST, ORGANIC MANURE, AND BACTERIAL CONSORTIUM

In the study real effect of vermicompost, organic manure, and the bacterial consortium on the nutrient level of soils was assessed at variable individual concentrations. The sets are prepared as indicated in Table 1.

Table 1

Composition and codes of the sets

Codes for the sets
Soil samples: R = red soil, B = black soil, L = loamy soil
RI A: 75 % soil + 25 % vermicompost
RI B: 66 % soil + 34 % vermicompost
BI A: 75 % soil + 25 % vermicompost
BI B: 66 % soil + 34 % vermicompost
LI A: 75 % soil + 25 % vermicompost
LI B: 66 % soil + 34 % vermicompost
RIII A: 75 % soil + 25 % organic manure
RIII B: 66 % soil + 34 % organic manure
RII Ai: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 4 % consortium

RII Aii: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 8 % consortium
RII Bi: 66 % soil + 34 % (17 % vermicompost+17 % organic manure) + 4 % consortium
RII Bii: 66 % soil + 34 % (17 % vermicompost+17 % organic manure) + 8 % consortium
BIII A: 75 % soil + 25 % organic manure
BIII B: 66 % soil + 34 % organic manure
BII Ai: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 4 % consortium
BII Aii: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 8 % consortium
BII Bi: 66 % soil + 34 % (17 % vermicompost+17 % organic manure) + 4 % consortium
BII Bii: 66 % soil + 34 % (17 % vermicompost+17 % organic manure) + 8 % consortium
LIII A: 75 % soil + 25 % organic manure
LIII B: 66 % soil + 34 % organic manure
LII Ai: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 4 % consortium
LII Aii: 75 % soil + 25 % (12.5 % vermicompost+12.5 % organic manure) + 8 % consortium
LII Bi: 66 % soil + 34 % (17 % vermicompost+17 % organic manure)
LII Bii: 66 % soil + 34 % (17 % vermicompost+17 % organic manure) + 8 % consortium

SOIL TESTING

All the soil sets were prepared and air-dried for ten days. All soil samples were tested by the standard kit of Hi-media K054 for the parameters such as pH, organic carbon (% oxidizable organic carbon), phosphate (kg/ha), potassium (kg/ha), ammoniacal nitrogen (kg/ha), nitrate and nitrogen (kg/ha).

RESULTS

COLLECTION OF SOIL

The soils were collected successfully from the said regions of Maharashtra state, India as shown in Fig. 1.

Fig. 1. Collected red, black, and loamy soils from Maharashtra state (left to right).

IDENTIFICATION OF *RHIZOBACTERIA* AND *AZOTOBACTERIA*

In the present study two bacterial species, each from red and black soil were identified as plant growth promoting *Rhizobacteria*. The isolate AB was identified as *Bacillus subtilis* by matching with 1317 nucleotide sequences of 16S rRNA (Fig. 2). The isolate AR was identified as *Bacillus cereus* by matching with 1327 nucleotide sequences of 16S rRNA (Fig. 3). The isolate RB was identified as *Acinetobacter radioresistens* by matching with 1314 nucleotide sequences (Fig. 4). The isolate RR was identified as *Pseudomonas putida* with 1260 nucleotide sequences of 16S rRNA (Fig. 5). The accession number was received for these isolates as AB (MW548665.1), AR (MW548666.1), RB (MW548667.1), and RR (MW548668.1).

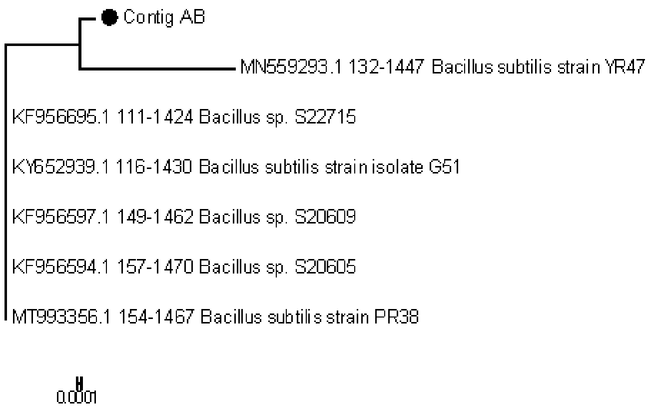

Fig. 2. Isolate AB was identified as *Bacillus subtilis* as per 16S rRNA gene sequence alignment.

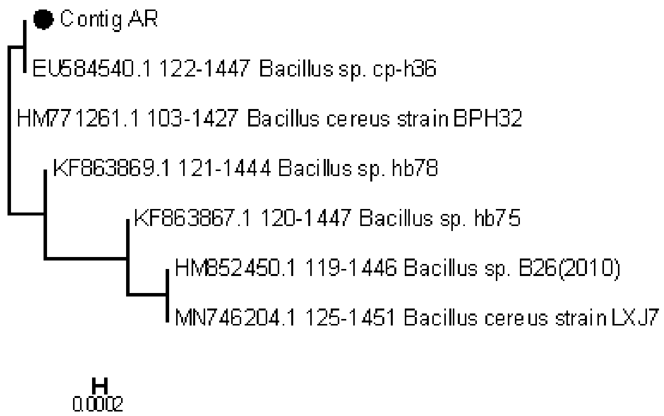

Fig. 3. Isolate AR identified as *Bacillus cereus* as per 16S rRNA gene sequence alignment.

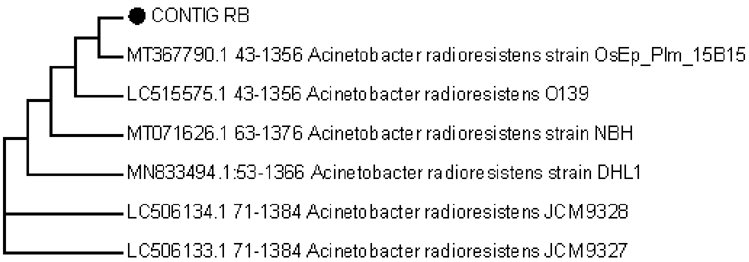

Fig. 4. Isolate RB was identified as *Acinetobacter radioresistens* as per 16S rRNA gene sequence alignment.

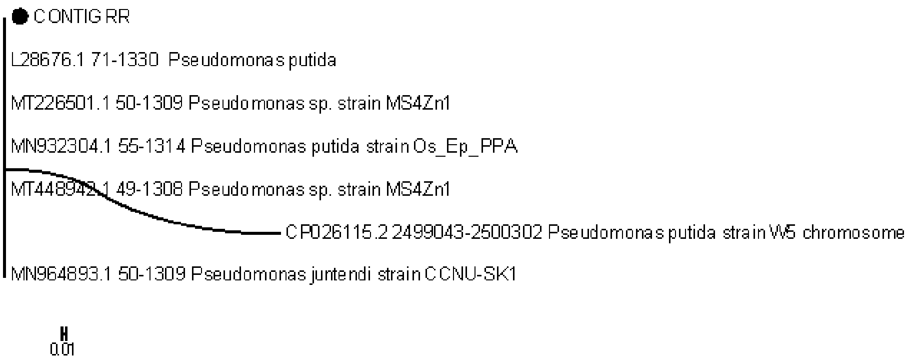

Fig. 5. Isolate RR identified as *Pseudomonas putida* as per 16S rRNA gene sequence alignment.

EFFECT OF VERMICOMPOST, ORGANIC MANURE AND BACTERIAL CONSORTIUM ON SOIL NUTRIENT LEVEL

pH of the soil

In a control set, the pH of the soil was initially recorded as red (8.40), black (7.55), and loamy soil (7.45) as in Table 2.

Upon vermicompost addition, no significant shift was recorded, and pH ranged between 7.82–8.10 in red soil, black soil with a range of 6.82–7.03, and loamy soil with 7.95–8.09 (Table 2).

The addition of organic manure imparts no significant pH shift as recorded in red soil, but in black soil, upon 34 % manure, it has shifted the pH by about 1.4 units towards the basic side, to record 8.91. A similar, picture was evident with loamy soil and pH shift by 1 unit towards alkalinity (Table 2).

In a set of vermicompost plus organic manure plus consortium, no significant pH shifts evident in any sets with red soil. In black soil, pH shifted by 1 unit towards alkalinity. In the case of loamy soil, a drastic pH shift by log two units were recorded towards alkalinity with pH 9.48 (Table 2).

Table 2

pH of the red, black, and loamy soil

Soil sample	pH chart reading	pH meter reading	Soil sample	pH chart reading	pH meter reading	Soil sample	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
RI A	9.0 to 9.5	8.10	**BI A**	7.5	6.82	**LI A**	9.0 to 9.5	7.95
RI B	9.0 to 9.5	7.82	**BI B**	8.0	7.03	**LI B**	9.0 to 9.5	8.09
RIII A	9.0	8.12	**BIII A**	7.5	7.01	**LIII A**	9.0	8.56
RIII B	9.0	8.50	**BIII B**	9.0	8.91	**LIII B**	9.0	8.73
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

Organic carbon

In a control set, red soil was recorded with 0.505–0.750 % oxidizable organic carbon, 1.00–1.50 % in black, and 0.750–1.00 % in loamy (Table 3). The addition of the only vermicompost increased the percent of oxidizable organic carbon in red soil, but the same effect was not observed with organic manure and consortium added with or without vermicompost. With black soil, a marginal decrease in % of oxidizable organic carbon with a value of 0.750–1.00 % was recorded in all the sets (Table 3). With loamy soil, % of oxidizable organic carbon increased with the addition of organic manure and vermicompost solely but remained closed to the control upon the addition of bacterial consortium (Table 3).

Table 3

Available organic carbon in a 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
RI A	0.750–1.00	**BI A**	0.750–1.00	**LI A**	1.00–1.50
RI B	1.00–1.50	**BI B**	1.00–1.50	**LI B**	1.00–1.50
RIII A	0.300–0.500	**BIII A**	0.300–0.500	**LIII A**	0.300–0.500
RIII B	0.300–0.500	**BIII B**	0.300–0.500	**LIII B**	0.300–0.500
RII Ai	0.300–0.500	**BII Ai**	0.300–0.500	**LII Ai**	0.300–0.500
RII Aii	0.300–0.500	**BII Aii**	0.300–0.500	**LII Aii**	0.300–0.500
RII Bi	0.300–0.500	**BII Bi**	0.300–0.500	**LII Bi**	0.300–0.500
RII Bii	0.300–0.500	**BII Bii**	0.300–0.500	**LII Bii**	0.300–0.500

Phosphate

In a control set, the level of phosphate was recorded 22 to 56 kg/ha in red, black, and loamy soils.

In all experimental sets, treatment of vermicompost, organic manure with bacterial consortium did not change the level of phosphate (Table 4).

Table 4

Available phosphate in red, black, and loamy soil

Samples	Range (kg/ha)	Samples	Range (kg/ha)	Samples	Range (kg/ha)
Red soil control	22 to 56	**Black soil control**	22 to 56	**Loamy soil control**	22 to 56
RI A	22 to 56	**BI A**	22 to 56	**LI A**	22 to 56
RI B	22 to 56	**BI B**	22 to 56	**LI B**	22 to 56
RIII A	22 to 56	**BIII A**	22 to 56	**LIII A**	22 to 56
RIII B	22 to 56	**BIII B**	22 to 56	**LIII 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

In a control set levels of potassium (kg/hectare) were noted as <112, >392, and 112 to 280 for red, black, and loamy soil, respectively. In red soil, the addition of organic manure improved potassium significantly, while other sets were not recorded with any changes (Table 5). Black soil remained low in potassium with the addition of the organic manure, vermicompost, or inoculation of bacterial consortium as compared to the control (Table 5). Loamy soil fortification failed to increase the level of potassium in all experimental sets and remained to be 112 to 280 kg/ha (Table 5).

Table 5

Available potassium in a 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
RI A	Below 112	**BI A**	Below 112	**LI A**	112 to 280
RI B	Below 112	**BI B**	112 to 280	**LI B**	112 to 280
RIII A	112 to 280	**BIII A**	Below 112	**LIII A**	112 to 280
RIII B	112 to 280	**BIII B**	Below 112	**LIII 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

In control sets, the level of ammoniacal nitrogen was noted to be 15, 15, and 73 kg/ha for red, black, and loamy soils, respectively. In an experimental set, red soil did not record significant change with any treatment. A similar picture was evident with black and loamy soil, with no significant improvement in the level (Table 6).

Table 6

Available ammoniacal nitrogen in a red, black, and loamy soil

Samples	**Range (kg/ha)**	**Samples**	**Range (kg/ha)**	**Samples**	**Range (kg/ha)**
Red soil control	About 15	**Black soil control**	About 15	**Loamy soil control**	About 73
RI A	About 15	**BI A**	About 15	**LI A**	About 15
RI B	About 15	**BI B**	About 15	**LI B**	About 15
RIII A	About 15	**BIII A**	About 15	**LIII A**	About 73
RIII B	About 15	**BIII B**	About 15	**LIII 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 control set, the level of nitrate nitrogen was noted to be 04 kg/ha in red and black soils, while higher about 20 kg/ha in loamy soil. In experimental sets, inoculation of vermicompost to black soil increased nitrate nitrogen to 50 kg/ha. Similarly, the addition of organic manure increased nitrate nitrogen levels. Other sets remained unaffected (Table 7).

Table 7

Available nitrate nitrogen in a 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
RI A	About 04	**BI A**	About 50	**LI A**	About 04
RI B	About 04	**BI B**	About 50	**LI B**	About 04
RIII A	About 10	**BIII A**	About 50	**LIII A**	About 04
RIII B	About 10	**BIII B**	About 20	**LIII B**	About 04

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

In the present study, vermicompost was found to be effective to improve the overall nutrition level of red soil for organic carbon (0.505–0.750 %); for black soil also improved the recorded organic carbon (0.750–1.00 %), and lastly of nitrate-nitrogen (50 kg/ha). In the case of loamy soil, vermicompost improved organic carbon level significantly (1.00–1.50 % oxidizable organic carbon).

In similar studies, soil organic carbon dynamics improved many folds when wheat gram crop rotation was amended with vermicompost along with combination with organic fertilizers [14]. Rajkhowa *et al*. [12] mentioned the better effect of vermicompost once used with green gram at 75 % and 50 % concentration able to improve organic carbon and also N, P, K. Vermicompost improved water holding capacity, cation exchange capacity, increases organic carbon, as well as carbon content with clay loam, sandy loam, and red loam soil similar to our study [10].

In the present study, inoculation of organic manure (25 % and 34 %) was found to be increasing potassium (112 to 280 kg/ha) in red soil, while in black soil it improves nitrate nitrogen (20 kg/ha). In loamy soil, the addition of organic manure did not impart any positive effect.

In a similar study, the addition of organic manure to the soil was noted with improved potassium uptake when plants studied like maize [7], yellow poplar [6], and lentil plants in sandy soil [20]. Similarly, the combined use of organic manure, green manure, and fertilizer was also found to be improving N, P, and K levels in pearl millet [17].

In the present study, inoculation of *Pseudomonas putida*, *Acinetobacter radioresistens*, *Bacillus cereus*, and *Bacillus subtilis* isolated from the soil did not impart any positive effect once used in 4 % and 8 % consortiums but also not proven negative in response once used in combination with organic manure and vermicompost or alone. The overall study put forward the success of vermicompost and organic manure in improving soil nutrition while the use of *Rhizobacteria* and *Azotobacter* species in consortium proving to be neutral in action for soil nutrient ion level change.

CONCLUSION

In the study positive effect of fortification by the organic manure and vermicompost was prominently reported that improves organic carbon, N, P, and K in the red and black soil. The further study did not record any positive effect of the *Rhizobacteria* when used in 4 % and 8 % consortium fortified with organic manure and vermicompost.

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