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Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of *Pogostemon cablin* (patchouli) Benth

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ABSTRACT

Pre-treatment of patchouli lignocellullosic distillation waste with a consortium of microbes viz. *Trichoderma harzianum* – ATCC PTA-3701, *Pseudomonas monteilii* – HQ995498, *Bacillus megaterium* – ATCC 14581 and *Azotobacter chroococcum* – MTCC 446 significantly enhanced the bio-degradation of cellulose, hemicelluloses and lignin by 58.44%, 29.44% and 65.23%, respectively and improved the yield of vermicompost by 15%. Application of bioinoculant-enriched vermicompost (BEVC) in patchouli reduced the percent disease index (PDI) of *Rhizoctonia* root-rot by 36.36% and improved the essential oil yields by 59.74%, 37.70% and 37.96 as compared to plots receiving untreated (not enriched) vermicompost (VC), bioinoculant-enriched compost (BEC) and chemical fertilizers (CF), respectively. Furthermore, BEVC application resulted in a marked improvement in physical (bulk density and water holding capacity) and chemical properties (pH, percent organic carbon, available N, P and K) of the soil. In conclusion, BEVC from distillation waste can be safely utilized as a bio-organic input unambiguously particularly in situations where the use of chemical fertilizers and pesticides is restricted like organic agriculture.

1. Introduction

In India, about three million tons of distillation waste (plant material left after extraction of essential oil) is generated annually. This waste rots in the fields during rains, posing a sequel of environmental and health problems (Kalra et al., 2002). The farm generated agro-waste is used in landfill and small amount is used as fuel (Kumar and Shweta, 2011). Disposal of solid waste has become a major problem due to shortage of dumping sites and strict environmental laws. As a result, emphasis is now on aerobic composting, that converts waste into organic manure, rich in plant nutrients and humus (Sharma et al., 1999). Vermicomposting is a process of accelerated breakdown of organic lignocellulosic wastes by combined action of microorganisms (especially bacteria and fungi) and earthworms in a mesophillic environment (Dominguez, 2004). The resultant vermicompost can be utilized as plant growth media or soil conditioner (Edwards and Arancon, 2004; Singh et al., 2012a). Biodegradation and bioconversion of lignocellulosic wastes has greater importance in production of quality vermicompost rich in beneficial microbes, essential plant nutrients, humus (Singh and Sharma, 2002) and simultaneous reduction in vermicomposting period by accelerating the biodegradation process (Kumar and Shweta, 2011). Lignocelluloses are composed of 30–56% cellulose, 10–27% hemicelluloses, 3–30% lignin, and 3.6–7.2% protein (Emtiazi et al., 2001) though their composition may vary with plant. Though most of the organic wastes can be converted into nutrient rich vermicompost, utilization of distilled waste is a better option (Kalra et al., 2007). Delignification of lignocellulosic material by fungi and bacteria improves the digestibility of agricultural wastes, wood or straw for animal feed and reduces cost for pulp and paper industries (Sinegani et al., 2005; Hu and Yu, 2005; Johansson and Nyman, 1993; Vares et al., 1995). Pre-treatment with bacterial inoculants such as *Bacillus shackletonni*, *Streptomyces thermovulgaris*, *Ureibacillus thermosphaericus* and ligno-cellulolytic fungus like *Trichoderma* spp. are generally used to improve the composting process by reducing the cellulose and lignin levels of the lignocellulosic waste (Pérez et al., 2002; Vargas-García et al., 2007; Chandra et al., 2009a,b, 2010). Few reports suggest that waste (wood waste, sugar cane waste, raw olive pomace, pepper plant waste and crop residues) decomposition can be easily achieved by microbial

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inoculation prior to vermicomposting (Kumar et al., 2010; Kumar and Shweta, 2011; Haddadin et al., 2009; Vargas-García et al., 2007; Singh and Sharma, 2002). The resultant vermicompost has advantages in terms of higher amounts of essential nutrients (N, P and K) and useful microbes (Singh and Sharma, 2002; Kumar and Shweta, 2011). Biodegradation can also be hastened by synthesis of auxins like indole acetic acid and gibberellins; vitamins like thiamine, riboflavin, pyridoxin, cyanocobalamin, nicotinic and pantothenic acid and microbes by the *A. chroococcum*, which may influence the growth of other inoculated microflora (Subba Rao, 1982). This process holds promise as an agro-waste management for effective recycling of organic wastes to the soil and eco-friendly way of converting waste into nutrient-rich composts (Bano et al., 1987). Vermicompost with desirable aesthetics, high level of plant growth hormones and soil enzymes improves microbial population. It tends to hold more nutrients over longer periods with no or minimal adverse impacts on environment (Ndegwa and Thomson, 2001). It can also be used as a bioremedial measure to reclaim problem soils, especially acid soils, because of the near-neutral to alkaline pH of vermicompost and its ability to suppress labile aluminium (Mitchell and Alter, 1993).

Patchouli oil ranks high among essential oils (Rekha et al., 2009). There is no synthetic replacement for this oil, which further enhances its value (Ramachandra et al., 2002). Production of patchouli oil in India is low (20 t annum^{-1}) and to meet its domestic demand India annually imports over 200 t oil from Indonesia, Malaysia and Singapore (Jhunjhunwalla, 2006). Because of the high domestic demand, the cultivation of patchouli is picking up and as a result, it is expected that higher amount of patchouli distillation waste would be generated at farmer's field. The growers of patchouli crop can be further benefited if the patchouli distilled waste is rapidly converted into enriched vermicompost. In general, conversion of patchouli distillation wastes into vermicompost using vermitechnology requires 95–100 days (Kalra et al., 2002). Further, vermicompost produced from distillation waste has been found to be an effective carrier of various bioinoculants (Kalra et al., 2010; Singh et al., 2012a). Considering the uses and demand of patchouli oil, there is a need to develop a complete agro-technology (cultivation and waste utilization) package for this crop to improve the yields and soil health and reduce the gap between demand and supply. An effort was made to produce an enriched organic fertilizer by pre-treating the distillation waste with efficient bioinoculants useful as cellulase producers/growth promoters and suppressors of soil-borne pathogens into the vermicomposting process. The objective of the present study was to produce a bioinoculants-enriched vermicompost (BEVC) utilizing patchouli de-oiled waste which apart from being a rich organic source of nutrients and beneficial microbes, would suppress many soil-borne diseases including *Rhizoctonia* root-rot/wilt causing extreme losses in patchouli, improve biomass yields and health of soil.

2. Materials and methods

2.1. Microbial cultures

CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India, has a large collection of bioinoculants which have been screened for their growth promoting and disease suppressing activities in medicinal and aromatic plants (MAPs). Four microbial isolates: (a) an efficient growth promoter – *Pseudomonas monteilii* (a native strain CRC1) (HQ995498, MTCC9796) (Singh et al., 2013) and also cellulase producer [(clear area of 18 mm on M9 medium agar amended with 10 g carboxy methyl cellulose (CMC) and 1.2 g yeast extract/l]; (b) a P solubilizer – *Bacillus megaterium* (ATCC 14581); (c) an efficient N fixer – *Azotobacter chroococcum* (MTCC 446); (d) *Trichoderma harzianum* (ATCC no. PTA-3701) [a strain developed by CSIR-CIMAP useful as growth promoter in patchouli (Puttanna et al., 2010) and an efficient cellulase producer (clear area of 12 mm on M9 medium agar with CMC) (Chandra et al., 2009a,b)], found promising in our preliminary screening for growth promotion and biomass production of patchouli (unpublished data), were used in vermicomposting process utilizing distillation waste of patchouli.

2.2. Selection of compatible bioinoculants

Compatibility test between *Bacillus* and fluorescent *Pseudomonas* isolates was performed on nutrient agar (NA, M001, HiMedia, Mumbai, India) medium. *Bacillus* colonies were streaked on the centre of the plate, followed by spraying of 24 h old culture of fluorescent *Pseudomonas* and *Azotobacter* using an atomizer in different Petri dishes (Jain et al., 2011). The same test was performed with *Pseudomonas* and *Azotobacter* individually streaked on the centre of the Petri dish. No zone of inhibition was observed at the point of intersections indicating the compatibility among the strains. All the three strains were further checked for their compatibility with *Trichoderma* isolate on potato dextrose agar (PDA, M096, HiMedia, Mumbai, India) medium. A 5 mm diameter mycelial plug from actively growing culture of *Trichoderma* was kept at the centre of the plate and *Bacillus*, *Azotobacter* and *Pseudomonas* isolates were streaked on either side. Overgrowth of *Trichoderma* on bacterial streaks without a zone of inhibition showed compatibility of *Trichoderma* with bacterial isolates. Based on their compatibility, *T. harzianum* (ATCC no. PTA-3701), bacterial strains *P. monteilii* (HQ995498, MTCC9796), *B. megaterium* (ATCC 14581) and *A. chroococcum* (MTCC 446) were selected for biodegradation of patchouli distillation wastes. All the four bioinoculants, found promising in the present study, are kept at CSIR-CIMAP-Microbial Culture Collection Repository for further use.

2.3. Molecular characterization (16S rRNA) of selected bacterium (CRC1)

Bacterial genomic DNA was isolated from overnight grown cells using standard procedures (Chachaty and Saulnier, 2000; Awasthi et al., 2011). 16S rDNA amplification and their sequence analysis of newly isolated strain CRC1 was carried out as described earlier by Singh et al. (2013) using the nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Further 16S rRNA gene sequence of strain CRC1 was submitted to NCBI Genbank (HQ995498).

2.4. Experimental setup for in vitro and pot study

2.4.1. Multiplication of bioinoculants

T. harzianum was mass multiplied on potato dextrose broth at 28 ± 2 °C for 7 days. Mycelial mat was separated from broth, homogenized and suspended in 500 ml of 100 mM of phosphate buffer to achieve colony forming unit (CFU) of 1.2×10^7 ml^{-1} buffer suspension. The bacterial cultures *P. monteilii*, *B. megaterium*, and *A. chroococcum* were multiplied in nutrient broth (*A. chroococcum* on Jensen's broth) for 36 hours at 210 rpm in an incubator shaker at 30 °C. The bacterial suspensions were centrifuged at 8000 rpm for 10 minutes. The supernatants were discarded and the pellets containing bacterial cells were suspended in 100 mM phosphate buffer. The CFU in this suspension was 2.5×10^8 ml^{-1} for *P. monteilii*, 1.8×10^8 ml^{-1} for *B. megaterium* and 2.3×10^7 ml^{-1} for *A. chroococcum*.

Table 1
Chemical analysis of patchouli distillation waste prior to inoculation of bioinoculants.

Parameter	
pH	7.01
TOC	39.2
TKN	1.90
TP	0.21
TK	0.70
Cellulose	54.5
Hemicellulose	24.1
Lignin	18.3

All values are mean of five observations; all values are given in percentage except pH.

2.4.2. Collection of distillation waste of patchouli and enrichment with bioinoculants

The distillation waste of patchouli was collected from field distillation unit of CSIR-CIMAP, Research Centre, Bangalore, which contained 85 ± 5% moisture. The patchouli distillation waste had a pH of 7.0 ± 0.1, total organic carbon (TOC) of 39.2 ± 1.0%, total Kjeldhal nitrogen (TKN) of 1.9 ± 0.1%, total phosphorus (TP) of 0.21 ± 0.01% and total potassium (TK) of 0.70 ± 0.1% (Table 1) on dry weight basis. Distillation waste (1 kg), transferred into autoclavable HiDispo Bags (HiMedia, Mumbai, India, size: 50 cm × 35 cm) was sterilized for 1 hour. Bioinoculants were transferred aseptically (10 ml culture^{-1} kg^{-1} distilled waste) in their six respective treatments namely *T. harzianum*, *P. monteilii*, *B. megaterium*, *A. chroococcum*, *T. harzianum* + *P. monteilii* + *B. megaterium* + *A. chroococcum* and un-inoculated control.

Inoculated substrate with different bioinoculant treatments and an un-inoculated control were replicated five times and incubated for 7 days at 28 ± 2 °C in a B.O.D incubator. Bioinoculants population was enumerated in their respective treatments on semi selective medium using serial dilution technique. Population (CFU g^{-1} inoculated substrate) of various bioinoculants as observed after 7 days incubation was: *T. harzianum* (5.37×10^6), *P. monteilii* (6.32×10^6), *B. megaterium* (7.0×10^6) and *A. chroococcum* (6.1×10^5) while *T. harzianum* + *P. monteilii* + *B. megaterium* + *A. chroococcum* had *T. harzianum* (5.36×10^6), *P. monteilii* (6.1×10^6), *B. megaterium* (6.72×10^6) and *A. chroococcum* (5.72×10^5). Later, inoculated substrate was mixed (1:10 w/w) with freshly distilled, but cooled waste of patchouli and the mixture was subjected to vermicomposting and composting (without worms) process for 80 days. Generally, the commercial distillation of patchouli herb is carried out for 6–8 h at high steam pressure (30 psi) which completely sterilizes the material. If inoculated immediately after removal of cooled de-oiled herb, the waste can be considered for growing bioinoculants cultures. The experiments were conducted twice to confirm the results but only mean data of two experiments have been discussed.

2.4.3. Collection and multiplication of earthworms

Earthworms (*Eisenia fetida*), obtained from vermicomposting unit of CSIR-CIMAP, Lucknow, India, were mass multiplied in cow dung. Cow dung was added as a worm-bedding material together with the patchouli distillation waste.

2.4.4. Vermicomposting in pot conditions

The bio-inoculated and un-inoculated substrate of each replicated treatment was kept individually in separate sterile earthen pots (diameter 30 cm) in completely randomized design. There were five replicates. The pots were kept in a glass house at ambient temperature (28–32 °C). To keep the material moist, sprinkling of sterile distilled water was done with fine rosecan on alternate days. The epigeic species of earthworm (25 numbers of adult clitellate *E. fetida*) was introduced, in each pot. The substrate was turned-over every week to avoid thermophillic stage. The pots were harvested when the compost was ready by its physical appearance, as judged by development of dark brown to black uniform granular structure. Watering was stopped at this stage. Two days later, the vermicompost/compost was removed from the pot along with worms and uniformly spread on plastic sheet under shade. The total biomass of vermicompost was determined after sieving (2 mm sieve) the produced vermicompost and the number of adults worms separated were counted. Samples were collected from sieved vermicompost (about 10 g) of each replicated treatment for chemical and biological analysis on dry weight basis.

2.5. Field study

Patchouli var. 'Johore' nursery was raised from terminal stem cuttings (5 months old crop) in polyethylene bags (7.5 cm × 14 cm) filled with a mixture of soil and sand in the ratio 1: 1 (v/v). The experiment was conducted at CSIR-CIMAP, Research Centre, Bangalore, India. Bangalore is located at latitude 12°58′ N, longitude 77°35′ E and at an altitude of 930 m above mean sea level. The climate is semi-arid tropical. The soil was a red sandy loam (*Kandiustalf*) with a pH of 6.1, electrical conductivity 0.05 dS m^{-1}, water holding capacity 46%, bulk density 1.61 g cm^{-3}, percent organic carbon 0.45, available N 175 kg ha^{-1}, Olsen's P_2O_5 11.2 kg ha^{-1} and exchangeable K_2O 101.2 kg ha^{-1}. The bioinoculants-enriched vermicompost (BEVC) and bioinoculants-enriched compost (BEC), which contained four bioinoculants (*T. harzianum*, *P. monteilii*, *B. megaterium* and *A. chroococcum*), overall found superior both in terms of its nutrient status and bioinoculants population as compared to vermicompost/compost produced by individual bioinoculant, was used in the field experiment in the present study. The field trials were composed of five treatments: BEVC, BEC, vermicompost (VC), recommended dose of chemical fertilizers (CF) and control replicated five times adopting randomized complete block design (RCBD). The initial soil samples were collected to determine the initial levels of bioinoculants population. The 45-day old rooted cuttings in the nursery were transplanted with a spacing of 60 cm × 45 cm in 3.6 m × 3.6 m raised beds in fields continuously cultivated with patchouli crop for last three years where *Rhizoctonia* root-rot/wilt was a consistent problem (Narayanappa et al., 1984). The patchouli crop was maintained in the same plots with same treatments for the period of 2 years. In treatments involving BEVC, BEC, VC and CF, the recommended dose of total N requirement (66 kg ha^{-1} harvest^{-1}) was supplied through their respective vermicompost/compost and therefore different amounts of vermicompost/compost were added depending on their N content. However, the chemical fertilizer treated plots received recommended dose of fertilizers N P K (66:50:50 kg ha^{-1} harvest^{-1}) through urea, single super phosphate and muriate of potash. The urea was applied in two split doses every alternate month. Ten plants were randomly tagged for growth/severity of root-rot observations from each plot and the mean value of ten plants were taken for statistical analysis from each plot. Plant height and number of primary branches were recorded at the time of harvesting. Re-transplanting (three times) of the patchouli rooted cuttings was done following same methods during the period of 2 years. The harvesting of the crop was done after 155–165 days of transplanting and biomass yield was recorded. Severity of stem/root-rot disease was measured on a 0–4 scale of Kesavan and Chowdhary (1977) where 0 = no symptoms, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 ≥ 75% stem/root affected by rot. Based on the root disease symptoms score of each treatment, the percentage disease index (PDI) was calculated as follows:$\text{PDI} = \left(\frac{\text{Sum of numerical grading recorded}}{\text{Number of roots observed} \times \text{highest numerical rating}}\right) \times 100$

2.6. Physical, chemical and microbiological analysis

The rhizosphere soil samples of each crop were collected after harvesting from a depth of 0–15 cm at 5 random points adjacent to plant roots with the help of soil auger for each replicated treatments for a period of 2 years. Pooled soil samples (5 random points) were stored in sealed plastic bags under refrigeration (4 °C) prior to assay of chemical and microbiological properties of soil.

Undisturbed soil cores collected from each plot with soil sampling rings of known volume were weighed and then dried in an oven and reweighed for bulk density assays (Liu et al., 2007). Soil water holding capacity was determined as the method described by Sampson and Allen (2000). The pH was determined in a 1:10 (w/v) vermicompost/distillation waste/rhizo-soil:water suspension. The patchouli distillation waste (before and after inoculation of bioinoculants) and BEVC were analyzed on oven dry basis for percent total organic carbon [TOC (%)] by Walkley-Black method (Nelson and Sommers, 1996) and total Kjeldhal nitrogen (TKN), total phosphorus (TP) and total potassium (TK) by the methods described by Jackson (1973). Soil samples were analyzed for TOC (%), available N (kg ha^{-1}), available P (Olsen's P_2O_5, kg ha^{-1}) and available K (exchangeable K_2O, kg ha^{-1}) following Jackson (1973). Cellulose, hemicellulose and lignin were fractionated sequentially by Dutta's method (1981). Distillation waste/bioinoculants-eriched vermicompost/vermicompost/rhizo-soil were used for the determination of microbial load (CFU g^{-1}) in triplicate on dry weight basis. *Trichoderma, Pseudomonas, Bacillus* and *Azotobacter* populations in the distillation waste/vermicompost/rhizo-soil were determined by serial dilution technique (10^{-3} folds for fungi and 10^{-4} folds for bacteria) in 0.85% NaCl (w/v) (Denin, 1963) under in vitro conditions using *Trichoderma* selective medium (Elad and Chet, 1983), King's B medium (King et al., 1954), *Bacillus* isolation and cultivation medium for P solubilizers (Pikovskaya, 1948) and Jensen's medium (Jensen and Petersen, 1954), respectively.

2.7. Essential oil estimation of herb

A sample of the herb was shade dried for three days by spreading in shade and the oil content was determined using Clevenger's apparatus (Langenau, 1948). The oil samples were analyzed for major constituents using Varian CP 3800 gas chromatograph. The chromatograph was fitted with a CP–5 SIL 30 m × 0.25 mm column and programmed 100 °C (2 °C), 8 °C, 200 °C (3 min.). The carrier gas was nitrogen at a flow rate of 0.4 ml min^{-1} and the injector and the flame ionization detector were maintained at 250 °C and 300 °C, respectively. 0.2 μl of samples were injected with a split ratio of 1:80. Peaks were identified by co-injection with authentic pure samples. The percentages of the main components of patchouli oil, namely, β-patchoulene, caryophyllene, α-guaiene, seychellene, α-δ-patchoulene, α-bulnesene and patchouli alcohol were determined.

2.8. Statistical analysis

The collected data were subjected to statistical analysis by analysis of variance method (ANOVA), suitable to completely randomized design (CRD) for pot experiment and randomized complete block design (RCBD) for field experiment, with the help of software ASSISTAT Version 7.6 beta (2012). The experimental data of the two trials for pot experiments and four trials for field experiments had similar variance values, so the data were combined for further analyses. Significant differences among treatments were based on the *F*-test in ANOVA and treatment means were compared using least significant difference (LSD) at $P \leq 0.05$. The standard error (SE) of the mean in vertical bar charts was computed with Sigma Plot 11 (http://www.sigmaplot.com). The results and discussion are based on the average of the trials during the 2-year period.

Table 2
Analysis of cellulose, hemicelluloses and lignin left in the vermicompost harvested after 80 days.

Treatments	Cellulose (%)[a]	Hemicellulose (%)[a]	Lignin (%)[a]
THVC	24.15cd	16.05ef	4.21d
THC	24.82c	16.82de	4.88cd
PMVC	23.08d	17.05de	4.11d
PMC	23.78cd	17.85cd	4.45cd
BMVC	26.12b	18.25bc	5.01bc
BMC	26.54b	19.74ab	5.31b
AZVC	26.71b	18.11cd	5.18bc
AZC	27.23b	18.97bc	5.45b
BEVC	14.74e	14.1g	3.14e
BEC	15.21e	14.97fg	4.12d
VC	35.47a	20.01ab	9.03a
C	36.12a	20.89a	9.67a

THVC = *Trichoderma harzianum* enriched vermicompost, THC = *Trichoderma harzianum* enriched compost, PMVC = *Pseudomonas monteilii* enriched vermicompost, PMC = *Pseudomonas monteilii* enriched compost, BMVC = *Bacillus megaterium* (P solubilizer) enriched vermicompost, BMC = *Bacillus megaterium* enriched compost, AZVC = *Azotobacter* enriched vermicompost, AZC = *Azotobacter* enriched compost, BEVC = Bioinoculants (TH + PF + BM + AZ) enriched vermicompost, BEC = Bioinoculants (TH + PF + BM + AZ) enriched compost, VC = un-inoculated microbe vermicompost, C = un-inoculated microbe and earthworm compost;

[a] Average of two trials (ten observations for each treatment) during the 2-year period; the mean values in vertical columns followed by the same letters do not differ statistically between themselves at $P \leq 0.05$.

3. Results

3.1. 16S rRNA gene sequence analysis-based identification of selected bacteria

The BLAST analysis of 16S rRNA gene sequence of isolate CRC1 revealed that the bacterium belongs to the genus *Pseudomonas*. The isolate CRC1 has shown maximum similarity (99%) with *Pseudomonas monteilii* strain WAPP53 (Accession no. FJ905913), therefore isolate CRC1 was designated as *P. monteilii* (Singh et al., 2013). The 16S sequence of isolate CRC1 was submitted to Genbank (NCBI) under the Accession number HQ995498. The identification of strain CRC1 was also confirmed by CSIR-IMTECH (Institute of Microbial Technology), Chandigarh, India, and the bacterium is deposited with MTCC (Microbial Type Culture Collection and Gene Bank – an international Depositary Authority) as strain no. 9796.

3.2. Pre-treatment of de-oiled lignocellulosic patchouli waste for rapid production of enriched vermicompost in pot conditions

The initial composition of patchouli de-oiled waste is presented in Table 1. Preliminary experiments indicated that inoculation of distillation waste with plant growth promoting/cellulase producing microbes like *T. harzianum, P. monteilii, B. megaterium* and *A. chroococcum* yield a vermicompost rich in the respective microbe (un-published data). Further, it was observed that vermicomposting of distillation wastes of patchouli pre-inoculated with all four microbes (*T. harzianum, P. monteilii, B. megaterium* and *A. chroococcum*) together resulted in faster decomposition of lignocellullosic waste in terms of cellulose, hemicelluloses and lignin bio-degradation by 58.44%, 29.44% and 65.23%, respectively (Table 2), resulting in higher yields (15%) of granular vermicompost recovered through 2 mm sieves (Fig. 1) and higher population (21%) of earthworms (data not presented). The pH of BEVC was near neutral (7.02). As a result of higher degradation in BEVC, the total organic carbon (TOC) was significantly reduced by 21% as compared to VC (Table 3). Also, total Kjeldhal nitrogen (TKN), total

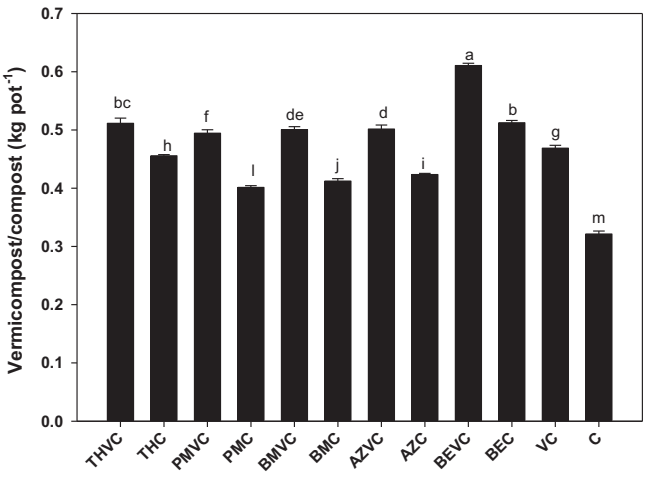

Fig. 1. Effect of pre-treatment of bioinoculants for the production of enriched vermicompost/compost. Error bars are presented as standard error of mean (±SE), Different letters above the error bars show significant difference at $P \leq 0.05$, THVC = *Trichoderma harzianum* enriched vermicompost, THC = *Trichoderma harzianum* enriched compost, PMVC = *Pseudomonas monteilli* enriched vermicompost, PMC = *Pseudomonas monteilii* enriched compost, BMVC = *Bacillus megaterium* (P solubilizer) enriched vermicompost, BMC = *Bacillus megaterium* enriched compost, AZVC = *Azotobacter* enriched vermicompost, AZC = *Azotobacter* enriched compost, BEVC = Bioinoculants (TH + PF + BM + AZ) enriched vermicompost, BEC = Bioinoculants (TH + PF + BM + AZ) enriched compost, VC = un-inoculated microbe vermicompost, C = un-inoculated microbe and earthworm compost.

phosphorus (TP) and total potassium (TK) increased significantly in BEVC by 20%, 32% and 3%, respectively as compared to VC (Table 3).

3.3. Effect of BEVC, BEC, VC and CF on soil health, incidence of Rhizoctonia root-rot/wilt, growth and yield of patchouli under field conditions

The initial population (CFU g^{-1} soil) of *Azotobacter, P. monteilii*, *B. megaterium* and *T. harzianum* in the experimental plots was 1.85×10^4, 8.5×10^3, 2.2×10^4 and 8.5×10^3, respectively.

Table 3
Production and chemical analysis of enriched vermicompost/compost harvested after 80 days.

Treatments	TOC (%)[a]	TKN (%)[a]	TP (%)[a]	TK (%)[a]
THVC	18.822de	1.993cd	0.231bc	0.717ab
THC	19.002cd	1.672f	0.211cd	0.705ab
PMVC	20.582cd	2.079bc	0.239bc	0.722ab
PMC	20.893bc	1.779de	0.209cd	0.682e
BMVC	20.385cd	2.044cd	0.295a	0.715ab
BMC	20.775bc	1.742ef	0.254b	0.685de
AZVC	20.334cd	2.445a	0.232bc	0.723ab
AZC	20.884bc	2.005cd	0.192d	0.693cd
BEVC	17.805f	2.327ab	0.299a	0.725a
BEC	18.505ef	2.097bc	0.258b	0.625f
VC	22.597b	1.939cd	0.227bc	0.703bc
C	25.346a	0.939g	0.127e	0.403g

THVC = *Trichoderma harzianum* enriched vermicompost, THC = *Trichoderma harzianum* enriched compost, PMVC = *Pseudomonas monteilii* enriched vermicompost, PMC = *Pseudomonas monteilli* enriched compost, BMVC = *Bacillus megaterium* (P solubilizer) enriched vermicompost, BMC = *Bacillus megaterium* enriched compost, AZVC = *Azotobacter* enriched vermicompost, AZC = *Azotobacter* enriched compost, BEVC = Bioinoculants (TH + PF + BM + AZ) enriched vermicompost, BEC = Bioinoculants (TH + PF + BM + AZ) enriched compost, VC = un-inoculated microbe vermicompost, C = un-inoculated microbe and earthworm compost, TOC = Total organic carbon, TKN = Total Kjeldhal nitrogen, TP = Total phosphorus; TK = Total potassium,

[a] Average of two trials (ten observations for each treatment) during the 2-year period; the mean values in vertical columns followed by the same letters do not differ statistically between themselves at $P \leq 0.05$.

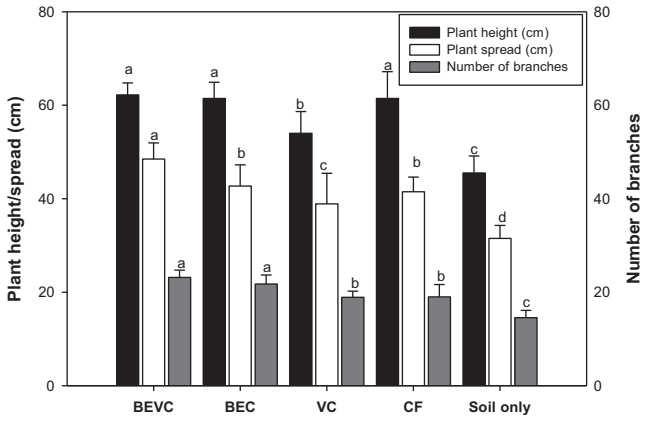

Fig. 2. Effect of enriched vermicompost/compost/chemical fertilizers on growth characteristics of patchouli in field conditions. Error bars are presented as standard error of mean (±SE), Different letters above the error bars show significant difference at $P \leq 0.05$, BEVC = Bioinoculants-enriched vermicompost, BEC = Bioinoculants-enriched compost, VC = Vermicompost, CF = Chemical fertilizers.

Plant height enhanced significantly (15.2%) in plots treated with BEVC and BEC/CF (13.7%) treated plots compared to VC treated plots (Fig. 2). The highest plant spread was observed with BEVC (24.7%) followed by BEC (9.8%) and CF (6.7%) treated plots compared to VC treated plots (Fig. 2) Number of branches were significantly higher in BEVC (22.5%) and BEC (15.1%) treated plots compared to CF and VC treated plots (Fig. 2). Over all, plant height, plant spread and number of branches increased in all the treatments (BEVC, BEC, VC and CF) over control. The percent disease index (PDI) of *Rhizoctonia* root-rot/wilt ranged from 7–13% in various treatments and was significantly lower in plots receiving BEVC and BEC; the severity of *Rhizoctonia* root-rot/wilt in BEVC treated plots was reduced by 48% and 61% compared to VC and CF treated plots, respectively (Fig 3). VC also reduced the disease severity by 26% compared to CF treated plots (Fig. 3). The shade dried herb yield of patchouli was significantly higher (54%) in BEVC treated plots compared to VC alone plots and 32% higher than CF treated plots (Table 4). There was a significant difference in shade dry herb yield (an increase of 35%) in BEVC treated plots as compared to BEC treated plots (Table 4). The content of essential oil varied from 2.20 to 2.30% (on shade dry basis). The yields of essential oil improved by 59.74%

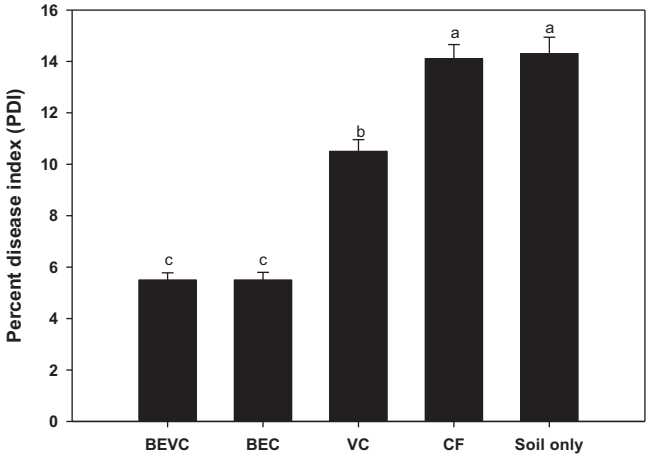

Fig. 3. Effect of enriched vermicompost/compost/chemical fertilizers on PDI of patchouli in field conditions. Error bars are presented as standard error of mean (±SE), Different letters above the error bars show significant difference at $P \leq 0.05$, BEVC = Bioinoculants-enriched vermicompost, BEC = Bioinoculants-enriched compost, VC = Vermicompost, CF = Chemical fertilizers.

Table 4
Effect of bioinoculants-enriched vermicompost, compost, vermicompost and chemical fertilizers on biomass and essential oil yield of patchouli.

Treatments	[a]Biomass/essential oil yield		
	Shade dry herb yield (t ha^{-1})	Essential oil (%)	Essential oil yield (kg ha^{-1})
BEVC	1.28a	2.30a	29.44a
BEC	0.95c	2.25b	21.38b
VC	0.83d	2.22c	18.43c
CF	0.97b	2.20c	21.34b
Soil only	0.55e	2.21c	12.16d

BEVC = Bioinoculants-enriched vermicompost, BEC = Bioinoculants-enriched compost, VC = Vermicompost, CF = Chemical fertilizers; the mean values in vertical columns followed by the same letters do not differ statistically between themselves at $P \leq 0.05$.

[a] Mean data of four trails during 2-year period.

in plots treated with BEVC as compared to VC (not enriched) and an increase of 37.70% and 37.96% when compared with BEC and CF treated plots, respectively (Table 4). The quality of essential oil was not affected in any of the treatments (data not presented). The content of patchouli alcohol, a major chemical constituent, ranged from 45.16 to 48.43%. Other main constituents were seychellene (4.24–4.30%), caryophyllene (2.31–2.40%), α-bulnesene (8.01–8.08%), α-guaiene (5.78–6.07%), β-patchoulene (1.21–1.28%) and α-δ-patchoulene (3.97–4.15%).

Application of BEVC also improved the physical status of soil with a reduction in bulk density by 13.58% (Table 5). On the other hand, water holding capacity of soil increased by 8.2% over plots treated with CF (Table 5). However, differences were not significant among BEVC, BEC and VC treated plots. Another significant important change in pH was observed in plots treated with BEVC where the soil pH improved from 6.1 to 6.39 (Table 5). There was no significant change in pH in plots applied with CF. Soil fertility status like percent total organic carbon, available N, available P and available K were significantly higher in BEVC treated plots; an increase of 19.2 and 24.9%, 13.5 and 2.95%, 19.1 and 26.9%, 14.9 and 20.3% (Table 5) compared to VC and CF treated plots, respectively.

The bioinoculants (*Azotobacter, P. monteilii*, *B. megaterium* and *T. harzianum*) populations (CFU g^{-1} soil) were significantly higher in plots receiving BEVC compared to VC and CF treated plots after harvesting of patchouli crop grown over 2-year period. The final population of *Azotobacter, P. monteilii*, *B. megaterium* and *T. harzianum* in the BEVC treated rhizosphere after 2-year period improved by 34.21–142.85%, 71.43–554.54%, 52.73–236% and 96.77– 454.55%, respectively, compared to VC and CF treated plots (Table 6).

Table 6
Effect of bioinoculants-enriched vermicompost, compost, vermicompost and chemical fertilizers on rhizospheric bioinoculants population.

Treatments	[a]Root zone bioinoculants population (CFU × 10^4 g^{-1} soil)			
	Azotobacter	Pseudomonads	P solubilizers	*Trichoderma*
BEVC	5.1a	7.2a	84a	6.1a
BEC	4.8b	6.5b	73b	4.5b
VC	3.8c	4.2c	55c	3.1c
CF	2.1d	1.1d	25d	1.1d
Soil only	0.5e	0.4e	12e	0.2e

BEVC = Bioinoculants-enriched vermicompost, VC = Vermicompost, CF = Chemical fertilizers; the mean values in vertical columns followed by the same letters do not differ statistically between themselves at $P \leq 0.05$.

[a] Mean data of four trails during 2-year period.

4. Discussion

Organic manures and plant beneficial microbes are vital components for improving soil health and yields in agricultural systems. Delivering both the components in sufficient quantities/numbers will be definitely beneficial particularly in organic fields (Singh et al., 2012a,b).

4.1. Pre-treatment of de-oiled lignocellulosic patchouli waste for rapid production of enriched vermicompost

Recycling of distillation wastes of medicinal and aromatic plants may reduce the need for fossil fuel based fertilizer, and help in sustaining and restoring soil fertility in terms of available nutrients and major physical and chemical characteristics of the soil (Kalra et al., 2012; Singh et al., 2012b).

Pre-treatment of crop residues, specially lignocellulosic waste, significantly reduces the composting period and improve the quality vermicompost (Singh and Sharma, 2002; Kumar and Shweta, 2011). An attempt was made towards effective recycling of distillation waste of patchouli and further enhancing its value by incorporating beneficial microbes (*T. harzianum, P. monteilii, B. megaterium* and *A. chroococcum*) during vermicomposting process for production of BEVC; superior in terms of accelerated biodegradation by producing lignocellulolytic enzyme and richness in both nutrients and desirable beneficial microbes.

The vermicompost/compost had relatively neutral pH. This may be due to the secretion of NH_4^+ ions and activity of calciferous glands in earthworms. NH_4^+ ions temporarily reduce the pool of H^+ ions (Haimi and Huhta, 1987). The calciferous glands contain carbonic anhydrase which catalyze the fixation of CO_2 as $CaCO_3$, thereby preventing the fall in pH (Kale et al., 1982). Naik et al. (2008) reported that approximately 31% *P. monteilii* strains are efficient cellulase producers which could be exploited for the management of enormous agricultural waste lying in the field which is supported by the present study. Chandra et al. (2009a,b) explained the role of six *Trichoderma* spp. for the rapid biodegradation of lignocellulosic distillation waste. *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp. are known to produce cellulase enzyme (Lynch et al., 1981). Padmavathiamma et al. (2008) observed faster decomposition of banana leaves during vermicomposting process with *Eudrilus*

Table 5
Effect of bioinoculants-enriched vermicompost, compost, vermicompost and chemical fertilizers on physical and chemical properties patchouli rhizospheric soil.

Treatments	[a]Mean physical and chemical properties of patchouli rhizospheric soil						
	pH	Bulk density (g^{-1} cm^3)	Water holding capacity (%)	Total organic carbon (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
BEVC	6.39a	1.38e	48.85a	0.621a	300.05a	17.560a	136.99a
BEC	6.40a	1.40d	49.12a	0.601b	290.75b	16.891b	120.54b
VC	6.27b	1.42c	47.15b	0.521c	264.30c	14.755c	119.24b
CF	6.08c	1.62b	45.15c	0.497d	291.58b	13.835d	113.90c
Soil only	6.10c	1.64a	45.25c	0.501d	170.50d	11.051e	102.55d

BEVC = Bioinoculants-enriched vermicompost, VC = Vermicompost, CF = Chemical fertilizers; the mean values in vertical columns followed by the same letters do not differ statistically between themselves at $P \leq 0.05$.

[a] Mean data of four trails during 2-year period.

eugineae worms. Organic wastes fragmented by earthworms have a much greater surface area and can support greater microbial activity (Edwards et al., 2010) which in turn may help in production of higher amount of cellulases, hemicellulases, pectinases and ligninases leading to rapid biodegradation of lignocellulosic waste. Granular vermicompost, produced from the essential oil bearing crops, are efficient carrier for the N-fixer (*Rhizobium meliloti*, Rmd 201) since it could maintain higher populations of 5.9×10^8 CFU g^{-1} vermicompost for 180 days (Kalra et al., 2010), a value acceptable and suitable for inoculation (Smith, 1992; Ben Rebah et al., 2007). In an another study, Singh et al. (2012a) observed that pre-inoculation of bioinoculants in vermicompost, as a rooting medium for patchouli cuttings, could be a better carrier for transferring propagules of arbucular mycorrhizal (AM) fungi (*Glomus aggregatum*, *G. fasciculatum*, *G. intraradices* and *G. mosseae*) and bioinoculants (*P. fluorescens*, *B. megaterium* and *A. chroococcum*). The optimal performance of the vermicompost is due to its higher nutrient content and high water-holding capacities, which are the two key characteristics of good carriers (Smith, 1992). Satchell and Martein (1984) found an increase of 25% in P content in paper waste sludge, after worm activity. They attributed this increase in P content to direct action of worm gut enzymes and indirectly by stimulation of microflora during vermicomposting process. Increase in total phosporus during vermicomposting is probably due to mineralization of organic matter (Edwards and Lofty, 1972) and CO_2 evolution (Haddadin et al., 2009). Garg et al. (2006) also demonstrated that microbial flora enhanced the total potassium; acid production by the micro-organisms seems to be prime mechanism for solubilizing the insoluble potassium. Organic manures and plant beneficial microbes are important for improving soil health and sustainability in agricultural systems and BEVC rich in both nutrients and beneficial microbes will be an organic input of choice for organic farming. Vermicomposts are known to provide a slow, balanced nutritional release pattern to plants, particularly in terms of release of plant available N, soluble K, exchangeable Ca, Mg and P (Edwards and Fletcher, 1988; Edwards, 2004) which are subsequently used by plants efficiently. Vermicompost application generally improves the soil environment particularly soil aeration, encouraging the proliferation of roots, which in turn draw more water and nutrient from distant areas and helps to introduce and sustain beneficial micro-organisms into the rhizosphere (Padmavathiamma et al., 2008) in sufficient numbers for a longer period.

4.2. Effect of BEVC, BEC, VC and CF on soil health, incidence of Rhizoctonia root-rot/wilt, growth and yield of patchouli under field conditions

Bioinoculants-enriched vermicompost (BEVC) was evaluated for achieving higher yields particularly in situations where chemical inputs are restricted. BEVC was compared with BEC, VC and CF continuously for 2-year involving 4 harvests.

During field study, plant growth parameters improved significantly by beneficial bio-inoculants. This has been successfully shown in industrially important aromatic crop like patchouli (Singh et al., 2012a), horticultural crop like tomato (Al-Karaki, 2006) and in medicinal plant *Coleus forskohlii* (Singh et al., 2009). The application of BEVC containing higher amount of nutrients and beneficial microbes (*T. harzianum*, *P. monteilii*, *B. megaterium* and *A. chroococcum*) in sufficient numbers could have increased the nutrient availability and also reduced the disease severity resulting in better growth and yield of crop. Puttanna et al. (2010) observed that the growth and yield of patchouli crop was adversely affected in soil alone treatment (without vermicompost) due to low nutrient status of soil and low colonizing ability of microbes in poor soil. Therefore, the use of BEVC could be a balanced organic fertilizer for optimum growth and herb yield of the crop. Singh et al. (2009, 2011, 2012b,c) demonstrated that use of efficient bioinoculants along with VC or VC alone ($5 t\ ha^{-1}$) for reduced disease severity and higher yields in *C. forskohlii*. Mishra et al. (2000) have earlier successfully demonstrated the use of eco-friendly bioagents (*T. harzianum*, *Glomus virens* and *G. aggregatum*) in controlling patchouli root-rot. Improvement in yields and reduction in disease severity with BEVC may be a suitable alternative to chemical fertilizers and fungicides which may help in reducing environmental and health hazards incurred by the use of harmful chemicals. The success of bioinoculants depends on the selection of potential bioinoculants/antagonist, method and mode of application, the right environment and other factors (Singh et al., 2012a). The efficient bioionculants applied along with nutrient rich vermicompost performed well because of the presence of vermicompost which probably provided optimal growing environment (near neutral pH, nutrients, good water holding capacity) for the bioinoculants. A relatively lower yield noticed in the present study with VC alone suggests the beneficial effects of added bioinoculants. Quality of essential oil was not affected by the treatments, however, yield of essential oil improved by the application of enriched vermicompost/compost. The similar trends were observed during previous study where patchouli plants rooted in vermicompost along with microbes like AM fungi (*Glomus aggregatum*, *G. fasciculatum*, *G. intraradices* and *G. mosseae*) and plant growth promoters (*Pseudomonas fluorescens*, *Bacillus subtilis*, *B. megaterium* and *Azotobacter*) when transplanted into pots and fields yielded significantly higher essential oil yield (Singh et al., 2012a).

Bioinoculants enriched vermicompost could be an excellent source of organic fertilizer to sustain productivity and fertility soil. Willson et al. (2001) demonstrated the use of organic manures for the better management and sustainability of organic soils. Kalra et al. (2012) studied the effect of menthol mint vermicompost for the improvement of soil properties. The use of organic amendment has been reported to increase soil organic matter, provide nutrients and improve microbial activity (Lee et al., 2004). Higher nutrient concentration in bioinoculants enriched vermicompost (BEVC) might be the reason for supporting threshold population of bioinoculants thereby higher availability of nutrients (N, P and K) to the test crop patchouli. The enriched vermicompost could be an excellent source of organic nutrients especially under conditions of organic agriculture preventing loss of yields generally observed in transition to organic farming practice (Acs et al., 2007).

5. Conclusion

The present study revealed that the above ground biomass of patchouli could be exploited in two ways: (i) for the production of essential oils (economic part) and (ii) the generated de-oiled waste (un-economic part) can be further utilized to produce bioinoculants enriched vermicompost. In this way, the produced distillation waste in large quantities from the patchouli crop, which contains major essential nutrients exhausted from the soil, can be biodegraded after pre-treating with bioinoculants (*T. harzianum, P. monteilii, B. megaterium* and *A. chroococcum*) and subsequently vermicomposted with the help of epigeic species of earthworm (*E. fetida*). The resultant bioinoculants enriched vermicompost would be an optimal organic bio-input for supply of nutrients, enriching the soil with beneficial microbes and improving soil health. BEVC could significantly reduce the disease and improve the yields of patchouli herb and essential oil. This technology could be referred as zero waste technology where the produced biomass herb (after extraction of essential oil) is again recycled (involving beneficial microbes and earthworms) containing the same amount of essential nutrients (as only secondary metabolites are removed) is added to the soil after vermicomposting process. Application of enriched vermicompost therefore may reduce the use of chemical fertilizers

and fungicides and with improvement in soil pH, bulk density and water holding capacity, bioinoculants enriched vermicompost may also reduce the number of irrigations further reducing the cost of inputs.

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