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ORIGINAL RESEARCH ARTICLE

Cooperative interaction of *Glomus intraradices* with plant growth-promoting rhizobacteria promotes plant development and essential oil yield of *Pogostemon cablin* and reduces disease occurrence under organic field conditions

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Abstract

In this study, two efficient plant growth promoters coupled with potent antagonists viz. *Pseudomonas monteilii* strain-CRC1, *Cedecea davisae* strain-CRC2 and AM Fungi named *Glomus intraradices* (GI) were assessed individually and in combination for their potential to increase yield and essential oil yield as well as lessen the severity of the disease caused by *Rhizoctonia solani* in *Pogostemon cablin* (patchouli). In field trials, nine treatments were used: CRC1, CRC2, GI, CRC1 + CRC2, CRC1 + GI, CRC2 + GI, CRC1 + CRC2 + GI, un-inoculated vermicompost, and uninoculated soil as control, with five replications in randomised complete block design, where *Rhizoctonia* root-rot/wilt was a persistent problem. As compared to the control, the plants inoculated with CRC1 + CRC2 + GI performed best and significantly increased the plant height (87%), plant spread (50%), branch count (67%), herbs yield (67%), essential oil yield (69%) as well as reduced the percent disease index (68%) and percent wilt incidence (87.5%). Moreover, the Patchouli alcohol, a key component of its essential oil, was found to be markedly enhanced by 10% in CRC1 + CRC2 + GI inoculated plants. Furthermore, 43, 27 and 191% of higher uptake of NPK were observed in CRC1 + CRC2 + GI inoculated plants, respectively. After harvesting, a considerable abundance of CRC1, CRC2, and GI in the rhizosphere soil was observed. The results of this experiment indicate that higher herb yields and other observed plant attributes could be due to improved nutrient (NPK) uptake by the patchouli plants. The management of wilt disease and the production of high-quality essential oils in patchouli both can be accomplished with the help of the established consortium.

Keywords AM fungus · Organic cultivation · Patchouli · PGPR · Vermicompost

Introduction

Over 25% of the biodiversity on Earth is made up of soil microorganisms that have interacted with soil, plants, and animals in ecosystems around the planet for millions of years (Fierer 2017; Wagg et al. 2014). Plants and soil bacteria may interact in a variety of ways, and the plant may be positively or negatively impacted depending on the species. Soil microorganisms, including mutualists and pathogens, regulate the presence of various species and the growth of certain plants through recurring interactions (Li et al. 2020). This symbiont or pathogen community of plants is made up of a wide range of bacteria, archaea and fungi (Hussain and Khan 2020).

Rhizosphere microorganisms including plant growth-promoting rhizobacteria (PGPR) and Arbuscular

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mycorrhizal fungi (AMF) are often considered to have the ability to accelerate plant growth through direct or indirect interactions with plant roots (Artursson et al. 2006). PGPR has significant plant-associated microbiome constituents such as *Azospirillum, Pseudomonas, Azotobacter, Klebsiella, Enterobacter, Alcaligens, Arthrobacter, Burkholderia*, *Microbacterium*, *Bacillus*, etc. (Singh et al. 2009; 2012 a; 2012b; 2013a; 2013b; 2018; Agnolucci et al. 2020; Soni et al. 2022; 2023). These PGPRs can improve plant growth through fixing nitrogen, solubilizing and mineralizing phosphorus and other nutrients, siderophores formation, phytohormones production, induced systemic resistance (ISR), improving tolerance/resistance from various abiotic and biotic stresses, maintaining the soil's health via nutrient recycling and performing other vital tasks like soil formation and the breakdown of organic materials etc. (Wallenstein 2006; Saxena et al. 2020; Soni et al. 2014a, b, 2022, 2023). AMF maintains symbiotic relationships with more than 70% of terrestrial plants and through their arbuscules, where the exchange of vital nutrients and sugars takes place between host plants and fungi (Wagg et al. 2019). AMF can significantly improve the fitness of a host plant by increasing the exhaustion zone by collecting additional water and nutrients through a dense hyphal network (Ezawa and Saito 2018; Field and Pressel 2018), tolerance to drought, heavy metals, and diseases (Li et al. 2019; Soni et al. 2014a, b, 2022; Singh et al. 2013a, 2018), enhances plant quality by fostering the synthesis of secondary metabolites/bioactive compounds (organosulfides, polyphenols, phytosterols, stilbenes, vitamins, lignins, and terpenoids, including carotenoids), which can be essential for plant tolerance to abiotic and biotic stresses or beneficial to human health due to their antioxidant properties (Gianinazzi et al. 2008, 2010).

The rhizobacteria and hyphal network of AM fungus can communicate with each other in the rhizosphere and symbiotically improve plant growth (Barea et al. 2017; Rasmann et al. 2017; Yuan et al. 2021; Soni et al. 2014a, 2022). For the production of their fundamental building blocks, bacteria depend on lignin and cellulose-hydrolyzed fungi (Roman et al. 2006). Fungal hyphae build "fungal hyphae highways" for bacteria to transport substrates by forming hyphal networks to link soil patches (Barea et al. 2002; Warmink et al. 2011). Fungi in turn receive nutrition from bacteria. Moreover, several bacteria have been found to increase the amount of AM fungal colonisation and to boost spore germination and hyphal growth rate (Bharadwaj et al. 2008b; Nazir et al. 2010; Soni et al. 2014a, 2022). Some of the hypothesised mechanisms for mycorrhization stimulation include the production of volatile compounds that can help AM spores germinate, nitrogen availability through nitrogen fixation, solubilization of soil phosphate sources, detoxification of the fungal microhabitat, change in pH, and level of siderophores (Bharadwaj et al. 2012; Soni et al. 2022). The capacity of these organisms to produce cell wall-degrading enzymes, which may weaken the cell walls of the roots and facilitate the AM fungi's penetration of the roots, has also been investigated as a possible mechanism (Bharadwaj et al. 2008a, b; Nazir et al. 2010).

Most plant-microbe interactions in soil depend on the release of different chemicals from roots that can influence the development and activity of microbes (Hartmann et al. 2009). The hyphal exudates and deposition of AM fungal mycelial products found in the root exudates of mycorrhizal plants have the potential to act as substrates for bacterial growth and exert a direct influence on the bacterial communities in the myco-rhizosphere. AM colonisation can increase the overall number of aerobic bacteria in the rhizosphere (Krishnaraj and Sreenivasa 1992) without having any effect on it (Waschkies et al. 1994) or reducing it (Ames et al. 1984). Several researchers also assessed the function of AMF concerning *G. intraradices*. According to Filion et al. (1999), mycelial exudates of *G. intraradices* can either promote or prevent the growth of other fungi and bacteria. According to Toljander et al. (2007), *G. intraradices* mycelial exudates altered the composition of the bacterial population in addition to promoting bacterial growth and vitality. The size and makeup of particular microbial groups have frequently been used to describe the environmental impact of microbial inoculants in soil. However, these methods do not offer a thorough understanding of how inoculants affect the health of the soil ecosystem (Doyle and Stotzky 1993).

Patchouli (*Pogostemon cablin* Benth), a member of the Lamiaceae family, is one of the most important aromatic and medicinal herbs. Several bioactive substances, such as terpenoids, phytosterols, flavonoids, organic acids, lignins, glycosides, alcohols, pyrone, and aldehydes, have been found in patchouli. Patchouli alcohol, patchoulene, patchoulene epoxide, pogostone, and pachypodol are of particular significance among the many chemicals. The pharmacological effects of these substances include anti-peptic ulcer, antimicrobial, anti-oxidative, anti-inflammatory, and influence on ischemia/reperfusion injury, analgesic, anticancer, antidiabetic, anti-hypertensive, and immunoregulatory effects (Junren et al. 2021).

Patchouli oil is one of the most popular and demanded essential oils (Rekha et al. 2007). This oil cannot be replaced by a synthetic, which increases its value (Ramachandra et al. 2002). Patchouli oil is produced in India; however, to fulfil the domestic demand, India imports oil from Indonesia, Malaysia, and Singapore (Jhunjhunwalla 2006; Srivastava et al. 2022). Indonesia was the main supplier, accounting for more than 80% of India's total import value of patchouli essential oil, which was roughly US$70,319,999 up till 2016 (https://www.zauba.com/

importanalysis-patchouli+oil-report.html; Srivastava et al. 2022).

Patchouli is prone to a variety of ailments, the two most detrimental of which are collar rot and wilt. *Rhizoctonia solani* has been identified as the pathogen responsible for collar rot and wilt and causes more than 20% of the economic losses (Narayanappa et al. 1984). Due to the wide variety of organisms they are connected with, soil-borne illnesses are complicated. Patchouli wilt has also been linked to *Fusarium oxysporum*, according to reports (Gogoi et al. 2017). Typically, synthetic compounds are used to control phytopathogenic organisms. Their broad usage is thought to have contributed to the emergence of pathogens that are resistant to these pesticides (Elad et al. 1992), endangering the stability of crop output. Increased concentrations may result in decreased soil biological activity, which could lead to agricultural fields losing their fertility (Van Zwieten et al. 2010).

The enormous local demand has led to an increase in patchouli cultivation in India and other countries. In another study, we showed that the de-oiled patchouli waste could be successfully bio-converted into enriched vermicompost using efficient bioinoculants viz. *Trichoderma harzianum*, *Pseudomonas monteilii*, *Bacillus megaterium* and *Azotobacter chroococcum* (Singh et al. 2013c). The biomass yield and growth of patchouli were both dramatically boosted by the use of improved vermicompost. An additional investigation discovered that inoculating patchouli plants with particular bioinoculants (N-fixers, AM fungus, and *Pseudomonas*) significantly boosted the herb biomass yield (Singh et al. 2012a). Also, *P. monteilii* and *Cedecea davisae* are established as negative regulators of certain plant pathogens (Singh et al. 2009, 2013a) but the interaction study is lacking in patchouli.

Considering the aforementioned aspects the present study was designed to investigate the interaction between bioinoculants (N-fixers and pseudomonad) and AM fungus (*G. intraradices*) and identify a synergistic combination of a PGPR and AM fungus which could improve the plant growth, and oil yield, soil health and able to suppress *Rhizoctonia* collar-rot/wilt under organic field conditions.

Materials and methods

Microbial cultures

CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India had a vast collection of bioinoculants (26° 89′ N latitude, 80° 98′ E longitude). These bioinoculants have been tested for their ability to promote plant development and combat disease (MAPs). Based on growth promotion, yield-enhancing, and disease-suppressing activities on medicinal and aromatic plants as described in our earlier studies (Singh et al. 2013a, b, c), as well as patchouli pot studies, two native bacterial isolates, were chosen: CRC1 (*Pseudomonas monteilii*-HQ995498; MTCC9796) and CRC2 (*Cedecea davisae*-HQ995499; MTCC9797 (unpublished data). The Microbial Culture Collection of CSIR-CIMAP provided the AM fungus (*Glomus intraradices*; GI), which was recommended in our previous study for better growth and yield of patchouli (Singh et al. 2012a).

Selection of compatible bioinoculants

A compatibility test between CRC1 and CRC2 was carried out by following the methods described by Jain et al. (2011). The 24-hour-old culture of CRC2 was spread on a nutrient agar medium (Himedia, India) containing Petri-dishes and then the CRC1 culture was streaked at the centre of Petri-dishes. The plates were incubated for 48 h at 28°C. The absence of a zone of inhibition at the intersections indicates that the two strains are compatible. The compatibility among two different domains of bioinoculants i.e., bacteria (CRC1 and CRC2) and AM fungus (GI) was also screened out by observing the root colonizing ability and AM spores' population under pot conditions (unpublished data).

Multiplication of bioinoculants

The bacterial culture (CRC1) was multiplied in nutrient broth (Himedia, India) and N-fixer (CRC2) on Jensen's broth (Himedia, India) for 36 h at 210 rpm on an incubator shaker (Excella E24R–New Brunswick Scientific, Eppendorf, India). The bacterial suspension was centrifuged at 8000×g for 10 min. The supernatants were discarded and the pellets containing bacterial cells were suspended in 500 mL of 100 mM phosphate buffer, pH 7.0. The CFU (colony forming unit) in this suspension for bacterial strains was maintained between 2.1 and 2.8×10^8 mL^{-1}.

An inoculum of the AM fungus *G. intraradices* (Singh et al. 2012a) was propagated on maize roots (*Zea mays* L.) for ten weeks in a 1:1 volume mixture of sterilised sand and soil (4.5 kg) with low phosphorus content (6.9 kg ha^{-1}), and it was then allowed to dry for two weeks. A mixture of AM fungus propagules (spores and mycelium) from a dry maize pot culture was used as the inoculum, which was based on the colonised roots and the sand-soil fraction. The roots in the pot culture were taken out of the soil, cut into segments measuring 1 cm, and thoroughly mixed with the sand soil mixture. After that, the mixture was kept at 5 °C until usage. The inoculum potential of the AM fungus used in this experiment was determined using Liu and Luo's methodology to

be 3.9 ± 1.3 infecting propagules g^{-1} of the sand-soil mixture. This is the quantity of inoculums that can infect roots under a typical set of conditions (Liu and Luo 1994).

Collection and multiplication of earthworms

Earthworms (*Eisenia fetida*), acquired from the vermicomposting facility of the CSIR-CIMAP, were multiplied in large numbers in cow dung. The waste from the patchouli distillation was mixed with cow dung to serve as worm-bedding.

Production of quality vermicompost

CSIR-CIMAP has developed and patented a technology to produce high-quality vermicompost (VC) from aromatic oil crop distillation waste (Kalra et al. 2002, 2010; Singh et al. 2012b). To keep the de-oiled patchouli waste moist, tap water was sprayed across it with a hose pipe on alternate days. The epigeic earthworm (adult clitellate *Eisenia fetida*) was transferred into vermicomposting units/pits. Every week, the substrate was turned over to ensure uniformity. After 80 days, the compost was ready to harvest, as indicated by the formation of a consistently sized, dark brown to black granular structure. At this point, the supply of tap water was stopped. The vermicompost was taken out of the pits with the worms two days later and evenly placed on plastic under the cover of shade. By using a 2 mm sieve to recover the granular vermicompost, the worms and their cocoons were separated and employed in the subsequent batch of vermicomposting. Before application in organic plots, the vermicompost was kept in the shade in airtight polyethene bags to preserve the moisture content (around 45%). Every year, samples of sieved vermicompost (approximately 50 g) were taken, and NPK analysis was performed as described by Jackson (1973). On a dry weight basis, the vermicompost contained 1.21% N, 0.67% P, and 0.87% K.

Field study

Patchouli var. 'Johore' nursery was raised from terminal stem cuttings (5-month-old crop) in polyethene bags (7.5 cm×14 cm) filled with a mixture of soil and sand in a ratio of 1: 1 (v/v). Holes were punched in the polyethene bags suitably to provide drainage of excess water if any. AM fungi inoculum (10 g pot^{-1}) was placed adjacent to the stem cuttings of patchouli. However, for bio-inoculants treatment stem cuttings were dipped in their respective treatment for half an hour before planting and the respective culture suspension (5 ml $cutting^{-1}$) was also poured into each treatment. The experimental trials were conducted in the certified organic farm (Ecocert) at CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR–CIMAP), Research Centre, Bangalore, India. Bangalore is located at latitude 12°58′ N, longitude 77°35′ E and 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, percent organic carbon 0.33, available N 183 kg ha^{-1}, Olsen's P_2O_5 13.2 kg $ha^{-1}$and exchangeable K_2O 111.5 kg ha^{-1}.

A picture of efficient bioinoculants CRC1, CRC2, GI and vermicompost, field trial of experiment is depicted in Fig. 1. The field trials were composed of nine treatments (based on our previous findings): CRC1 (*Pseudomonas monteilii*–HQ995498, MTCC9796), CRC2 (*Cedecea davisae*–HQ995499, MTCC9797), GI (*Glomus intraradices*), CRC1 + CRC2, CRC1 + GI, CRC2 + GI, CRC1 + CRC2 + GI, un-inoculated vermicompost, and un-inoculated soil as control with five replications in randomized complete block design. The initial soil samples were collected to determine the initial levels of the bioinoculant population. The 50–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 the last two years where *Rhizoctonia* collar-rot/wilt (> 20% disease incidence) was a consistent problem. In all the treatments (except soil only) the recommended dose of total N requirement (66 kg $ha^{-1}harvest^{-1}$) was supplied through vermicompost as a nutrient supplement based on their N content whereas plots receiving vermicompost only (no bioinoculants) served as control (Singh et al. 2013c). Five plants were randomly tagged for growth/disease severity observations from each net plot and the mean value of five plants was taken for statistical analysis from each plot. Plant height, number of primary branches and wilt incidence were recorded at the time of harvesting. Percent wilt incidence (PWI) (yellowing and drooping of leaves) was observed in each replicated plot before harvest (Singh et al. 2013a).

$$PWI = \frac{Numbers\,of\,wilted\,plants}{total\,number\,of\,plants} \times 100$$

Re-transplanting of the patchouli-rooted cuttings was done following the same methods for 2 years. The harvesting of the crop was done after 155–160 days of transplanting and biomass yield was recorded. The severity of collar/root-rot disease was measured on a 0–4 scale, where 0 = no symptoms, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 ≥ 75% collar/root affected by rot. Based on the collar/root disease symptoms score of each treatment, the percentage disease index (PDI) was calculated (Kesavan and Chowdhary 1977).

$$PDI = \frac{\sum of\,numerical\,grading\,recorded}{number\,of\,roots\,observed \times Highest\,numerical\,rating} \times 100$$

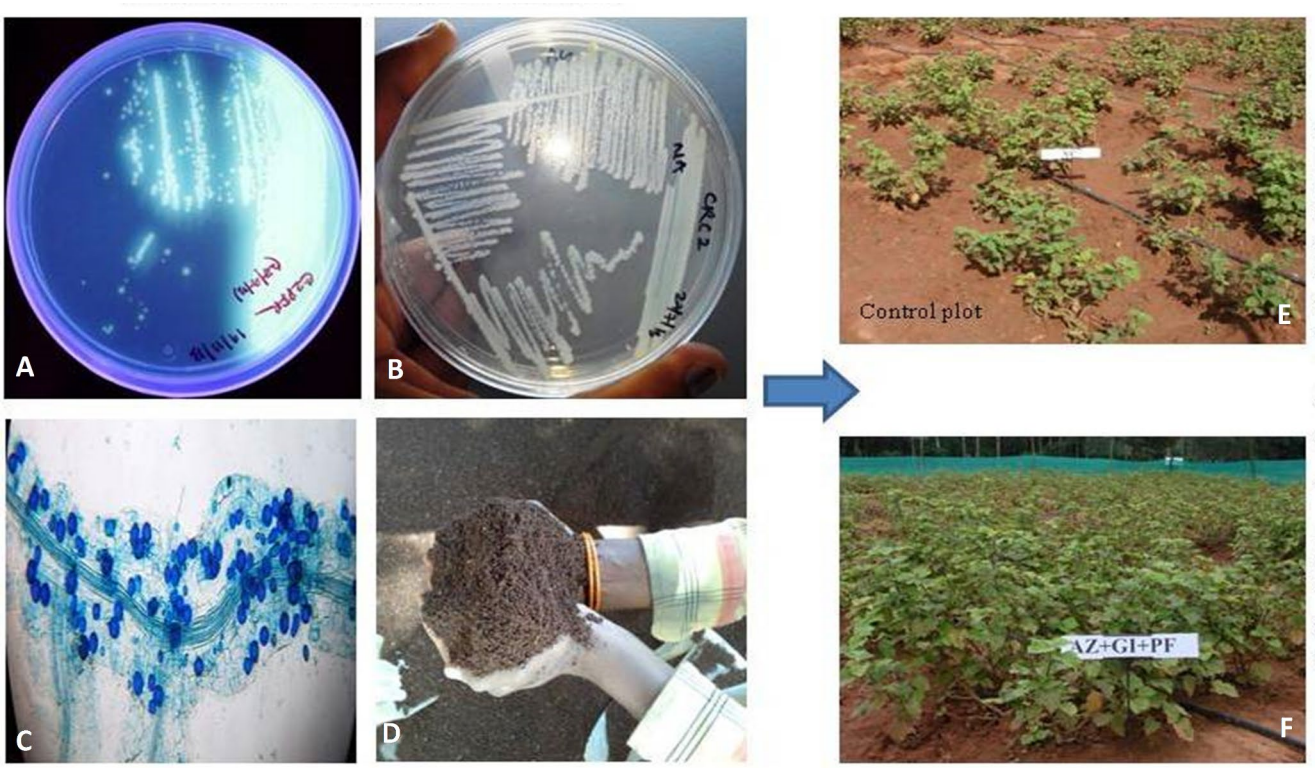

Fig. 1 Efficient bioinoculants **(A)***P. monteilii* str CRC1 **(B)** *C. davisae* str CRC2 **(C)***G. intraradices*; GI and **(D)** Vemicompost, applied in experimental plot (E) Control plot (F) Treated plot

Oil extraction

The harvested plants were shade-dried for 4 days to complete the removal of moisture content. Afterwards, the essential oil was extracted by hydro-distillation process using Clevenger's apparatus (Langenau 1948). The leaves were kept in a round bottom flask and filled with plenty of water to evade overheating. Due to the heat effect, the generated vapour (mixture of essential oil and water) was condensed and collected in other flasks. The pure oil was obtained by water elimination from the mixture using rotavapour. The yield of essential oil was found by the following equation.

$$Y = \frac{W1 \times 100}{W2}$$

Where W1 is the weight of oil collected (g) and W2 is the weight of the plant material (g) used.

Essential oil analysis

The oil samples were analyzed for major constituents using a 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, and 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 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 calculated.

Physical, chemical and microbiological analysis

The rhizosphere soil of each sample was taken after harvesting from a depth of 0–15 cm at 5 random sites near plant roots using a soil auger. Five points of soil were obtained from each plant site, mixed well, sieved, and pooled to form one sample. The sample was kept in sealed plastic bags stored in the refrigerator at 4 °C for further physic-chemical and microbiological analysis.

The pH was determined in a 1:10 (w/v) rhizospheric soil: water suspension. Soil organic carbon was analyzed following the method of Walkley and Black (1934). The NPK analysis in plant samples was carried out according to the procedure of Jackson (1973). To examine the AM fungi colonization, fine roots from host plants were cut into 5 mm size sections, cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman 1970). The

percentage of root length colonized by mycorrhizal fungi was calculated as reported by Mcgonigle et al. (1990). Positive counts for mycorrhizal colonization included the presence of aseptate hyphae/vesicles/ arbuscules. Wet sieving and decanting procedures were used for the isolation and estimation of AM fungal spores from the soil (Gerdemann and Nicolson 1963). Pseudomonads and N–fixer populations [colony forming unit (CFU) g^{-1}] in the root zone soil were determined by serial dilution (10^{-2} folds) with 0.85% saline solution (Denin 1963) using King's B medium (King et al. 1954) and Jensen's medium (Jensen and Petersen 1954) in triplicate, respectively. King and Jensen's medium was supplemented with different concentrations of antibiotics (CRC1: 25 µg mL^{-1} medium rifampicin, strain CRC2: 10 µg mL^{-1} medium streptomycin sulphate) for estimating the selective population of pseudomonads and N-fixers found to be tolerant to particular antibiotics.

Statistical analysis

The collected data were subjected to statistical analysis by analysis of variance method (ANOVA), suitable to randomized complete block design (RCBD) for field experiment, with the help of β-version of software ASSISTAT 7.6. The experimental data of the two trials of 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 the 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 software. The

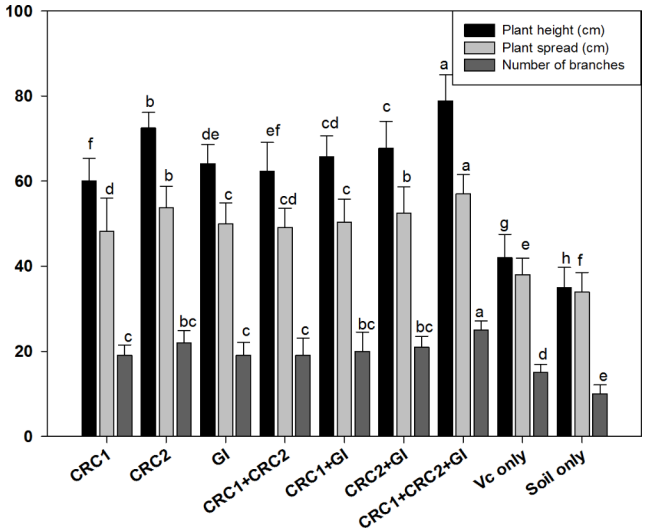

Fig. 2 Effect of bioinoculants and AM fungus (alone or in-combination) on growth characteristics of patchouli. VC, vermicompost; CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*. Error bars shown as standard error of mean (SE). Different letters above the error bars indicate a significant difference at $P \leq 0.05$

results and discussion are based on the average of the trials during the 2 years.

Results

Effect of bioinoculants on growth characteristics of patchouli

Plants treated individually or in combination with bioinoculants or AM fungus significantly improved the growth parameters of patchouli compared to un-inoculated VC control (Fig. 2). Maximum increase in plant height (88%), plant spread (50%) and the number of branches (67%) was achieved when the patchouli nursery was treated with microbial consortium CRC1 + CRC2 + GI followed by CRC2 alone (73, 41 and 47%, respectively) and dual inoculation with CRC2 + GI (61, 38 and 40%, respectively) compared to un-inoculated VC control plants (Fig. 2).

Effect of bioinoculants on shade dry biomass, oil yield, oil quality and incidence of Rhizoctonia collar-rot/wilt of patchouli

The wilt incidence in patchouli plants significantly declined from 55 to 80%, however, the maximum reduction in PWI was observed in CRC1 + CRC2 + GI (Fig. 3A). The infection site was the collar region. The disease progressed upwards to the shoot and downwards to the root. The collar/root of the plant became almost brown to black. The percent disease index (PDI) of *Rhizoctonia* collar-rot ranged from 4.50 to 18.50% in various treatments. The severity of *Rhizoctonia* collar-rot was significantly reduced (68%) in treatment with CRC1 + CRC2 + GI and CRC2 + GI followed by CRC2 (67%). VC alone could also reduce the disease severity by 23% compared to un-inoculated soil-only plots (no vermicompost or bioinoculants) (Fig. 3B). Shade dry biomass (23–67%) and oil yield (23–69%) were significantly higher when the patchouli nursery was treated with various bioinoculants/AM fungus (individually or in-combination) compared to un-inoculated VC control plants (Table 1); Consortium of beneficial microbes CRC1 + CRC2 + GI being most effective yielded considerably higher herb and oil yields (an increase of 67% and 69%, respectively) followed by CRC2 alone (34 and 35%, respectively) and dual inoculation ofCRC2 + GI (31 and 33%, respectively) (Table 1). The content of essential oil varied from 1.94 to 1.96% (on shade shade-dry basis) but no significant differences were observed among the treatments (Table 1). Also, the quality of essential oil was not affected in any of the treatments (Table 2).

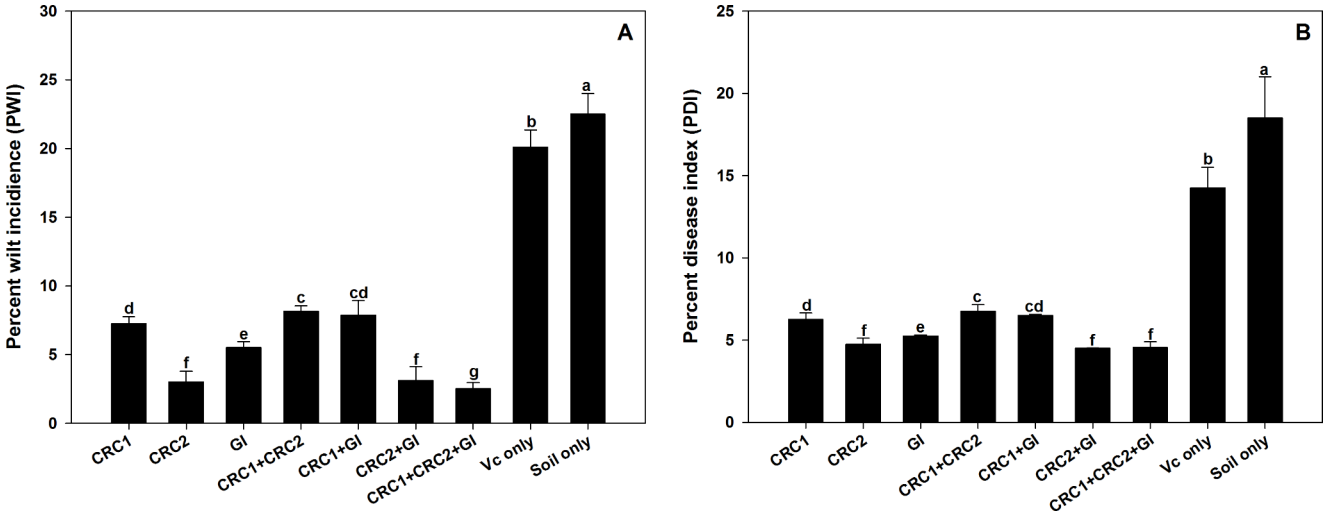

Fig. 3 Effect of bioinoculants and AM fungus (alone or in-combination) on **(A)** Percent wilt incidence (PWI) and **(B)** Percent disease index (PDI) of patchouli. VC, vermicompost; CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*. Error bars shown as standard error of mean (SE). Different letters above the error bars indicate a significant difference at $P \leq 0.05$

Table 1 Effect of bioinoculants and AM fungus (alone or in-combination) on biomass and essential oil yield of patchouli

Treatments	Shade dry herb yield (t ha^{-1})	Oil percent	Oil yield (kg ha^{-1})
CRC1	0.99c	1.95a	19.38d
CRC2	1.08b	1.95a	21.12b
GI	1.04bc	1.95a	20.29c
CRC1 + CRC2	1.03bc	1.95a	20.09c
CRC1 + GI	1.05b	1.95a	20.50bc
CRC2 + GI	1.06b	1.95a	20.71bc
CRC1 + CRC2 + GI	1.35a	1.95a	26.31a
VC only	0.81d	1.95a	15.78e
Soil only	0.51e	1.94a	9.95f

Values in each column followed by different letters are significantly different at $P \leq 0.05$. CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*

Effect of bioinoculants on chemical properties of soil and nutrient uptake by patchouli

Application of bioinoculants or AM fungus individually or in combination significantly improved the percent of total organic carbon (2–13%) (Table 3). However, the maximum increase was observed when the patchouli plants were treated with CRC1 + CRC2 + GI (13%) followed by CRC1 alone (8%) compared to vermicompost control plants (Table 3). Another significant change in pH was observed in patchouli plants treated with microbial consortium CRC1 + CRC2 + GI where the soil pH improved from 6.10 to 6.13 (Table 3). Also, a marked improvement from 6.01 to 6.10 in soil pH was noticed with VC alone compared to un-inoculated soil alone treatment (no vermicompost or bioinoculants) (Table 3).

An increase in the nutrient (NPK) uptake was noticed in bioinoculants or AM fungus-treated patchouli plants but the

Table 2 Gas Chromatography (GC) profile of patchouli oil in field condition

Treatments	Mean chemical composition						
	β-Patchoulene	Caryophyllene	α- Guaiene	Seychellene	α,δ Patchoulene	α-Bulnesene	Patchouli alcohol
CRC1	1.15a	3.21a	9.29a	5.19a	5.05a	10.57a	42.50a
CRC2	1.19a	3.27a	8.94a	5.29a	5.10a	10.32a	41.26a
GI	1.20a	3.83a	8.92a	5.15a	5.19a	10.98a	42.69a
CRC1 + CRC2	1.19a	3.10a	9.01a	5.20a	5.30a	10.11a	42.19a
CRC1 + GI	1.18a	3.28a	9.13a	5.44a	5.53a	10.82a	42.83a
CRC2 + GI	1.19a	3.87a	8.99a	5.55a	5.89a	10.25a	42.13a
CRC1 + CRC2 + GI	1.21a	3.67a	8.79a	5.74a	5.46a	10.11a	42.17a
VC only	1.20a	3.10a	8.55a	5.65a	5.14a	10.99a	42.55a
Soil only	1.21a	3.29a	8.99a	5.66a	5.69a	10.55a	42.50a

Values in each column followed by different letters are significantly different at $P \leq 0.05$. CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*

Table 3 Effect of bioinoculants (CRC1 and CRC2) and AM fungus alone or in-combination on chemical properties and nutrient uptake of patchouli

Treatments	Chemical properties of soil and nutrient uptake (kg ha^{-1})				
	pH	TOC (%)	N	P	K
CRC1	6.11ab	0.52b	41.20c	13.90d	17.00bc
CRC2	6.11ab	0.51bc	43.50b	13.80d	16.90bc
GI	6.08c	0.50 cd	39.80d	14.80c	17.80ab
CRC1 + CRC2	6.09bc	0.51bc	43.90b	13.90d	17.50b
CRC1 + GI	6.10bc	0.47f	41.50c	15.01bc	17.80ab
CRC2 + GI	6.09bc	0.49de	44.60ab	15.10b	18.10ab
CRC1 + CRC2 + GI	6.13a	0.54a	45.40a	15.80a	18.90a
VC only	6.10bc	0.48ef	31.70e	12.50e	15.90c
Soil only	6.01d	0.39 g	28.50f	10.10f	13.10d

Values in each column followed by different letters are significantly different at P ≤ 0.05. CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*

maximum N, P and K uptake (43, 27 and 191%, respectively) were observed in patchouli cuttings treated with microbial consortium CRC1 + CRC2 + GI followed by dual inoculation of patchouli cuttings with CRC2 + GI (41, 21 and 14%, respectively) (Table 3) compared to un-inoculated VC control plants.

Root zone microbial population at harvesting time

The initial population (CFU g^{-1}soil) of N–fixers and fluorescent pseudomonads in the experimental plots were 0.4×10^3 and 0.5×10^3 respectively. On the other hand, the AM fungus population was 88 spores 100 g^{-1} soil. The bioinoculants (*P. monteilii*, *C. davisae*) or AM fungus (*G. intrardices*) populations were significantly higher in their respective treatments where the inoculation was done individually or in combination as compared to un-inoculated VC control plots (Table 4) but the highest population (CFU g^{-1} soil) of N–fixers and fluorescent pseudomonads were maintained in treatment with microbial consortium CRC1 + CRC2 + GI compared to un-inoculated VC control plants (Table 4). Significantly higher root colonization by AM fungus (65%) was observed in the case of CRC1 + CRC2 + GI followed by CRC1 + GI compared to un-inoculated VC control plants but the maximum population of AM fungus (317 spores 100 g^{-1} soil) was recovered in treatment with GI alone followed by CRC1 + CRC2 + GI (315 spores 100 g^{-1} soil)(Table 4).

Discussion

Organic manures and plant-beneficial microbes are vital components for improving soil health and yields in agricultural systems. Delivering both components will be beneficial, particularly in organic fields (Singh et al. 2012a, b; 2012c; Singh et al. 2018; Soni et al. 2014a, b, 2022, 2023). Disease control activity or plant growth promotion can be achieved when beneficial microorganisms are present above 1×10^5 microbial count g^{-1} of seed, root or soil (Raaijmakers and Weller 1998). Generally, the objective of nursery bio-inoculation (especially for patchouli vegetative cuttings) is not to achieve a growth response, but rather to establish a strong relationship of bioinoculants with the plant so that it can be effectively transferred to the field (Singh et al. 2012a). The pre-inoculation of vegetative cuttings in nurseries provides the introduced bacterial strains with a special advantage over the indigenous bacterial/fungal strains after transplanting in the field (Sorensen et al. 2008). Inoculation with AM fungi at very early stages has been found to result in higher crop uniformity, reduced transplant mortality (Waterer and Coltman 1988) and higher yields after transplanting to the field (Lovato et al. 1996).In our previous studies, we reported that even low levels of root colonization (< 18%) at the nursery stage helped spread new roots after transplanting to the organic field which might be adequate for the successful establishment of mycorrhizal plants, especially in the organic field conditions (Singh et al. 2012a, 2018).

Table 4 Mean population of bioinoculants in the root zone soil of patchouli at the time of harvesting

Treatments	Root zone bioinoculants population			
	N-fixers (CFU× 10^4 g^{-1} soil)	Pseudomonads (CFU× 10^4 g^{-1} soil)	AM spores (100 g^{-1} soil)	AM root colonization (%)
CRC1	1.1c	2.6b	125c	36c
CRC2	1.3ab	2.3e	119d	32de
GI	1.1c	2.4d	317a	58b
CRC1 + CRC2	1.4a	2.6b	121d	35 cd
CRC1 + GI	1.2bc	2.5c	312b	61b
CRC2 + GI	1.3ab	2.1f	316a	59b
CRC1 + CRC2 + GI	1.4a	2.7a	315a	65a
VC only	0.7d	0.6 g	101e	31e
Soil only	0.4e	0.5 h	89f	25f

Values in each column followed by different letters are significantly different at P ≤ 0.05. CRC1, *P. monteilii*; CRC2, *C. davisae*; GI, *G. intraradices*

Our past findings in medicinal and aromatic plants (Singh et al. 2012a, b; Soni et al. 2022) are consistent with the fact that AM fungi/bio-inoculants considerably increased plant growth characteristics. Our prior research has demonstrated that the dual inoculation of AM fungi (*G. fasciculatum* or *G. mosseae*) and plant growth-promoting bacteria (PGPR) like *P. monteilii* (strain CRC1) and *Bacillus subtilis* (strain DAz26) can significantly enhance the growth of medicinal plants like *C. forskohlii* and *Artemisia annua* (Awasthi et al. 2011; Singh et al. 2013a).

Plant growth parameters were significantly improved by AM fungi/bio-inoculants which are supported by earlier findings (Singh et al. 2012a, b; Soni et al. 2022), in medicinal and aromatic plants. Our earlier findings have established that dual inoculation of AM fungi (*G. fasciculatum* or *G. mosseae*) along with plant growth-promoting bacteria (PGPR) such *as P. monteilii* (strain CRC1) and *Bacillus subtilis* (strain DAz26) can effectively improve the growth of medicinal plants like *C. forskohlii* and *Artemisia annua* (Awasthi et al. 2011; Singh et al. 2013a). Additionally, patchouli plants grew faster in vermicompost made from distillation waste that was enriched with the microbial consortium *T. harzianum*, *P. monteilii*, *B. megaterium*, and *A. chroococcum* (Singh et al. 2013c). Increased systemic resistance, the generation of plant hormones, enzymes, or antibiotics, a decrease or weakening of pathogens, and the application of AM fungi or bio-inoculants (alone or in combination) contributed ultimately to the growth parameters of the plants (Zahir et al. 2004).

By increasing nutrient availability through N-fixation or P solubilization/mobilization and reducing disease severity, the use of beneficial microorganisms (*P. monteilii*, *C. davisae*, and *G. intrardices*) individually or in combination could have improved patchouli crop development and yield. According to Puttanna et al. (2010), the low nutritional level of the soil and the low capacity of the microorganisms to colonise in poor soil had a negative impact on the growth and yield of the patchouli crop in the soil alone treatment (without vermicompost and bioinoculants). The application of efficient bioinoculants in the proper combination with an organic fertiliser (vermicompost as a nutrient supplement) may be helpful for the growth and yield of patchouli crops. Additionally, more diverse microbial populations in vermicompost (Singh et al. 2012b) may help to inhibit the growth of *R. solani*. Our earlier studies showed the advantages of using vermicompost and effective bioinoculants in reducing disease severity and increasing yields in *C. forskohlii* (Singh et al. 2011, 2012a, b, 2018). The effectiveness of using eco-friendly bio-agents (*T. harzianum*, *G. virens*, and *G. aggregatum*) in reducing patchouli collar rot was previously demonstrated by Mishra et al. (2000). Microbial consortiums like CRC1 + CRC2 + GI may be a good substitute for chemical fertilisers and fungicides, improving yields and reducing the severity of disease while reducing the risks to the environment and human health associated with the use of dangerous chemicals. The choice of prospective bioinoculants/antagonists, method, modes, appropriate combinations, environment, and other elements all play a role in the bioinoculant's performance (Singh et al. 2012a). The effective bioinoculants worked well when vermicompost was added because it provided ideal growth conditions for bioinoculants (near-neutral pH, nutrients, and strong water-holding capacity) (Soni et al. 2022). Vermicompost alone produced a lower yield in the current study, which points to the positive benefits of bioinoculants inoculated at nursery raising time, either alone or in combination.

The treatments had no effect on the essential oil's quality, however using bioinoculants or the AM fungus alone or in conjunction with vermicompost increased the essential oil yield. Similar patterns were seen in a previous study where patchouli plants rooted in vermicompost and supplemented with microbes like AM fungi (*G. aggregatum*, *G. fasciculatum*, *G. intraradices*, and *G. mosseae*) and plant growth promoters (*P. fluorescens*, *B. subtilis*, *B. megaterium*, and *Azotobacter*) when transplanted into pots and fields produced significantly more essential oil yield (Singh et al. 2012a).

The nutrient uptake (NPK) by patchouli was considerably improved by either a single or combined inoculation of *P. monteilii*, *C. davisae*, and *G. intrardices*. The maximum uptake of NPK was seen in plants inoculated with CRC1 + CRC2 + GI. Similar outcomes were noted in plants such as the *Eucalyptus hybrid*, *Ficus benjamina*, and *C. forskohlii* that had been exposed to a favourable microbial and fungal consortium (Srinath et al. 2003; Singh et al. 2013a). *C. davisae* has also demonstrated improved N uptake capacity in basil crops, making it a suitable substitute for N-fixation in patchouli (Singh et al. 2013b). According to Naik et al. (2008), P. *Monteilii* is a P-solubilizer, which may have helped *C. forskohlii* absorb phosphorus more effectively. The AM fungus (*G. intrardices*) and *P. monteilii* play a crucial role in solubilizing inorganic phosphate, which could then be transported to the plants by the hyphae of the mycorrhizal fungi. This means that *P. monteilii* could be an incredibly useful microbe in organic or sustainable farming where phosphorus is a major bottleneck. Additionally, its antagonistic behaviour toward plant pathogens (such as *F. chlamydosporum* and *R. solani*) and compatibility with N-fixers and AM fungi to form a powerful microbial consortium may be helpful in organic farms for managing nutrients and diseases.

In the treatment with CRC1 + CRC2 + GI, higher levels of pseudomonad and N-fixer populations, as well as more numbers of AM fungal spores and increased mycorrhizal

root colonisation, were observed. Higher populations of beneficial microorganisms in the soil under field settings may have an impact on the species makeup of the soil microbial community (Krishnaraj and Sreenivasa 1992). A link between AM root colonisation and rhizospheric bacterial populations was shown by Soni et al. (2014; 2022). According to Edwards et al. (1998), G. *mosseae* boosted the *P. fluorescens* population in the rhizosphere of tomato (*L. esculentum*) and leek (*A. porrum*), but the bacterium had no impact on the AM fungal population. In the present study, the increased population of AM fungi or fluorescent pseudomonad or N-fixers following co-inoculation of *P. monteilii*, *C. davisae* and *G. intrardices*, as compared to single inoculations, probably contributed to the better performance of patchouli in the presence of all the beneficial microbes. Another study by Duponnois and Plenchette (2003) found that the *P. monteilii* strain (HR13) promoted mycorrhization of *Acacia mangium* and *A. auriculiformis*. *P. monteilii* is hence referred to as a mycorrhiza helper bacterium (MHB). According to Srinath et al. (2003), MHB promotes AM development by secreting hydrolytic enzymes that enlarge cortical cells and create broader intercellular spaces, making it easier for AM fungus to infiltrate and ramify in the root system.

Conclusion

The current research unquestionably showed that native potential bioinoculants employed for nursery rearing may be successfully transferred to organic field environments. In conclusion, microbial consortia are composed of two different domains and have different functional activities viz. AM fungus *G. intraradices*- assist in nutrient uptake; growth promoter and antagonist *P. monteilii* (CRC1); and N-fixer *C. davisae* (CRC2) may be helpful to improve the biological management of nutrients and diminish collar rot/wilt in patchouli, induced by *R. solani*, acting as the potential antagonist/growth promoter and subsequently leading to yield enhancement. This approach could be used in a variety of vegetatively propagated and transplanted crops which may be adopted for safe and sustainable agriculture, especially in the case of medicinal and aromatic plants where the use of synthetic pesticides is strictly prohibited due to their residue and health concerns. Moreover, this study also proved that all the bioinoculants worked synergistically as indicated by their substantial population in the rhizosphere post-harvest of patchouli and therefore could be a source of inoculum of beneficial microbes for subsequent season crops.

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Authors contribution Rakshapal Singh: conceived the research, and designed experiments; Rakshapal Singh and Sumit K. Soni performed the experiments. Rakshapal Singh and Sumit K. Soni analyzed the data; Rakshapal Singh, Sumit K. Soni, and Anju Bajpai prepared the MS draft. Rakshapal Singh, Sumit K. Soni, and Anju Bajpai finally edited and prepared the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

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