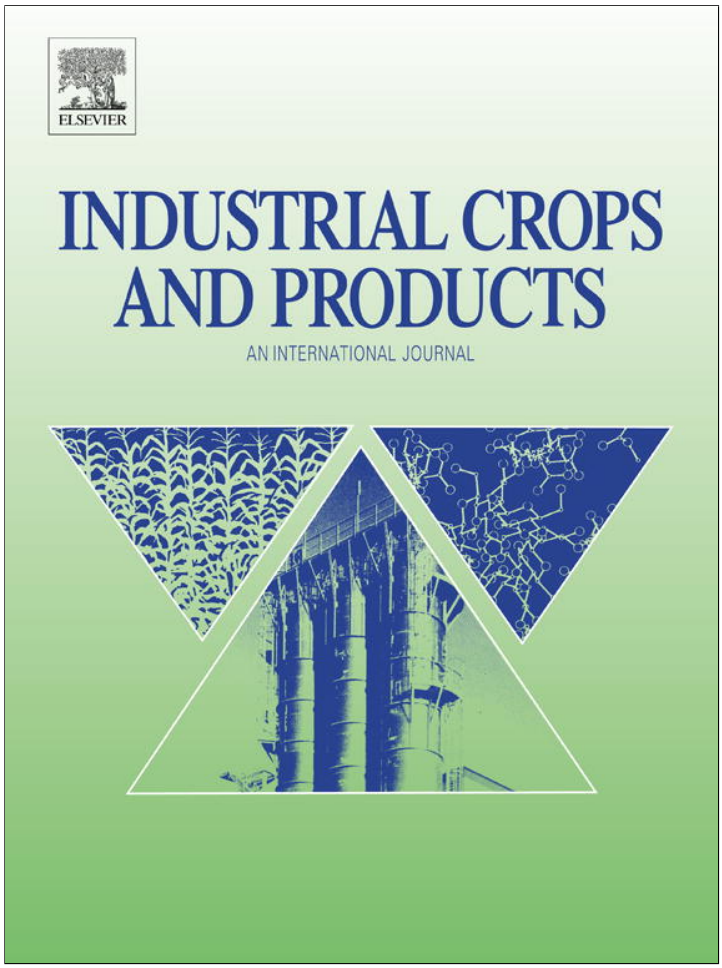

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Technology for improving essential oil yield of *Ocimum basilicum* L. (sweet basil) by application of bioinoculant colonized seeds under organic field conditions

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ABSTRACT

Two year field studies indicated that seed treatment of *Ocimum basilicum* var. CIM-Saumya with efficient bioinoculants (*Pseudomonas monteilii* – strain CRC1, *Cronobacter dublinensis* – strain CRC3 and *Bacillus* spp. – strain AZHGF1) can significantly improve the essential oil yield (45–56%); maximum essential oil yield was observed in plants inoculated with *P. monteilii* (56%) followed by *C. dublinensis* (49%) and *Bacillus* spp. (45%). The content of essential oil was also significantly improved (15%) when inoculated with *P. monteilii* compared to un-inoculated control. The higher concentrations of linalool (40.40%) and β-caryophyllene (14.15%) were observed in the plants inoculated with *P. monteilii. P. monteilii* also produced maximum biomass yield; an increase of about 55% followed by *C. dublinensis* (42%) and *Bacillus* spp. (30%). To the best of our knowledge this might be an exclusive report suggesting the use of bioinoculants for higher yields and disease management for organic growers of sweet basil.

1. Introduction

Sweet basil (*Ocimum basilicum* L.), one of the major essential oil producing species belonging to the to the family *Lamiaceae* (Grayer et al., 1996), is an annual herb, native to India and other regions of Asia (Klimánková et al., 2008) and commonly used in many kinds of food preparations in Mediterranean countries. Sweet basil is commonly cultivated in areas with warm climatic conditions, such as India, Greece, Morocco and Madagascar. In India, it is mainly cultivated in Uttar Pradesh, Haryana and Punjab, and has recently been introduced in the southern and western parts of India. Basil leaves containing essential oils of distinctive aroma can be used, both fresh and dried, to spice up various kinds of meals (Klimánková et al., 2008). Basil is an economically and industrially important plant. It's essential oil is synthesized and stored in glandular trichomes of leaves (Sangwan et al., 2001) and are used as flavourings agents in foods and beverages, as fragrances, as fungicides, or insecticides and in various pharmaceutical and industrial products (Grayer et al., 1996). The qualitative and quantitative improvement of essential oil production represents an area of high commercial interest (Copetta et al., 2006). The presence of essential oils and their composition determine the specific aroma of plants and the flavour of the condiment. Not only the type of cultivar, but also the agronomical practices and environmental conditions affect the composition of sensory important compounds (Jirovetz et al., 2003). Regardless of these factors, 1,8-cineole, methyl cinnamate, methyl chavicol, and linalool (Lee et al., 2005) are generally the main compounds responsible for the typical basil aroma. The area under cultivation of basil (>25,000 ha) in India accounts for annual production of about 250–300 t of essential oil (Varshney, 1997), which is still short of its demand in the country. Besides local demand, there exist good possibilities for its export in the world market.

A number of reports exists suggesting the use of bioinoculants such as plant growth promoting bacteria and arbuscular mycorhizal (AM) fungi for enhancing growth and yield of different medicinal and aromatic crops (Singh et al., 2009, 2012a,b,c; Awasthi et al., 2011) but only few reports suggest the usefulness of different microbes in sweet basil (Copetta et al., 2006; Rasouli-Sadaghiani et al., 2010). The crop is affected by vascular wilt/rot caused by *Fusarium oxysporum* f.sp. *basilici*. The pathogen has been reported to spread by aerial means as air-dispersed conidia and through infested seeds (Gamliel et al., 1996). It may therefore be desirable to select bioinoculants which could be useful as growth promoter as well as antagonists for suppressing disease, improving yields especially under organic field conditions (free from chemical pesticides and fertilizers). The aim of the present study was to evaluate the potential bioionculants for the treatment of seeds which would be useful in disease management (better seed germination with better survival) and in enhancing biomass, essential oil quality and yields of *O. basilicum*; and a successful technology to deliver

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sufficient number of bioinoculants along with seedlings at the time of transplanting to organic fields, when seeds were raised in nursery along with bioinoculants.

2. Materials and methods

2.1. Isolation of pathogen

Diseased basil plants (sweet basil var. CIM-Saumya) were collected from the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Bangalore. Sections (2–5 mm) of root and stem were taken, dipped into 70% ethanol, surface sterilized with NaOCl (1%) for 1 min, and rinsed in sterile distilled water. Sections were placed on peptone–pentachloronitrobenzene *Fusarium* selective medium (FSM) (Gamliel and Katan, 1991). Petri plates were incubated at 28 °C in a BOD incubator for 5–7 days and the fungus growing from the infected bits was isolated and its pathogencity was established (using Koch's postulates) using 30-day old seedlings in pots containing sterilized soil. The fungus (strain FO1) was identified as *Fusarium oxysporum* f.sp. *basilici* following the method described by Gamliel et al. (1996) and preserved in −80 °C with 50% glycerol in microbial culture collection of CSIR-CIMAP for further studies.

2.2. Isolation and selection of bacterial strains

The bacterial strains CRC1 and OS11 were isolated from the rhizospheric soil [red sandy loam (*Kandiustalf*) in nature and had pH 6.3, and contained 0.37% organic carbon, available N (Alkali permanganate extractable) 218 kg ha^{-1}, available P (0.5 M $NaHCO_3$ extractable) 10.5 kg ha^{-1}, exchangeable K (NH_4OAc extractable) 230 kg ha^{-1}] of medicinal plants *Coleus forskohlii* and *Ocimum sanctum*, respectively. Strain AZHGF1 was isolated from the rhizospheric soil (0–15 cm) of horse gram (*Macrotyloma uniflorum*) field and rest of the bacterial strains (CRC2, CRC3 and CRC4) were isolated from vermicompost. Granular vermicompost had major nutrient concentration of 1.01% N, 0.78% K, 0.65% K and pH 7.02. Soil/vermicompost suspension was obtained by shaking 10 g of soil sample in Erlenmeyer flasks containing 90 mL of 0.1 M $MgSO_4{\cdot}7H_2O$ buffer for 30 min at 200 rpm on a rotary shaker. Resulting suspensions were serially diluted and 0.1 mL aliquots of each dilution were spread onto King's medium B (KB) agar (g L^{-1}) (proteose peptone Difco: 20, K_2HPO_4: 1.5, glycerol: 10 mL, agar: 15, pH: 7.0), nitrogen free (NFB) media (g L^{-1}) (K_2HPO_4: 0.8, KH_2PO_4: 0.2, $FeCl_3{\cdot}6H_2O$: 0.05, $CaCl_2{\cdot}2H_2O$: 0.05, $NaMoO_4{\cdot}2H_2O$: 0.05, mannitol: 15, $MgSO_4{\cdot}7H_2O$: 0.05 and agar: 18, pH: 7.0) and Piskovskaya's agar (g L^{-1}) (yeast extract: 0.5, dextrose: 10, calcium phosphate: 5, ammonium sulphate 0.5, potassium chloride 0.2, magnesium sulphate 0.1, manganese sulphate 0.0001, ferrous sulphate 0.0001, agar: 15, pH: 7.0) in triplicates. After incubation at 28 °C for 2 days, fluorescent pseudomonads produced a yellow pigment that fluoresces under long wave ultraviolet light (366 nm) on King's B agar medium. Bacterial isolates grown on NFB medium further inoculated into culture vials containing 5 mL of NFB semi-solid culture medium (Döbereiner, 1995). About 7 days after incubation at 28 °C, the isolates that formed a pellicle, characteristic of free living diazotrophs, were streaked on NFB medium supplemented with 20 mg L^{-1} yeast extract. The isolates that grew on solid medium were then re-inoculated in the NFB medium. To assess the ability of bacteria to solubilize inorganic phosphate, isolates were transferred to the Petri dishes containing Piskovskaya's agar and incubated at 28 °C for 5–7 days. The colonies with a clear halo zone around, where the phosphate had been solubilized were considered inorganic phosphate solubilizers. A total of 16 bacterial strains exhibiting fluorescence, N fixing and P solubilzing abilities were obtained following above said procedures. Of these 6 bacterial strains (CRC1, CRC2, CRC3, CRC4, OS11 and AZHGF1) based on their strong antagonistic activity against *F. oxysporum* f.sp. *basilici* (strain CIMAP-FO1) under *in vitro* conditions (dual culture method) using modified potato dextrose agar (PDA) medium (g 500 mL^{-1} PDA (Hi Media)-19.5, peptone-1, yeast extract-0.5, agar-2.5) (Tiwari et al., 2010) and their growth promoting ability in pot conditions (un-published data) were selected.

2.3. Molecular characterization of the bacterial isolates

Bacterial genomic DNA was extracted from overnight grown cells using standard procedures (Chachaty and Saulnier, 2000), electrophoretically separated on 0.8% agarose gel in TAE buffer and visualized under UV (uvitec, Bangalore Genei, India) to check for integrity. The extracted DNA was quantified spectrophotometrically (Nanodrop ND1000). The universal primers (forward 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse 5′-ACGGCTACCTTGTTACGACTT-3′) described earlier (Awasthi et al., 2011) were used to amplify the 16S rRNA gene from the bacterial strain. Approximately 25 ng of bacterial genomic DNA and 10 pmol of forward and reverse primer, 0.6 U of Taq polymerase, 2.5 μL of 10× buffer (Bangalore Genei, India) were used for amplification in a Mastercyler gradient (Eppendorf) programmed as 94 °C for 5 min; 34 cycles of 94 °C for 1 min, 57.4 °C for 1 min, 72 °C for 2 min; 72 °C for 10 min; 4 °C for an infinite period. PCR products were checked in 1.26% agarose gels in TAE buffer stained with ethidium bromide (0.5 mg mL^{-1}) and visualized under UV. The PCR product was purified using PCR Cleanup Kit (Genaxy) according to the manufacturer's instructions and directly sequenced using the forward universal primer and Big Dye® Terminator *v*3.1 cycle sequencing kit (Applied Biosystems, USA) on a 3130xl Genetic Analyzer (Applied Biosystems, USA) using the manufacturer's protocol. Sequence analysis was carried out using the nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) to identify and download the nearest neighbour sequences from the NCBI database. All the sequences were aligned using ClustalW alignment tool. ClustalW was accessed through the MEGA, version 5 software (Tamura et al., 2011). The phylogenetic tree was constructed by using Bootstrapped neighbor-joining tree method using 1000 replication. Further the partial sequence of 16S rRNA gene sequence of strain CRC1, CRC2, CRC3, CRC4, AZHGF1 and OS11 determined in this study were submitted to NCBI GenBank data base under the accession number HQ995498; HQ995499; HQ995500; HQ995501 and JN700924, QG461751 respectively.

2.4. Preparation of bio-inoculums

The bacterial cultures (CRC1, CRC4, and OS11) were multiplied in nutrient broth and N-fixers/diazotrophs (CRC2, CRC3, and AZHGF1) on Jensen's broth for 36 h at 210 rpm on an incubator shaker. The bacterial suspension was centrifuged at 8000 rpm 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 1.8 and 2.5 × 10^8 mL^{-1}.

2.5. Seed treatment

Approximately 0.135 g (containing 100 no.) seeds of *O. basilicum* cv. CIM-Saumya were surface sterilized by gently shaking in 1% NaOCl solution for 3 min and rinsed six times for 5 min in sterile deionized water. For each treatment (consisting of three replicates), seeds were soaked in their respective culture suspension (containing ≈2.0 × 10^8 bacterial cells mL^{-1}) in an individual Erlenmeyer flask for half an hour and seeds treated with sterile phosphate buffer

(without culture) served as control. The treated and untreated seeds were sown in plastic pots (10 cm in diameter and 15 cm long; 100 seeds pot^{-1}), holding approximately 550 g potting mixture consisting of sterile soil and vermicompost (1:1/10, v/v), replicated three times in completely randomized design (CRD). The respective culture suspension (5 mL pot^{-1}) was also poured in each treatment. The soil used for seed germination was red sandy loam (*Kandiustalf*) in nature and had pH 6.1, 0.43% organic carbon, available N (Alkali permanganate extractable) 221 kg ha^{-1}, available P (0.5 M $NaHCO_3$ extractable) 9.3 kg ha^{-1}, exchangeable K (NH_4OAc extractable) 218 kg ha^{-1}. The vermicompost used in the pot and field experiment was produced from mixture of distillation waste (plant-spent de-oiled herb) of aromatic grasses (*Cymbopogon winterianus* and *C. flexuosus*) in a vermicomposting unit for 90 days using adult clitellate *Eudrilius eugineae*, epigeic species of earthworms, at our certified organic farm. The vermicompost contained 1.05% N, 0.65% P and 0.71% K. Watering was carried out with fine rose can. The pots were maintained up to 30 days. Before transplanting to fields, number of seedlings was counted for calculating percent seed germination and 10 nursery seedlings were randomly selected from each pot for seedling height and number of leaves. The rhizospheric soil samples of seedlings from each replicated treatments were collected to estimate the population build up of seed treated/inoculated bioinoculants.

2.6. Field trials

The experiment was conducted in a certified (ECOCERT) organic farm at the CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Bangalore (India), located at 12°58′N77°35′E and 930 m above mean sea level. The chemical characteristics of experimental field soil were same as in pot experiment. The treatments were imposed in plots (2.7 m width × 2.7 m length) with 3 replications arranged in randomized block design (RBD). The initial soil samples (200 g) were collected with the help of soil auger (0–15 cm) from five points of each replicated treatments. The soil samples were pooled, mixed and subsample was used to record the initial microbial population (beneficial and pathogenic) prior to transplanting and application of bio-inoculants. The soil had uniform infestation of *Fusarium* [CFU $5.3 \pm 0.1 \times 10^4$ g^{-1} soil] throughout the field. The seedlings (30-day-old) of *O. basilicum* var. CIM-Saumya were gently removed from potting medium. Each seedling contained approximately 8–10 g of rhizospheric soil (wet) as carrier of inoculums for respective bioinoculants. Transplanting was done into planting holes having a depth of 10–12 cm and dia of 8–10 cm by placing individual seedlings on flat beds at a spacing of 45 cm × 45 cm. There were six rows per plot. Transplanting of *O. basilicum* crop was done in similar manner in same plots and treatments for second cropping year also. The total N requirement (80 kg ha^{-1}) was fulfilled by the application of vermicompost used as organic nutrient supplement for supporting growth of the plants. Each bed contained 36 plants, only 16 net plants (non-peripheral plants to avoid border effect) were considered for various observations in seven treatments: plots receiving seedlings raised in nursery from the seeds treated with (i) *Pseudomonas monteilli* (CRC1) (ii) *Cedecea davisae* (CRC2) (iii) *Cronobacter dublinensis subsp. dublinensis* (CRC3), (iv) *Advenella* spp. (CRC4) (v) *Bacillus subtilis* (OS11) (vi) *Bacillus* spp. (AZHGF1) and (vii) plots receiving untreated seedlings (control). All the plots were supplemented with equal doses vermicompost (approximately 8 t ha^{-1}) irrespective of bioinoculant treatments and control.

2.7. Plant growth observations and incidence of wilt in field

Randomly tagged 5 out of 16 net plants from each plot were considered for growth parameters (plant height and plant spread). Plant growth parameters such as plant height (measured from soil surface to the growing tip of the plant) and plant spread were recorded at each harvest during both the years, however, mean plant height and plant spread over harvests and years, is presented in Fig. 4. Percent wilt incidence (PWI) (yellowing and drooping of leaves, browning of vascular tissues of stem) was assessed in the field with net plants of each replicated plots before harvest; PWI = (Numbers of wilted plants/total number of plants) × 100.

2.8. Harvesting

The crop was harvested two times during each cropping year. The first harvesting (at the cutting height of 10 cm from ground) was done after 90 days of transplanting and the second harvest after 60 days of first harvest. Fresh shoot biomass was recorded from net plots. For the estimation of essential oil content in fresh herb, 200 g green shoot biomass was collected just before harvesting from each plot and was hydro-distilled in a Clevenger's hydro distillation apparatus. To obtain oil yield (kg ha^{-1}), fresh herb yield of the plot was multiplied with corresponding oil content (v/w %) and 0.9 (approximate specific gravity of oil) and the resultant yields were extrapolated to per hectare basis. Fresh shoot and root biomass sample (100 g each) was dried in a hot air oven at 80 °C for 24 h to determine the moisture content and nutrient concentration. From the moisture content and biomass yields, dry matter yields were calculated. Nutrient concentration (NPK) in dried root and shoot was determined by Jackson (1973). The total uptake by plants was determined by considering nutrient concentration and total dry matter yield. At the time of harvesting, rhizospheric soil samples (200 g) were also collected with the same manner (as in initial soil sampling) to determine microbial population (both beneficial and pathogenic) from each bed. After harvesting, disease severity was measured taking into account the stems and roots harvested from each replicated net plots.

2.9. Percent disease index (PDI)

Disease severity was measured on a 0–4 scale where 0 = healthy plants with no symptoms and 4 = >75% roots affected by rot (brown and black discoloration of roots and basal stems) and black lesion along the stems (Gamliel et al., 1996). Based on the scoring the percentage disease index (PDI) was calculated as follows:

$$\text{PDI} = \left(\frac{\text{Sum of numerical grading recorded}}{\text{Number of roots observed} \times \text{highest numerical rating}} \right) \times 100$$

2.10. Gas chromatography (GC) analysis for O. basilicum

GC analysis of essential oil samples were carried out for their chemical constituents, *viz.* methyl chavicol and linalool using Varian CP-3800 with star work station chromatography data system fitted with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m × 0.25 mm; film thickness 0.25 μm) with 100% dimethyl polysiloxane. Nitrogen was the carrier gas at 0.5 mL min^{-1} constant flow rate. The column was initially held at 120 °C for 2 min then heated to 240 °C at a rate of 80 °C min^{-1} held for 3 min. The injector and detector temperature were 250 °C and 300 °C respectively. Samples (0.2 μL) were injected with a 20:80:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_6–C_{19}). Peak areas and retention times were measured by an electronic integrator. The relative percentages of individual components were determined by the peak area percentages.

2.11. Microbial population estimation

Population of fluorescent pseudomonads, P solubilizing bacterium, *Bacillus* and N-fixer populations (CFU g^{-1} soil) in the root zone soil were determined by serial dilution technique with 0.85% saline solution using King's B medium (King et al., 1954), P solubilizing bacterium zone formation and cultivation medium (Pikovskaya, 1948), *Bacillus* isolation and cultivation medium (Claus and Berkeley, 1986) and NFB medium (Döbereiner, 1995), respectively. NFB medium was supplemented with different concentration of antibiotics (strain CRC2: 10 μg mL^{-1} medium streptomycin sulphate, strain CRC3: 30 μg mL^{-1} medium rifampicin, strain AZHGF1: 5 μg mL^{-1} medium kanamycin) for estimating selective population of N-fixers/diazotrophic organisms found to be tolerant to particular antibiotics and concentrations. The density of *Fusarium* population was estimated by using *Fusarium* selective medium (FSM) (Gamliel and Katan, 1991).

2.12. Statistical analysis

The collected data were subjected to statistical analysis for 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). Significant differences among treatments were based on the *F*-test in ANOVA and means were calculated using Duncan's multiple range test under a significance level of $P \leq 0.05$. There were two trials conducted for pot and field experiments. The experimental data of two trials had a similar variance value; hence the data were combined for further analysis. The results and discussion are based on the average of the trials of each experiment. The standard error (SE) of means in vertical bar charts was computed using Sigma Plot 11.

3. Results and discussion

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

The BLAST analysis of the 16S rRNA gene sequence from the CRC1, CRC2, CRC3 and CRC4 isolate revealed that the bacteria belong to the genus *Pseudomonas*, *Cedecea*, *Cronobacter* and *Advenella*, respectively and another two isolates OS11 and AZHGF1 belong to the genus *Bacillus* and their phylogenetic position in relation to other related species is given in Fig. 1. Phylogenetic analysis showed that isolate CRC1, CRC2, CRC3, CRC4, OS11 and AZHGF1 has a maximum similarity with *P. monteilii* strain WAPP53 (accession no. FJ905913), *C. davisae* str. NBRC 105,702 (accession no. AB682275), *C. dublinensis* str. G3977 (accession no. HQ880440415), *Advenella spp.* Str. SS-2009-PON8 (accession no. FN646615), *B. subtilis* (accession no. AJ276351) and *Bacillus* sp. str. PCA-2 (accession no. FN666529), respectively; therefore isolate CRC1, CRC2, CRC3, CRC4, OS11 and AZHGF1 were designated as *P. monteilii*, *C. davisae*, *C. dublinensis subsp. dublinensis, Advenella* spp., *B. subtilis* and *Bacillus* spp., respectively. This identification of the isolates was later confirmed by CSIR-IMTECH (Institute of Microbial Technology), Chandigarh, India, and the isolates CRC1, CRC2, CRC3, CRC4 and OS11 are deposited with Microbial Type Culture Collection (MTCC), Chandigarh, India with MTCC nos. 9796, 9797, 9798, 9799 and 10,010 respectively.

3.2. Effect of potential bioinoculants on seed germination and growth rate

The germination of seeds improved (35–48%) significantly when the seeds were treated with *P. monteilii*, *C. dublinensis subsp. dublinensis*, *B. subtilis* and *Bacillus* spp. compared to un-inoculated control. Maximum seed germination (48%) was observed with *P. monteilii* (data not presented). Treatment of cotton seeds with *P. fluorescens* having strong inhibitory effect against *Rhizoctonia solani*, significantly increased the seedling survival from 30 to 79% (Howell and Stipanovic, 1979); this antagonism exhibited by the fluorescent pseudomonads could be possibly the result of the production of an antifungal antibiotic pyrrolnitrin. Seedling growth characteristics improved significantly with *P. monteilii*, *C. dublinensis subsp. dublinensis and Bacillus* spp; an increase of 54%, 38% and 36% in seedlings height and 80%, 60% and 40% higher in number of leaves, respectively (data not presented). Lifshitz et al. (1987) found that increased growth promotion and yield of canola (rapeseed) seedlings can be achieved by the inoculation with plant growth promoting bacteria (PGPR). Similarly sugarbeet seeds when treated with fluorescent *Pseudomonas* spp. resulted in higher growth and yield (Suslow and Schroth, 1982). Improved growth of seedlings by plant growth-promoting rhizobacteria has been observed in many crops (Weller and Cook, 1986; Enebak et al., 1998). Increases in growth (shoot length and number of leaves) observed in nursery plants/seedlings due to application of bio-inoculants might be the result of combination of effects like production of plant hormones, enzymes or antibiotics, enhanced availability of nutrients, increased systemic resistance and decrease in population or weakening of pathogens (Zahir et al., 2004). Such improvement in plant growth parameters by bio-inoculants in medicinal and aromatic plant has also been noticed (Singh et al., 2009, 2012a,b).

3.3. Microbial population build-up at the time of transplanting

At the time of transplanting microbial population of seed treated/inoculated bioinoculants was estimated in the rhizosphere of nursery seedlings. The population (CFU g^{-1} potting medium) of *P. monteilii*, *C. davisae*, *C. dublinensis* subsp. *dublinensis*, *Advenella* spp., *B. subtilis* and *Bacillus* spp. ranged from 7.9×10^6 to 9.5×10^6 in the rhizospheric soil of seedlings but did not differ significantly among the treatments (data not presented).

Generally, the objective of nursery/seed inoculation 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 (Sylvia, 1989; Singh et al., 2012a). The pre-inoculation of seeds in sterilized substrates in nurseries, provides the introduced bacterial strains a special advantage over the indigenous bacterial/fungal strains after transplanting in field (Sorensen et al., 2008). Our earlier studies have indicated that the vermicompost produced from distillation waste medicinal and aromatic plants supports the growth of bioinoculants (Kalra et al., 2010; Singh et al., 2012a). Therefore, the present study was designed to evaluate the usefulness of beneficial bioinoculants as seed treatment in vermicompost as a part (1/10 v/v) of potting medium for effective multiplication and transfer to the fields along with basil seedlings for improving productivity under organic farm conditions. Delivery and maintaining the population of bioinoculants in sufficient numbers is important. 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). In the present study we observed that the population of bioinoculants was far more than the thresholds limit (10^5 microbial count g^{-1}).

3.4. Effect of potential bioinoculants on growth characteristics of O. basilicum under organic field conditions

The growth parameters (plant height and spread) of basil crop under organic field conditions were significantly influenced by all seed treated bioinoculants compared to un-inoculated control.

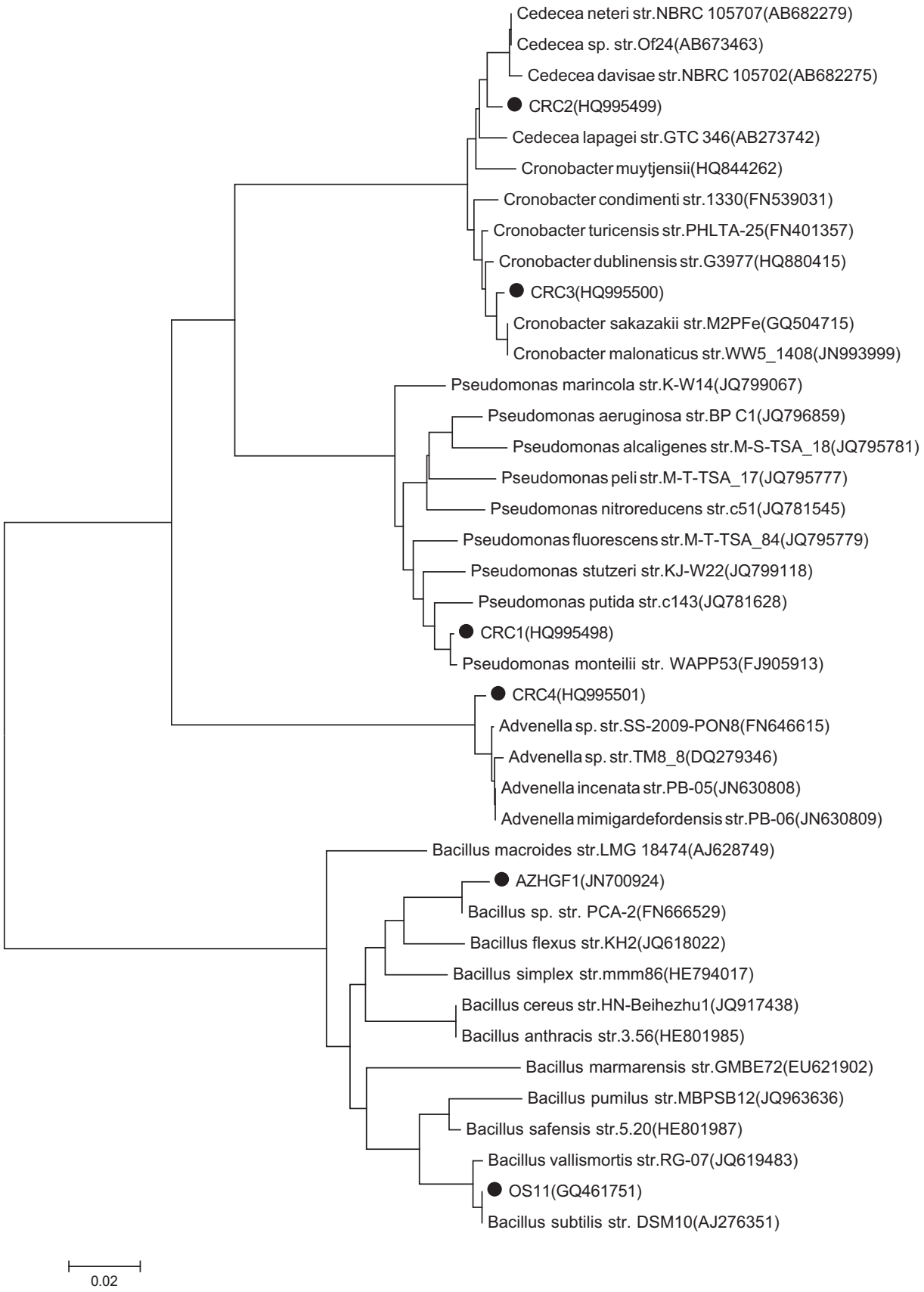

Fig. 1. Phylogenetic tree constructed from the 16S rRNA gene of strains CRC1, CRC2, CRC3, CRC4, AZHGF1 and OC11and related organisms constructed using neighbour-joining algorithm from an alignment of 798 nucleotides. Accession numbers of corresponding sequences are given in parentheses and scale bar represents 1 base substitution per 20 nucleotide positions. The bootstrap probabilities calculated from 1000 replications.

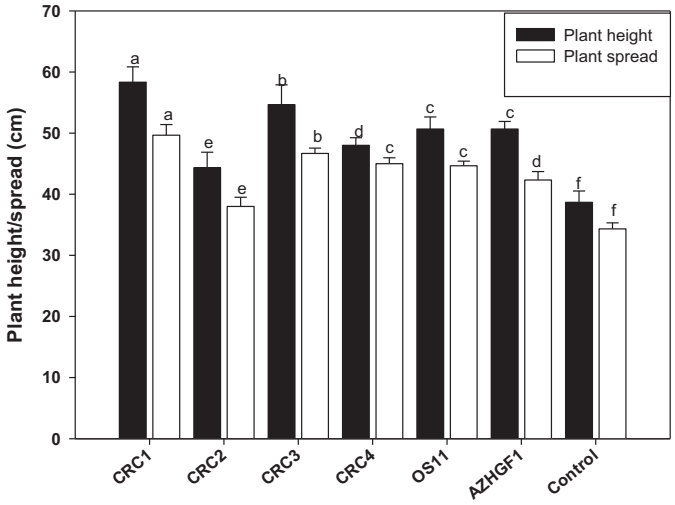

Fig. 2. Effect of potential bioinoculants (CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *C. dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp.) on growth characteristics of *O. basilicum* in organic field conditions. Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \leq 0.05$.

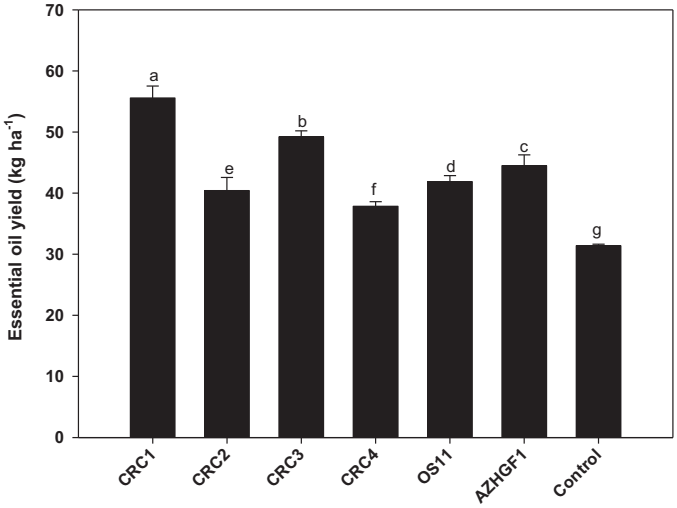

Fig. 4. Effect of potential bioinoculants (CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp.) on essential oil yield of *O. basilicum* in organic field conditions. Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \leq 0.05$.

Maximum increase in plant height (51%) and spread (47%) was achieved when the seeds were treated with *P. monteilii* followed by *C. dublinensis subsp. dublinensis* (41% and 35%, respectively) (Fig. 2). In our earlier experiments, we observed that plant growth parameters in *C. forskohlii* and *Pogostemon cablin* improved significantly by the application of PGPRs and AM fungi (Singh et al., 2009, 2012a,b). *P. monteilii* (strain CRC1) has earlier been shown to play a significant role in improving growth and reducing the disease incidence in medicinal plant *C. forskohlii* (Singh et al., 2012c).

3.5. Effect of potential bioinoculants on biomass yield of O. basilicum under organic field conditions

Total biomass yield of two harvests over the years was significantly higher when seeds were treated with various bioinoculants compared to un-inoculated control (Fig. 3); *P. monteilii* being most effective yielding higher (55%) herb followed by *C. dublinensis subsp. dublinensis* (42%) and *Bacillus* spp. (30%) (Fig. 3). Singh et al. (2012c) observed that inoculation of *P. monteilii* in *C. forskohlii* plants significantly improved the root and shoot biomass. In another study of Singh et al. (2012a) plant growth promoting bacteria and AM fungi significantly improved the shade dried herb biomass of patchouli (*P. cablin*). Inoculation of *O. basilicum* roots with PGPRs have been earlier found to improve growth and biomass yield (Ordookhani et al., 2011). Tiwari et al. (2010) reported that inoculation of endophytic bioinoculant *B. subtilis* significantly improved the growth and fresh biomass yield of *O. sanctum*. Treatment of seeds in nursery containing good amount of vermicompost appear to be a simple and economical way of multiplication and establishing a strong relationship of bioinoculants in the root rhizosphere and subsequently effective transfer to the fields.

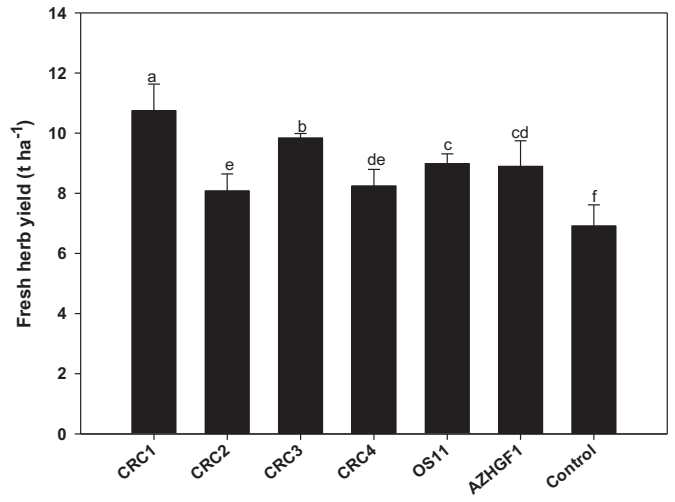

Fig. 3. Effect of potential bioinoculants (CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp.) on biomass yield of *O. basilicum* in organic field conditions. Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \leq 0.05$.

3.6. Effect of potential bioinoculants on content of essential oil, yield and quality of O. basilicum under organic field conditions

Only *P. monteilii* could significantly improve (15%) the content of essential oil (data not presented). A significant increase in essential oil yield (42–56%) could be achieved with bioinoculants; maximum being observed in plant treated with *P. monteilii* (56%) followed by *C. dublinensis* (49%) and *Bacillus* spp. (45%) (Fig. 4). The quality of essential oil which is basically considered by major constituents like methyl chavicol, linalool and caryophyllene, no significant differences were observed in the case of methyl cahvicol. However, the significant increase in the concentration of linalool and caryophyllene was observed in plants inoculated with *P. monteilii* strain CRC1 (Table 1). Singh et al. (2012a) also reported that inoculation with PGPRs and AM fungi though significantly enhanced the essential oil yield but no significant differences were observed in essential oil content of industrially important aromatic crop like patchouli. Ordookhani et al. (2011) reported that inoculation with PGPR enhanced the accumulation of essential oil of basil. In yet another study on medicinal plant *C. forskohlii*, inoculation with PGPR and AM fungi improved the forskolin content which is the active constituent of *C. forskohlii* tuberous-roots (Singh et al., 2009, 2012b,c).

Table 1
Gas chromatography (GC) profile of essential oil of sweet basil.

Treatment	Linalool	Methyl chavicol	β-Caryophyllene
CRC1	32.80a	63.02a	1.59a
CRC2	27.33b	64.90a	1.31c
CRC3	27.78b	63.27a	1.38bc
CRC4	25.76bc	64.54a	1.44b
OS11	27.31b	64.51a	1.32c
AZHGF1	32.10a	64.47a	1.33c
Control	23.36c	64.74a	1.39bc

CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp. values in vertical column followed by different letters are significantly different at $P \leq 0.05$.

3.7. Effect of potential bioinoculants on nutrient uptake of *O. basilicum* in organic field conditions

An increase in the nutrient (NPK) uptake was noticed in bioinoculants treated seedlings of basil plants but the maximum N, P and K uptake (42, 58 and 51%, respectively) was observed in seedlings treated with *P. monteilii* followed by *C. dublinensis* (45, 47 and 31%, respectively) (Fig. 5). In our earlier studies it was demonstrated that inoculation with PGPRs significantly improved the uptake of major nutrients (Singh et al., 2009, 2012a,b,c). The other study (Ordookhani et al., 2011) also provided a clear evidence for improving uptake of nutrients by the inoculation with PGPRs.

3.8. Effect of potential bioinoculants on percent wilt index (PWI) and percent disease index (PDI) of *O. basilicum* under organic field conditions

It was clearly demonstrated from the present 2-year field experiments that the inoculation with efficient PGPRs could significantly reduce the wilt and root rot incidence in *O. basilicum* (Fig. 6) under organic field conditions. Maximum reduction in the incidence and severity of the disease in terms of PWI and PDI was observed in plots with *P. monteilii* treated seedlings 93 and 83% followed by *C. dublinensis* (86 and 77%) and *Bacillus* spp. (85 and 77%), respectively (Fig. 6). In our recent study, *P. monteilii* was found to be a potent antagonist against *Fusarium* in medicinal crop *C. forskohlii* (Singh et al., 2012c). Singh et al. (2009, 2012b) clearly demonstrated the use of PGPRs not only improved the growth and yield of *C. forskohlii* but also significantly reduced the disease severity.

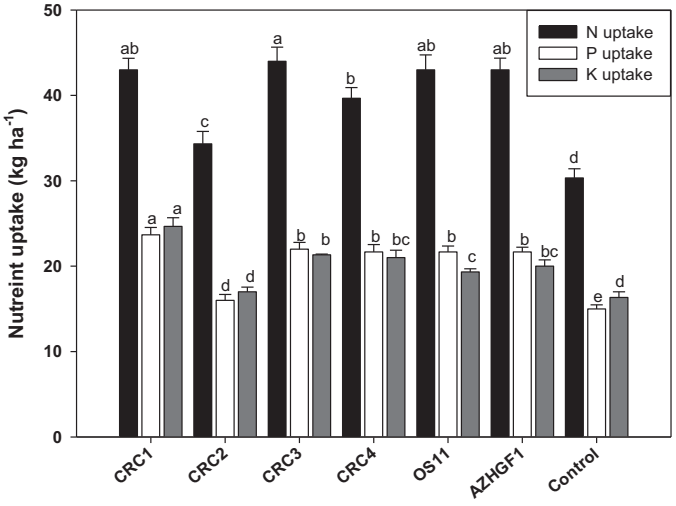

Fig. 5. Effect of potential bioinoculants (CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp.) on major nutrient uptake of *O. basilicum* in organic field conditions. Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \leq 0.05$.

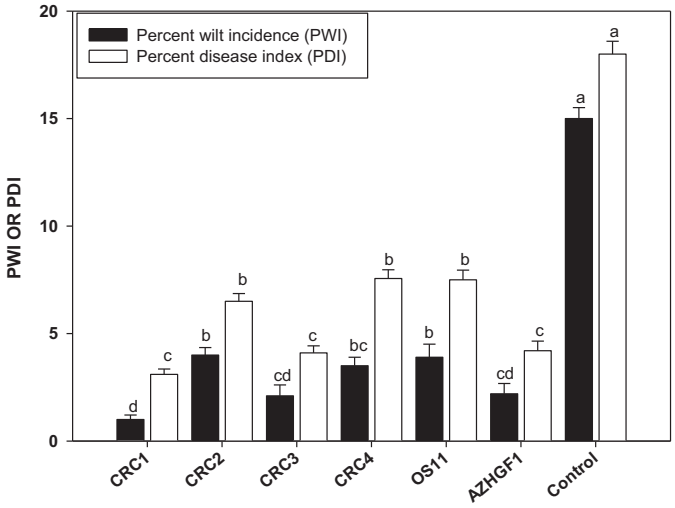

Fig. 6. Effect of potential bioinoculants on (CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp.) on PWI and PDI of *O. basilicum* in organic field conditions. Error bars shown as standard error of mean (SE), different letters above the error bars show significant difference at $P \leq 0.05$.

3.9. Effect of potential bioinoculants on mean population of bioinoculants and Fusarium in the rhizosphere of *O. basilicum* in organic field conditions

The microbial population (CFU g^{-1} soil) of fluorescent pseudomonads, N-fixers, P solubilizers, *Bacillus* and *Fusarium* were 3.5×10^4, 5.7×10^4, 1.8×10^4, 140×10^4 and 5.4×10^4, respectively, before transplanting. The population of fluorescent pseudomonads, N-fixers, P solubilizers and *Bacillus* in the rhizosphere was significantly enhanced by all the treatments compared to un-inoculated control. However, the maximum population of fluorescent pseudomonads, N-fixers, P solubilizers, *Bacillus* were noticed in *P. monteilii* (180%), *C. dublinensis* (51%), *Advenella* spp. (89%) and *Bacillus spp.* (21%), respectively, over control (Table 2). *Fusarium* population (CFU g^{-1} soil) was reduced significantly by the use of bioinoculated seedlings whereas maximum decrease was observed with *P. monteilii* (58%) followed by *C. dublinensis* (54%) and *Bacillus* spp. (49%) (Table 2). Singh et al. (2009, 2012b,c) earlier showed that the use of efficient bioinoculants significantly reduced the pathogen (*Fusarium* and *Ralstonia*) levels in *C. forskohlii* fields.

Table 2
Mean population of bioinoculants and *Fusarium* in rhizospheric soil of *O. basilicum* at harvesting.

Treatments	Rhizospheric population (CFU × 10^4 g^{-1} soil)				
	Fluorescent pseudomonads	N-fixers	P solubilizers	*Bacillus*	*Fusarium*
CRC1	9.8a	7.2c	3.2ab	165ab	2.3e
CRC2	5.5d	7.0d	3.1bc	160b	4.5b
CRC3	8.5b	8.8a	3.5ab	167a	2.5de
CRC4	6.5c	7.1cd	3.6a	151c	3.5c
OS11	6.2c	6.2e	2.8c	169a	3.6c
AZHGF1	8.0b	8.1b	2.9c	171a	2.8d
Control	3.5e	5.8f	1.9d	141d	5.5a

CRC1 *Pseudomonas monteilii*; CRC2 *Cedecea davisae*; CRC3 *Cronobacter dublinensis subsp. dublinensis*; CRC4 *Advenella* spp.; OS11 *Bacillus subtilis* and AZHGF1 *Bacillus* spp. values in vertical column followed by different letters are significantly different at $P \leq 0.05$.

4. Conclusion

The present studies undoubtedly indicated that native potential bioinoculants used for seed treatment for raising nursery could be successfully maintained, multiplied and transferred to organic fields in adequate numbers resulting in improved biological management of nutrients and plant diseases leading to yield improvement. This technology could be implemented in many seed germinated transplanted crops and could be adopted for safe and sustainable agriculture especially in medicinal plants where the use of chemicals is restricted because of health and residue considerations.

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