Microbial Inoculants Assisted Growth of *Chrysopogon zizanioides* Promotes Phytoremediation of Salt Affected Soil

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Abstract Restoration of salt-affected soil through cultivation *Chrysopogon zizanioides* is a promising approach. The two way benefit of such an approach is that reclamation of salt-affected soil concomitant to improve plant growth and increased yield of essential oil produced in the plants roots. Earlier studies showed physiological changes and reduced growth of *C. zizanioides* under salinity. In the present study, plant growth promoting microorganisms viz. *Pseudomonas monteilii*, *Bacillus megaterium*, *Azotobacter chroococcum* and *Rhizophagus intraradices* were used as bio-inoculants for cultivation of *C. zizanioides* under salt-affected soil. Bio-inoculants in combination with vermicompost significantly increased the growth and productivity of *C. zizanioides* under salt-affected soil, and simultaneously improved soil health. When compared to control, the soil physico-chemical and biological properties of bio-inoculants treated plants was significantly improved. The reclamation of salt-affected soil was evident by the significant decrease in the level of soil pH (11.0%), electrical conductivity (23.5%), sodium adsorption ratio (15.3%), and exchangeable sodium percent (12.4%) of bio-

inoculants treated plants. The improvement of soil cation exchange capacity indicated the decrease in soil salinity. Whereas increase in the microbial count (four-fold), AMF spores (447 spores), dehydrogenase (six-fold), acid (two-fold) and alkaline phosphatase (five-fold) activities in rhizosphere soil of bio-inoculant treated plants indicated the improved biological properties. A positive correlation of plant biomass production to soil organic carbon, total Kjeldahl nitrogen, available phosphorus and cation exchange capacity depicted improved nutrients content in rhizosphere soil of bio-inoculant treated plants. The findings of this study suggest that *P. monteilii* and *R. intraradices* with vermicompost can be effectively used as bio-inoculants for encouragement of phytoremediation in salt-affected soil.

Keywords Microbial inoculants · Phytoremediation · Plant growth · *Chrysopogon zizanioides* · Salt-affected soil

Introduction

Salt-affected soils are widespread in arid and semi-arid regions and occupy approximately 3.0% of total geographical area of world. The extensive agricultural practices with poor irrigation system and climatic changes had lead to increase in the percentage of salt-affected soil [1]. About 402 million hectare (Mha) area is classified as saline soil and 434 Mha land has been categorized as sodic soil [1]. These type of soils are characterized by high soil pH, electrical conductivity, sodium concentration (high exchangeable sodium percent and sodium absorption ratio) and least organic carbon [2, 3]. Soil salinization and sodication alters the physico-chemical and biological properties of the soil [1]. Excessive salts in soil decreases water

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and nutrients availability to plants and inhibits different metabolic processes resulting in stunted growth and productivity of the cultivating crops [4]. The other deleterious effect of salinity includes the reduction of soil microbial population and their activities [1, 5].

The physico-chemical properties of the salt-affected soils can be improved using good irrigation system, appropriate drainage system, cost-effective soil amendments, and suitable crop management practices [6]. The salt-affected soils are also being reclaimed and/or rehabilitated through afforestation and cropping with or without any fertilizers (chemical or organic), and biological amendments [2]. Phytoremediation is a promising and cost-effective method for the reclamation of salt-affected soil. Efficacy of phytoremediation depends on factors including soil characteristics, plant growth and plant biomass. Therefore, sufficient plant growth in salt-affected soil is the pre-requisite requirement for the reclamation of salt-affected soil, and this is a matter of concern because salinity stress inhibits plant growth and productivity [4]. Different methods including plant breeding, development of transgenic plants and crop cultivation in alliance to microbial inoculants [plant growth promoting rhizobacteria (PGPR) and Arbuscular mycorrhiza (AM)] have been used to reduce the deleterious effects of salinity to plants [7, 8]. PGPR increases plant growth by production of phytohormones and siderophores, phosphate solubilization and fixation of atmospheric nitrogen [9]. AM fungi exist in symbiotic association to plants and assist them to acquire immobile nutrients from nutrient deprived soils. Under the salt stress, AM fungi improve water and nutrient supply to plants and alleviate the adverse effect of salinity, and also improve the soil structure and aggregation [4, 10, 11].

The increase in human population has raised the demand of growing food crops and thus, cultivation of aromatic and medicinal crops has become a challenging task. The cultivation of aromatic crops in a degraded lands facilitates the restoration of degraded land and provide economic benefits. Grasses are considered more tolerant to environmental stresses and reclamation of the salt-affected soil through cultivation of different types of grasses have been reported earlier [1, 12]. *Chrysopogon zizanioides* commonly known as “vetiver or khus” is an aromatic grass having long and thick root system. The economic value of *C. zizanioides* is for its ability to produce essential oil in the secretory cells of the cortical sheet surrounding the endodermis of mature roots [13]. The vetiver essential oil is a complex mixture of sesquiterpene alcohols and hydrocarbons which is used as aroma ingredients in perfumery and cosmetics industry [13, 14]. Vetiver is a moderate salt tolerant plant which has been cultivated for restoration of salt-affected lands [15–17]. Conversely, studies have demonstrated reduced growth and physiological changes in

vetiver plants under high salinity [17]. Application of plant growth promoting microorganisms (bio-inoculants) would promote the cultivation of *C. zizanioides* under salt affected soil. The benefit of microbial assisted growth of *C. zizanioides* under high salinity includes reclamation of salt-affected soils and increased yield of economically important essential oils. Thus, objective of present study is to develop microbial inoculants for cultivation of aromatic grasses under high salinity. The microbial consortia described in this study efficiently increased the growth and productivity of *C. zizanioides* under high salinity, and rehabilitation of salt-affected soil was evident by improved physio-chemical and biological properties, and increased nutrient level of salt-affected soil.

Materials and Methods

Experiment Set Up

Salt-affected soil was collected from Khantari village (27°02'07.8"N, 80°59'50.5"E), Lucknow, Uttar Pradesh, India. The soil sample was processed and passed through 2 mm sieve. The soil was sterilized by autoclaving (polybags size, 14" × 20", Himedia BioHazards) at 121 °C for 1 h at three subsequent time interval. Physico-chemical properties of soil such as pH, electrical conductivity (EC_e , in saturated soil extracts), cation exchanged capacity (CEC), exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR) were analysed as described elsewhere [18]. In this study, the phosphate-solubilizing *Bacillus megaterium* (ATCC 14581), nitrogen-fixing *Azotobacter chroococcum* (MTCC 446) [19], indole acetic acid (IAA) producing *Pseudomonas monteilii* (HQ995498) [20] and AMF *Rhizophagus intraradices* were used as bio-inoculants for cultivation and growth promotion *C. zizanioides* under salt affected soil. The pure cultures of microorganisms used in this study were obtained from Microbial Technology Division, CSIR-CIMAP, Lucknow, India.

The experiment was conducted in completely randomised design (CRD) and all treatments replicated three times ($n = 3$). Vetiver slips with roots were obtained from the research farm of CSIR-CIMAP, Lucknow, India (26.5°N, 80.5°E). Before plantation, the rooted slips were washed to remove adhering soil particles and surface sterilized using 5% sodium hypochlorite for 10 min. The bacterial inoculum was prepared by growing cultures in 500 ml nutrient broth medium at 30 °C and 170 rpm. The cells were harvested from the exponential phase culture by centrifugation at 6000g for 10 min. The cell pellet was washed twice with sterile normal saline and re-suspended in 100 ml sterilized normal saline to adjust $A_{600\text{ nm}}$ to 1.0.

Surface sterilized slips were pre-treated (soaked) with the respective bacterial culture for 30 min. After treatment, slips were planted (1 slip pot⁻¹) in ceramic pots containing 12 kg autoclaved salt-affected soil and vermicompost. After plantation, specific bacterial suspension was poured into their respective treatments while normal saline was used for the control pot. The *R. intraradices* inoculum was propagated on maize roots (*Zea mays* L.) for 12 weeks in sterilized soil having low phosphorus content. The colonized roots were chopped into 1 cm segments and thoroughly mixed with the potting medium and stored at 5 °C until use. Five gram of AMF culture {containing soil, root segments, fungal spore (12–14 spore g⁻¹ soil) and mycelium} were mixed into the soil in respective treatments. Treatments were abbreviated as following: (1) Control (CS); (2) 5 t ha⁻¹ vermicompost (VC); (3) *B. megaterium* + 2.5 t ha⁻¹ vermicompost (BMV); (4) *P. monteilii* + 2.5 t ha⁻¹ vermicompost (PMV); (5) *Azotobacter chroococcum* + 2.5 t ha⁻¹ vermicompost (ACV); (6) *R. intraradices* + 2.5 t ha⁻¹ vermicompost (RIV); (7) *B. megaterium* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost (BMRIV); (8) *P. monteilii* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost (PMRIV); (9) *A. chroococcum* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost (ACRIV); (10) Inorganic fertilizer (N:P:K, 80:60:50) + *R. intraradices* (IFRI). Pots were carefully irrigated with sterile tap water whenever required to maintain soil moisture near field capacity throughout the experimental periods.

Evaluation of Growth and Yield of *Chrysopogon zizanioides*

After 12 months, the plants were harvested and weight of fresh biomass of shoots and roots were recorded. For extraction of essential oil, the freshly harvested root samples from each treatments were hydrodistilled in a Clevenger apparatus for 16 h. The essential oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at cool and dark place for further analysis. Essential oil yields were calculated by measuring the weight of essential oil (gram) per kilogram of fresh root. For quantification of total Na⁺ and K⁺ content fresh samples of shoots and roots were oven dried at 70 °C for 72 h for measurement of dry weight. The dried samples were ground to pass through a 0.2 mm mesh and 0.2 g plant sample were taken to which 10 ml di-acid (HNO₃ + HClO₄, 3:2 ratio) was added. This mixture was kept at Foss digester for digestion at 200 °C for 30 min. Total Na⁺ and K⁺ concentrations were determined in digested samples by Flame photometry, and phosphorus content was determined spectrometrically [21]. Likewise, the carbon

content in the biomass was evaluated with the help of CHNS analyzer (Euro Vector Elemental Analyzer 3000).

Arbuscular Mycorrhizal Fungi (AMF) Root Colonization

AMF root colonization was observed for each replicated treatments. AMF root colonization was determined by method described by Phillips and Hayman [22]. The fine roots were collected and washed properly with tap water. After washing, the roots were chopped into small pieces of 1.0 cm. About 1.0 g root pieces were incubated for 1 h in KOH solution (10% w/v) at 80 °C in a hot air oven. After heating, the roots were thoroughly rinsed with distilled water, dipped in 0.1 N HCl and again rinsed with distilled water. Cleared roots were stained with 0.05% trypan blue for observation of colonization (arbuscules, vesicles). Roots were de-stained and images were captured under a dissecting microscope at 40 × magnification [23].

Analysis of Post-harvest Soil

After 12 months growth, plants were uprooted and removed soil from the roots. Soil adhering to the root surfaces was collected, processed and used for evaluation of physico-chemical and biological properties. Soil organic carbon (SOC) was determined by rapid dichromate oxidation method [24], total Kjeldahl nitrogen (TKN) by micro Kjeldahl methods, available phosphorus (P) by Olsen's method [25] and available potassium content of the soil was determined using flame photometer by neutral normal ammonium acetate method [26]. For determination of total microbial population, were 0.1 ml serially diluted soil suspensions were spread on nutrient agar plates. Plates were incubated for 24 h and data were presented as colony forming unit (CFU) per gram of soil. Spores of AMF were extracted from 50 g of fresh soil sample by wet-sieving method [27]. Soil dehydrogenase (DHA), alkaline (ALP) and acid phosphatase (ACP) enzyme activities were determined as described elsewhere [28].

Data Analysis

Data (n = 3) were analysed using SPSS statistic software version 17.0 (IBM, Chicago) package for Windows and subjected to one-general linear model ANOVA (analysis of variance). The Tukey's test was used for post hoc comparisons to determine differences between means. Differences were considered significant at *P* < 0.05. The correlation coefficient (Pearson) was also performed among various observed parameters by using SPSS 17 software.

Results and Discussion

Plant Growth and Biomass Production Under Salt-Affected Soil

Phytoremediation of salt affected soil through cultivation of high value aromatic crop like vetiver provides greater economic benefits. However, soil salinity adversely affects the plant growth and productivity imposing restrictions to the phytoremediation based rehabilitation of degraded soil. The role of microbial inoculants in reclamation of deteriorated soils has been well documented [9]. This study describes the reclamation of salt-affected soil by bio-inoculants assisted cultivation of *C. zizanioides* which is an economically important aromatic plants. The experimental soil having high level of soil pH (9.90), EC_e (6.80 dS m^{-1}), ESP (50.70), SAR (17.60) and a lesser amount of amount of SOC (2.4 g kg^{-1} soil), AVP (6.0 $kg\ ha^{-1}$), TKN (0.02%) depicted high level salinity and nutrient deprived status of soil (Table S1). In this study, vermicompost which is a stabilized organic materials produced by the combined action of microorganisms and earthworms was used as a carrier material for application of bio-inoculants [29]. Salinity stress to bio-inoculant is significantly reduced when they are applied in combination to vermicompost, and this allows bio-inoculants to establish and proliferates under stressed environment. Earlier study have reported that application of bio-inoculant enriched vermicompost (BEVC) significantly reduced the percent disease index of *Rhizoctonia* root rot and improved essential oil yields of *P. cablin* [19]. Plant growth attributes under stressed soil was recorded in terms of shoot and root biomass, plant and root dry weight (Fig. 1). The increased plant growth in VC amended treatment (salt-affected soil amended with 5 t ha^{-1} VC) in

comparison to control treatment CS (only salt-affected soil) depicted stress mitigation and plant growth promotion ability of vermicompost in salt-affected area. Vermicompost contains higher amount of essential nutrients (N, P and K) required for plant growth and soil conditioning [20, 30]. In treatment VC, vermicompost was used in double amount with the view to the make a comparative analysis of plant growth in presence of vermicompost (5 t ha^{-1}), and bio-inoculants + 2.5 t ha^{-1} vermicompost (treatments BMV to ACRIV). The equivalent growth of plants in treatment containing bio-inoculants + 2.5 t ha^{-1} vermicompost and treatment VC (5 t ha^{-1} vermicompost) substantiated the role of bio-inoculant in plant growth promotion under salt-affected soil. Microbial inoculants used in this study increases plant growth by different mechanisms including synthesis of phytohormone, phosphate solubilisation and biological nitrogen fixation [9]. *Pseudomonas monteilii* produced indole 3-acetic acid required for root proliferation whereas *B. megaterium* is an efficient phosphate solubilizer. The nitrogen fixing *A. chroococcum* was used to increase the nitrogen content of soil [19, 20]. Likewise, AM fungi (*R. intraradices*) confer several benefits to plants including increased uptake of water, and acquisition of phosphorus and other immobile nutrients [20]. When compared to un-amended control (CS), the increase in plant biomass (shoot and root biomass) was observed in all treatments and maximum plant biomass was observed in treatment PMRIV (*P. monteilii* + *R. intraradices* + 2.5 t ha^{-1} vermicompost) followed by treatment BMRIV (*B. megaterium* + *R. intraradices* + 2.5 t ha^{-1} vermicompost). The elevated plant growth in treatment PMRIV and BMRIV in comparison to treatment RIV (*R. intraradices* + 2.5 t ha^{-1} vermicompost) could be accredited to the synergistic activity of *P. monteilii* and *B. megaterium* with *R. intraradices*. *P. monteilii* is an

Fig. 1 Growth attributes of *Chrysopogon zizanioides* plants in salt-affected soil. Bar indicates standard error of mean (SE, n = 3) and dissimilar letters above the bars indicate significant difference at $P < 0.05$ by using Tukey's test

efficient plant growth promoting rhizobacteria which produces indole 3-acetic acid (IAA) required for root proliferation [20]. Likewise, *B. megaterium* solubilized phosphate which was sequestered and eventually transferred to plant by *R. intraradices* (Fig. 1). When compared to control treatment CS, the essential oil yield was maximally observed in the plants harvested from treatment PMRIV. This observation could be accredited to the fact that *P. monteilii* present in treatment PMRIV is a good producer of IAA which lead to the development and proliferation of vetiver roots containing essential oils. This was further corroborated by observation that treatment PMRIV has maximum root biomass and hence yielded maximum essential oil (Figs. 1, 2, 3). When compared to treatments BMV, PMV and ACV (bio-inoculants + 2.5 t ha⁻¹ vermicompost), the increased plant growth in treatments BMRIV, PMRIV and ACRIV (bio-inoculants + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) depicted the role of AM fungi in plant growth under salt-

affected soil. *R. intraradices* provides mineral nutrients and water to host plant, and adsorbs potassium ions which eventually increases the plant growth [31].

Effect on Physico-Chemical Properties of Post-harvest Soil

Analysis of post harvest rhizosphere soil depicted the significant improvement in the physico-chemical properties of salt-affected soil (Table 1). A decrease in soil EC_e, SAR and ESP was observed in the rhizosphere soil of bio-inoculant treated plants. The pH of rhizosphere soil obtained from bio-inoculants treated plant was significantly reduced in comparison to rhizosphere soil collected from control treatment CS. The significant decrease in the soil pH of treatments BMRIV (*B. megaterium* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) and PMRIV (*P. monteilii* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) indicated improved plant growth due to the

Fig. 2 Uptake of phosphorus and potassium by *Chrysopogon zizanioides* plants at harvesting stage. Bar indicates standard error of mean (SE, n = 3) and dissimilar letters above the bars indicate significant difference at $P < 0.05$ by using Tukey's test

Fig. 3 Essential oil yield of *Chrysopogon zizanioides* plants in salt-affected soil. Bar indicates standard error of mean (SE, n = 3) and dissimilar letters above the bars indicate significant difference at $P < 0.05$ by using Tukey's test

Table 1 Physio-chemical properties of post-harvest soil of *Chrysopogon zizanioides*

Treatments	pH	EC _e (dS m ⁻¹)	CEC (meq 100 g ⁻¹ soil)	ESP	SAR	SOC (mg kg ⁻¹ soil)	TKN (mg kg ⁻¹ soil)	AVP (kg ha ⁻¹ soil)	AVK (kg ha ⁻¹ soil)
CS	9.8 ± 0.2 ^e	6.0 ± 0.30 ^b	13.1 ± 0.8 ^a	48.7 ± 1.6 ^d	16.9 ± 0.5 ^b	29.5 ± 2.1 ^a	5.6 ± 0.2 ^a	5.2 ± 0.2 ^a	372.3 ± 5.3 ^{bc}
VC	9.0 ± 0.8 ^{abcd}	5.2 ± 0.3 ^a	13.5 ± 0.9 ^{ab}	45.8 ± 1.7 ^{abc}	15.3 ± 0.9 ^a	49.5 ± 3.2 ^{ef}	6.9 ± 0.4 ^c	7.3 ± 0.7 ^e	373.8 ± 2.9 ^c
BMV	9.2 ± 0.3 ^{bcd}	5.3 ± 0.2 ^a	13.3 ± 0.7 ^{ab}	46.9 ± 1.5 ^{bcd}	15.7 ± 0.5 ^{ab}	42.4 ± 3.1 ^{cd}	5.7 ± 0.7 ^{ab}	6.0 ± 0.5 ^b	369.2 ± 6.6 ^{bc}
PMV	9.2 ± 0.2 ^{cd}	5.5 ± 0.3 ^{ab}	13.3 ± 1.1 ^{ab}	47.1 ± 2.5 ^{bcd}	16.0 ± 0.5 ^{ab}	41.1 ± 4.0 ^{cd}	5.6 ± 0.7 ^a	6.6 ± 0.2 ^d	344.1 ± 6.7 ^{ab}
ACV	9.1 ± 0.4 ^{abcd}	5.4 ± 0.5 ^a	13.3 ± 0.4 ^{ab}	46.4 ± 1.3 ^{abcd}	15.7 ± 0.7 ^{ab}	37.0 ± 3.1 ^{bc}	6.0 ± 0.5 ^a	6.2 ± 0.2 ^{bcd}	334.5 ± 6.7 ^a
RIV	8.9 ± 0.3 ^{abc}	5.4 ± 0.3 ^a	13.3 ± 0.7 ^{ab}	46.8 ± 1.5 ^{abcd}	15.8 ± 0.9 ^{ab}	44.0 ± 4.1 ^{de}	6.2 ± 0.4 ^{abc}	7.0 ± 0.5 ^e	329.5 ± 5.1 ^a
BMRIV	8.8 ± 0.5 ^{ab}	5.2 ± 0.5 ^a	13.4 ± 0.7 ^{ab}	45.1 ± 1.1 ^{ab}	15.4 ± 0.9 ^a	51.4 ± 6.0 ^{fg}	6.7 ± 0.2 ^{bc}	7.8 ± 0.5 ^f	356.0 ± 13.4 ^{abc}
PMRIV	8.8 ± 0.9 ^a	5.2 ± 0.2 ^a	13.8 ± 0.6 ^b	44.4 ± 1.6 ^a	14.9 ± 0.4 ^a	56.0 ± 5.0 ^g	6.9 ± 0.4 ^c	6.4 ± 0.3 ^{cd}	369.7 ± 8.9 ^{bc}
ACRIV	9.0 ± 0.9 ^{abc}	5.4 ± 0.2 ^a	13.4 ± 0.8 ^{ab}	46.8 ± 1.5 ^{abcd}	15.8 ± 0.8 ^{ab}	46.1 ± 5.2 ^{def}	6.1 ± 0.2 ^{abc}	6.1 ± 0.4 ^{bc}	356.2 ± 9.2 ^{abc}
IFRIV	9.3 ± 0.6 ^d	5.4 ± 0.2 ^a	13.3 ± 0.8 ^{ab}	47.7 ± 1.6 ^{cd}	15.8 ± 0.6 ^{ab}	33.4 ± 3.5 ^b	5.7 ± 0.1 ^a	6.3 ± 0.2 ^{bcd}	383.8 ± 6.0 ^c

± indicates standard error of mean (n = 3); the dissimilar letters indicate significant difference at $P < 0.05$ by using Tukey's test

EC_e, electrical conductivity in saturated soil extract, CEC cation exchange capacity, ESP exchangeable sodium percentage, SAR sodium absorption ratio, SOC soil organic carbon, AVP available phosphorus, TKN total Kjeldahl nitrogen

synergistic relationship between *B. megaterium* and *R. intraradices* (BMRIV), and *P. montellii* and *R. intraradices* (PMRIV) (Table 1). Organic acids produced by growing plants and microorganisms significantly reduces the soil pH [32–34]. The decreased pH level of rhizosphere soil is indicative for improved growth of bio-inoculants treated plant. The decrease in pH may be due to the release of organic substances by root exudates, decomposition of roots, microbial activities and uptake of excess salts from root zone [12]. Moreover, interaction of bio-inoculants with plant lead to the increase of the partial pressure of CO₂ resulting in the generation of H⁺ ion which further reduces the soil pH [3, 12]. During harvesting 30–40% of vetiver roots is left behind in the soil leading to the increased carbon content of rhizosphere soil [35]. The level of soil organic carbon in treatments BMV to ACRIV (2.5 t ha⁻¹ vermicompost) was comparable to treatment VC containing vermicompost in double amount (5 t ha⁻¹ vermicompost) (Table 1). This observation showed that application of bio-inoculants enhanced the root growth and greater amount of rhizodeposition contributing towards soil organic carbon. When compared to control CS, increased level of AVK was observed in the rhizospheric soil of all the treatments. Whereas no significant difference was observed in the TKN level of treatments ACV (*A. chroococcum* + 2.5 t ha⁻¹ vermicompost) and ACRIV (*A. chroococcum* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) when compared to control. Despite the nitrogen fixing abilities of *A. chroococcum* salinity stress and available nitrogen present in vermicompost inhibited nitrogenase enzyme of *A. chroococcum* [36]. The significant increase in AVP level in treatments BMRIV (*B. megaterium* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) could be attributed to the phosphate solubilization abilities of *B. megaterium* and phosphorus acquisition by *R. intraradices* [37, 38]. In salt-affected soil phosphorus is precipitated by ions like Ca²⁺, Mg²⁺ and Zn²⁺, and *B. megaterium* solubilized these inorganic phosphates, thereby, increasing the level of available phosphorus in rhizospheric soil. In both treatments BMV (*B. megaterium* + 2.5 t ha⁻¹ vermicompost) and BMRIV (*B. megaterium* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) the phosphorus is solubilized by *B. megaterium* but due to absence of *R. intraradices* in treatment BMV the AVP could not be sequestered efficiently, thus, AVP level of BMV is not comparable to BMRIV (*B. megaterium* + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) (Table 1). Symbiotic association of plants with AM fungi enables them to acquire immobile nutrients in rhizosphere region [39–41]. The synergistic behavior of bio-inoculants and *R. intraradices* was also corroborated by maximum increase in TKN and AVP level of treatments BMRIV, RIV, PMRIV and ACRIV (bio-

inoculants + *R. intraradices* + 2.5 t ha⁻¹ vermicompost) (Table 1). Congruent to this study, Al-Karaki [42], showed that AM fungi had a positive influence on the composition of mineral nutrients (particularly immobilized nutrients such as P) of plants under salt stress conditions.

Biological Properties of Post-harvest Soil

Soil microbial biomass and soil enzymes are two major bio-indicators used for monitoring soil health [43, 44]. In particular quantification of soil enzymes is considered helpful in prediction about ecosystem's function and interactions among different communities of ecosystem [43]. When compared to un-inoculated treatment CS and VC, increased microbial biomass (CFU) and higher dehydrogenases (DHA), alkaline phosphatases (ALP) and acid phosphatase (ACP) activities in treatments containing bio-inoculants (BMV to IFRI) indicated the successful establishment and proliferation of bio-inoculants in stressed soil (Table 2). On other side increased plant growth and improved physico-chemical properties of salt-affected soil of bio-inoculants treated plants (BMV to IFRI) indicated the growth and establishment of *C. zizanioides* in salt affected soil [33]. When compared to control treatment CS, CFU, soil respiration, DHA, ALP and ACP activities were higher in treatment PMRIV (*R. intraradices* + *P. monteillii* + 2.5 t ha⁻¹ vermicompost) followed by treatment BMRIV (*R. intraradices* + *B. megaterium* + VC 2.5 t ha⁻¹) (Table 2). The level of soil dehydrogenases (DHA) and phosphatases which are involved in recycling of carbon and phosphorus, respectively were significantly higher in treatment VC to IFRI. The maximum elevation in DHA activity was observed in PMRIV followed by BMRIV and ACRIV. This increase may be accredited to the maximum SOC content in treatment

PMRIV (*R. intraradices* + *P. monteillii* + VC 2.5 t ha⁻¹) followed by BMRIV (*R. intraradices* + *B. megaterium* + 2.5 t ha⁻¹ vermicompost) and ACRIV (*R. intraradices* + *A. chroococcum* + 2.5 t ha⁻¹ vermicompost) (Table 1). The decomposition of leaf litters, fine roots and root exudates integrate nutrients to the existing microorganisms and accelerate their growth and eventually soil enzyme activities [34]. Increase in soil dehydrogenase activities is accredited to microbial and plant growth. This is corroborated by significantly lower dehydrogenase activities in treatments CS and VC which lacked bio-inoculants. The low level of microbial count and enzymes activities observed in treatments CS and VC may be due to the entry of microorganisms from the environment and plants. The maximum number of AMF spore and root AMF colonization percent in PMRIV (*R. intraradices* + *P. monteillii* + 2.5 t ha⁻¹ vermicompost) depicted the improved biological properties of rhizosphere soil (Table 2). The maximum number of spore and colonization percent in plants of PMRIV is ascribed to the increased root growth in treatment PMRIV. The IAA producing *P. monteillii* present in the treatment PMRIV increased the root biomass eventually resulting in the increased number of AMF spore and percent of AMF colonization. Similar to our observation the presence of rhizobacteria *P. fluorescens* increased the AMF root colonization in a transgenic plant [45]. These types of bacteria are known as mycorrhiza helper bacteria [46].

Correlation Between Different Parameters of Plant Growth and Post-harvest Soil Properties

The significant positive correlation was observed among all the observed parameters of plant growth and post-harvest soil (ranged between $r = 0.92^{**}$ to 0.40^{*} ; Table S2). Shoot

Table 2 Biological properties of post-harvest soil of *Chrysopogon zizanioides*

Treatments	TMC (CFU × 10 ⁻⁵)	Soil respiration (CO ₂ -C mg kg ⁻¹ soil day ⁻¹)	AMF root colonization (%)	AMF spore (no. of 50 g ⁻¹ soil)	DHA (mg TPF kg ⁻¹ dry soil h ⁻¹)	ALP (μg p-n-phenol released g ⁻¹ soil ha ⁻¹)	ACP (μg p-n-phenol released g ⁻¹ soil ha ⁻¹)
CS	1.0 ± 0.1 ^a	5.6 ± 0.4 ^a	–	–	26.1 ± 1.1 ^a	5.0 ± 0.2 ^a	2.4 ± 0.5 ^a
VC	1.3 ± 0.3 ^a	8.0 ± 0.4 ^b	–	–	57.6 ± 0.8 ^b	14.3 ± 0.3 ^{bc}	4.5 ± 0.3 ^{bcd}
BMV	4.8 ± 0.2 ^d	10.4 ± 0.4 ^{cd}	–	–	104.2 ± 1.7 ^c	13.9 ± 0.2 ^{bc}	3.6 ± 0.4 ^{abc}
PMV	4.7 ± 0.2 ^{cd}	10.0 ± 0.3 ^c	–	–	91.8 ± 1.3 ^d	12.8 ± 0.6 ^b	2.6 ± 0.1 ^a
ACV	4.2 ± 0.2 ^{cd}	8.8 ± 0.3 ^{bc}	–	–	76.5 ± 3.5 ^c	15.7 ± 1.4 ^{bc}	3.5 ± 0.4 ^{abc}
RIV	1.0 ± 0.1 ^a	11.8 ± 0.2 ^{de}	80.8 ± 1.0 ^{ab}	353 ± 3.3 ^{ab}	132.7 ± 1.5 ^f	25.6 ± 0.4 ^e	3.6 ± 0.5 ^{abc}
BMRIV	3.1 ± 0.2 ^b	12.6 ± 0.4 ^e	81.8 ± 1.8 ^{ab}	400 ± 5.8 ^{bc}	158.8 ± 4.5 ^h	22.2 ± 0.8 ^d	3.9 ± 0.6 ^{abcd}
PMRIV	4.8 ± 0.2 ^d	17.7 ± 0.2 ^f	83.8 ± 1.0 ^b	447 ± 3.3 ^c	174.7 ± 1.2 ⁱ	27.1 ± 0.6 ^e	5.2 ± 0.3 ^d
ACRIV	3.8 ± 0.2 ^{bc}	12.2 ± 0.2 ^e	78.8 ± 3.1 ^{ab}	390 ± 2.9 ^{bc}	146.5 ± 2.0 ^g	17.0 ± 0.3 ^c	4.5 ± 0.2 ^{cd}
IFRIV	1.0 ± 0.1 ^a	10.4 ± 0.4 ^{cd}	72.7 ± 3.6 ^a	317 ± 1.7 ^a	103.4 ± 3.6 ^{de}	14.7 ± 0.7 ^{bc}	3.2 ± 0.1 ^{ab}

± indicates standard error of mean (n = 3); the dissimilar letters indicate significant difference at $P < 0.05$ by using Tukey's test

TMC total microbial count, AMF arbuscular mycorrhizal fungi, DHA dehydrogenase enzyme, ALP alkaline phosphatase enzyme, ACP acid phosphatase enzyme

Fig. 4 Linear regression analysis between cation exchange capacity (CEC) of salt-affected soil, and soil pH, exchangeable sodium percentage (ESP) and sodium adsorption ratio (SAR)

uptake ratio of $\text{Na}^+:\text{K}^+$ is significantly positively correlated to fresh root yield ($r = 0.87^{**}$) followed by essential oil yield ($r = 0.78^{**}$). Phosphorus uptake was positively correlated with root biomass ($r = 0.50^{**}$ and 0.57^{**}) and alkaline phosphatase activity (ALP) ($r = 0.46^*$). The AMF root colonization showed highly significant positive correlation with soil enzyme such as dehydrogenase (DHA) ($r = 0.83^{**}$) and alkaline phosphatase (ALP) ($r = 0.74^{**}$) activity (Table S2). Soil organic carbon (SOC) showed significantly positive correlation with AMFC ($r = 0.44^*$), ALP ($r = 0.82^{**}$), DHA ($r = 0.70^{**}$) and CEC ($r = 0.50^{**}$) while soil pH ($r = -0.79^{**}$), ESP ($r = -0.80^{**}$) and SAR ($r = -0.60^{**}$) showed significant negative correlation. Moreover, soil pH and soil exchangeable sodium percent (ESP) are negatively correlated with AMFC ($r = -0.50^{**}$ and -0.34), ALP

($r = -0.80^{**}$ and -0.76^{**}), DHA ($r = -0.75^{**}$ and -0.58^{**}), SOC ($r = -0.79^{**}$ and -0.80^{**}), TKN ($r = -0.53^{**}$ and -0.67^{**}) and AVP ($r = -0.70^{**}$ and -0.57^{**}). In this experiment the CEC of the rhizospheric soil was significantly negatively correlated with sodium adsorption ratio (SAR) ($r = -0.46^*$) and ESP ($r = -0.62^{**}$). The linear regression analysis showed that soil pH, ESP and SAR had dominant effect on soil CEC (Fig. 4). An improved CEC of the soil resulted in decreased salinity level of the soil. The exponential relationship between salinity parameters (soil pH, ESP and SAR) and soil CEC demonstrated that small increase in CEC could trigger great decrease in salinity parameters (soil pH, ESP and SAR). Improvement of soil CEC enhanced the availability of soil nutrients composition and diversity of microbial community [47].

Conclusion

This study emphasize on development of microbial inoculants for encouragement of phytoremediation in salt-affected soil. Under salinity stress, microbial inoculants including *P. monteilii*, *B. megaterium*, *A. chroococcum* and *R. intraradices* (AMF) significantly improved the growth of *C. zizanioides*. The physico-chemical and biological properties, and nutrient contents of rhizosphere soil of bio-inoculants treated plants was significantly improved indicating reclamation of salt-affected soil. This study suggests that combination of *R. intraradices* and *P. monteilii* can be used as potential bio-inoculant for cultivation of vetiver, and simultaneous recovery of soil health in salt-affected soil.

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Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

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