Grasas y Aceites 76 (1)
January-March 2025, 2264
ISSN-L: 0017-3495, eISSN: 1988-4214
https://doi.org/10.3989/gya.1204242.2264

Effects of inoculation with multi-trait rhizobacterial consortia on the growth, essential oil content and composition of Chrysopogon zizanioides (L.) Roberty under mediterranean conditions

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Submitted: 10 December 2024; Accepted: 27 February 2025; Published: 13 October 2025

SUMMARY: This study assessed the possible effects of chemical fertilizer (100 kg·ha^{-1} N, 50 kg·ha^{-1} P_2O_5, and 50 kg·ha^{-1} K_2O), a commercial liquid bio-fertilizer, and inoculation with four strains of multi-trait bacteria on various growth parameters of vetiver under Mediterranean conditions. The chemical composition of vetiver root essential oil was analyzed using GC-MS after hydrodistillation. Maximum dry biomass and root weights were observed with BF3 inoculation, followed by chemical fertilizer application. The highest essential oil ratios were found in BF1 and BF2 treatments, while treatments with superior plant characteristics had lower essential oil ratios. GC–MS analyses of the hydro-distillated essential oil of V. zizanioides allowed for the identification of 70 different components in the oil. The main components in the oil were khusimol (21.26-35.36%), followed by vetiverone (3.45-8.08%), β-vetivone (0.38-12.93%), α-gurjunene (0.02-9.68%), ɣ-muurolene (0.60-7.85%), β-vetivenene (0.60-3.41%), valerenal (0.58-3.16%), junenol (0.52-1.47%), and khusimene (0.81-1.95%).

KEYWORDS: *Bio-fertilizer; Chemical composition; Khusimol; Root yield; Vetiveria zizanioides.*

RESUMEN: ***Efecto de la inoculación con consorcios rizobacterianos multicaracterísticos sobre el crecimiento, contenido y composición de aceite esencial de Chrysopogon zizanioides (L.) Roberty en condiciones mediterráneas.*** Este estudio evaluó los posibles efectos de un fertilizante químico (100 kg·ha^{-1} N, 50 kg·ha^{-1} P_2O_5 y 50 kg·ha^{-1} K_2O), un biofertilizante líquido comercial y la inoculación con cuatro cepas de bacterias multirasgo sobre varios parámetros de crecimiento del vetiver en condiciones mediterráneas. La composición química del aceite esencial de la raíz de vetiver se analizó mediante cromatografía de gases-espectrometría de masas (GC-MS) después de la hidrodestilación. La biomasa seca máxima y el peso de las raíces se observaron con la inoculación de BF3, seguida de la aplicación de fertilizante químico. Las proporciones más altas de aceite esencial se encontraron en los tratamientos BF1 y BF2, mientras que los tratamientos con características superiores de la planta tuvieron proporciones más bajas de aceite esencial. Los análisis GC-MS del aceite esencial hidrodestilado de V. zizanioides permitieron la identificación de 70 componentes diferentes en el aceite. Los principales componentes del aceite fueron husimol (21,26-35,36 %), seguido de vetiverona (3,45-8,08 %), β-vetivona (0,38-12,93 %), α-gurjuneno (0,02-9,68 %), g-muuroleno (0,60-7,85 %), β-vetiveneno (0,60-3,41 %), valerenal. (0,58-3,16 %), junenol (0,52-1,47 %) y khusimene (0,81-1,95 %) que fueron los componentes más abundantes.

PALABRAS CLAVE: *Biofertilizante; Composición química; Khusimol; Rendimiento de raices; Vetiveria zizanioides.*

Citation/Cómo citar este artículo: Çakmakci R, Akçura S, Binbir U. 2025. Effects of inoculation with multi-trait rhizobacterial consortia on the growth, essential oil content and composition of Chrysopogon zizanioides (L.) Roberty under Mediterranean Conditions. Grasas Aceites 76 (1), 2264. https://doi.org/10.3989/gya.1204242.2264

1. INTRODUCTION

Vetiver, a perennial herbaceous perfume plant found in tropical and subtropical regions, is both wild and cultivated (Del Giudice *et al.*, 2008). One of its unique features is its ability to produce a complex volatile oil in its roots, consisting of sesquiterpene (monocyclic, bicyclic, tricyclic, and tetra-cyclic), terpenic ketones, sesquiterpene alcohols, and hydrocarbons. Vetiver is an aromatic perennial grass and major tropical perfume crop with fine essential oil-rich, thin fibrous roots. This oil finds wide application and is highly sought after in the perfume and cosmetic industry due to its fixative, fragrance, and skincare properties. The quality of the oil varies depending on genotype, environmental, genotype x environment interactions, and industrial factors (Vollú *et al.*, 2012), and creating a synthetic version is a challenging task due to its complex structure (Champagnat *et al.*, 2006). The economically important predominant aroma compounds used to evaluate the quality characteristics of its essential oil are khusimol, β-vetivone, and α-vetivone (Hartwig *et al.*, 2015).

The plant root rhizosphere contains free-living plant growth-promoting rhizobacteria (PGPR), which directly and indirectly promote root development and plant growth. Çakmakçı *et al*. (2006) identified growth-promoting bacteria from various plant rhizospheres and found that their beneficial effects on plant growth were strain-specific as well as varying depending on the inoculant type, soil organic matter content, growing stage, harvest date, and growth parameters evaluated. Bacteria use indirect stimulation to prevent plant diseases which inhibit plant growth and development. PGPR stimulate plant growth by a wide variety of mechanisms like biological nitrogen fixation, solubilization and mineralization of organic/inorganic P, K, Zn, and Fe, production of siderophore, ammonia, salicylic acid, and phytohormones like indole acetic acid (IAA), cytokinins, gibberellins, volatile organic compounds, recycling of essential elements and uptake of essential nutrients, and containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Orozco-Mosqueda *et al.*, 2022).

The use of free-living beneficial bacteria as an important agricultural input to increase crop production requires the selection of competent rhizobacteria with plant growth-promoting properties. Since PGPR is associated and specific to plant roots, and responses were strain-specific, there is a need to discover suitable local biofertilizer candidates to enhance plant growth and yield (Karagöz *et al.*, 2018). The effectiveness of bacteria can vary depending on environmental conditions, bacterial strains, plant species, and soil type (Akçura and Çakmakçı, 2023). Information on increasing or changing the yield, essential oil content, and components of medicinal and aromatic plants using multispecific bacteria is limited; in fact, to our knowledge, no research has addressed the effects of PGPR on growth, yield, essential oil content and components in vetiver. Vetiver, which has an attractive feature for soil-dwelling microorganisms with the aroma compounds in its roots, is one of the few plants from which essential oil is obtained through its roots. Indeed, the interaction between the fragrant sesquiterpenes in vetiver roots and the symbiotically living subterranean soil microorganisms has been noted as the source of these compounds (Adams *et al.*, 2008). However, there has been insufficient research conducted under Mediterranean conditions regarding the impact of plant growth-promoting bacteria on the root volatile oil components of vetiver. That is why a study was conducted in order to investigate the effect of plant-associated beneficial multi-trait bacterial formulations on the growth, yield, content and composition of essential oils of *Vetiveria zizanioides* under Mediterranean conditions in the field.

2. MATERIALS AND METHODS

2.1. Experiments and growth conditions

This research was conducted on the premises of the Medicinal Plants Research Center (BAÇEM) located in the Burhaniye district of Balıkesir, under the auspices of the Rural Services Department of Balıkesir Metropolitan Municipality. The experimental soil is characterized by a sandy-loam soil structure with pH 7.2, low salt levels (< 4 dS/m), very low organic matter content (0.73%), deficient in P, K, and N content, but sufficient in terms of most of the examined microelements (Table 1).

TABLE 1. Some soil properties at 0-30 cm depth of the experimental area

Soil properties	Value	Degree
Sand (%)	58	
Silty (%)	25	
Clay (%)	17	
Texture clas		Sandy-loam
pH (1:2,5 W/V H2O)	7.2	Slightly-alkaline
Water-soluble salt (ds/m)	0.26	Without salt
Lime (%)	1.63	Very little chalky
Organic matter (%)	0.73	Very little
Total nitrogen (%)	0.006	Very little
Available phosphorus (ppm)	5.45	Little
Available potassium (ppm)	20.51	Little
Available magnesium (ppm)	324.6	Sufficient
Available manganese (ppm)	3.35	Sufficient
Available iron (ppm)	8.9	More
Available zinc (ppm)	0.41	Sufficient
Available copper (ppm)	0.92	Sufficient

*Soil analyses were carried out at the Soil Fertilizer and Water Resources Central Research Institute

The monthly precipitation and average temperature values for the growing season between March 2021 and March 2022 for the Burhaniye District of Balıkesir Province, where the study was conducted, are presented in Figure 1 (BMD, 2022). The trial site typically experiences a Mediterranean climate, with hot and dry summers and mild and rainy winters. The highest rainfall was observed in December and February. The highest average temperatures were recorded in July and August (Figure 1).

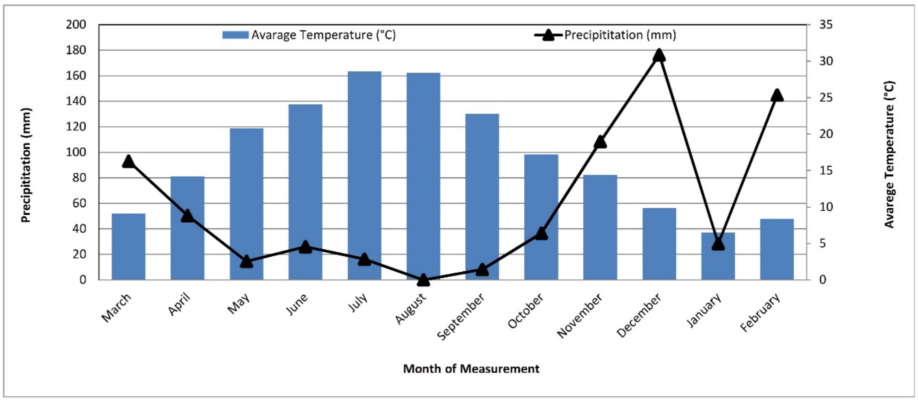

FIGURE 1. Monthly precipitation and average temperature values of the experimental site.

2.2. Bacterial strains and experimental details

The objective of this study was to evaluate possible effects of recommended mineral doses of NPK fertilizer (100 kg·ha^{-1} N, 50 kg·ha^{-1} P_2O_5, and 50 kg·ha^{-1} K_2O), one commercial liquid bio-fertilizer (CLBF), and N2-fixing, ACC deaminase-containing, and P-solubilizing bacteria-based bio-fertilizers in four strain combinations (BF1: ¼ Pseudomonas fluorescens RC09 + ¼ Bacillus subtilis RC11 + ¼ Bacillus megaterium RC33 + ¼ Pantoea agglomerans RC36; BF2: ¼ P. fluorescens RC77 + ¼ P. putida biotype B RC90 + ¼ B. subtilis RC631 + ¼ B. pumilus RC22; BF3: ¼ P. fluorescens RC84 + ¼ B. subtilis RC67 + ¼ B. megaterium RC34 + ¼ P. agglomerans RC16) on the various growth parameters of vetiver under Mediterranean conditions.

The parameters studied included plant height, root fresh and dry weight, dry biomass (above-ground plant dry weight), and essential oil content and composition in Vetiver. A total of six applications, including control (without fertilizer), were investigated in the study. The experiment was arranged as a completely randomized design with six replicates.

In this research, the bacterial strains used individually and in combination were selected from a collection of 786 bacterial isolates, previously sourced from various cultivated and wild plant root rhizospheres across Turkey, with a focus on the Çoruh Valley and Kaçkar Mountain regions, which exhibit plant growth-promoting characteristics. The isolates used in the study were obtained from rhizosphere soils of rye, wheat, wild garlic, hazelnut, wild blueberry, wild grape, wild strawberry, oregano, and wild raspberry in regions such as Yusufeli-Barhal Valley and Camili Biosphere Reserve Area in Artvin province, Aksu, Çamlıkaya, Çoruh, and Fırtına Deresi Valley in Rize province (Çakmakçı et al., 2006, 2020). Bacterial isolates were stored at -80 °C in the microbiology laboratory of Çanakkale Onsekiz Mart University, Faculty of Agriculture.

2.3. Field experiment

Rooted one-year-old cuttings of the ZFVZ-1 genotype, obtained from BAÇEM, were used as plant material for the research. The planting of the experiment was carried out in November 2020, while the fertilizer applications were performed in March 2021. A bacterial suspension of 20 ml per plant was applied around each plant, approximately 5 cm deep, in a circular manner. Regarding the chemical fertilizer application, 50 kg·ha^{-1} N, 50 kg·ha^{-1} P_2O_5, and 50 kg·ha^{-1} K_2O were applied before planting in November 2020, and the remaining half of nitrogen (50 kg·ha-1) was applied in March 2021. The plot size was set at 3 m wide and 5 m long, totaling 15 m². One-year-old rooted vetiver clones were planted in each plot, with a spacing of 1 m between rows and 1 m between plants, resulting in 3 rows and 15 plants per plot. Throughout the growing period of the experiment, drip irrigation was applied 5 times, providing approximately 70 mm of water each time. In the field experiment, the period from planting the clones to harvesting was 18 months. The harvesting and uprooting of plants were carried out using light machinery in March 2022. To mitigate edge effects during harvesting, plants in the middle rows of each plot were individually uprooted by creating a 2-meter soil profile using a small excavator.

2.4. Essential oil extraction

Fresh roots of *Vetiveria zizanioides*, over 2 years old, were washed and dried in the shade, and first, root yields were determined, followed by the extraction of essential oil. Approximately 150 g of vetiver root powder were subjected to hydrodistillation for 24 h in the Clevenger apparatus. The yields were based on the dry materials of plant samples.

2.5. GC–MS analysis

In the field trial of the research, plant root fresh weight, plant root dry weight, plant height, aboveground plant dry weight, and volatile oil ratios were determined. Before gas chromatography-mass spectrometry (GC-MS) analysis, all samples of volatile oil were frozen at -18 °C. The GC-MS device was employed to determine the aroma components of the volatile oil samples. The aroma components of the volatile oil samples were analyzed using a Shimadzu GC-MS-QP2020 NX GC-MS device with a Restek Rxi-MS column (30 m x 0.25 mm x 0.25 µm). After three minutes at 40 °C, the column temperature was raised to 240 °C and maintained there for ten minutes, at a rate of 5 °C·min^{-1} thereafter. Diluted volatile oil samples (1/100 hexane, v/v) of 1.0 µL were automatically injected in split mode (10:1). The GC-MS setup made use of helium as the carrier gas and electron ionization at 70 eV, covering a mass range of 40-330 m/z. In terms of GC-MS settings, we used 50 spectra per sample, 20,000 amu/s for the scan speed, and full scan mode. With a temperature of 250 °C for the contact and 200 °C for the ion source, we were ready to go. By utilizing a homologous series of C8-C24 saturated alkanes in hexane (1000 mg·L-1) from Merck Life Science, the retention index (RI) values were determined. Identification of the chemical compounds in the essential oils was achieved by comparing their RI and retention times (RT) with those of the C8–C24 saturated alkanes and matching their mass spectra against MS libraries (FFNSC 3, W9N11, and NIST11). Data collection and processing were performed using GC-MS solution software (version 4.50, Shimadzu). Peak assignment was based on two criteria: spectral similarity (over 85%) and retention index (RI) correspondence (±5 units tolerance) (Pandur et al., 2021). The essential oil mass spectra were identified using the libraries (version 4.0, Shimadzu), along with literature values (Adams, 2007). The normalization method was used to calculate the relative concentrations of components from the GC-MS peak regions without correction factors. Each of the percentages displayed below is the average of three injections of the vetiver root oil samples. In the same experimental setup, we also tested commercial vetiver oil (a gift sample from BACEM, Turkey) as a reference.

2.6. Statistical analysis

In evaluating the results obtained for all examined traits, variance analysis was first conducted according to the randomized complete block design. Subsequently, an LSD test was used for comparing statistically significant means for each trait. All statistical analyses were performed using the SAS JMP software (SAS Institute, 2017).

Biplots of PGPR x Trait were created to visually evaluate the changes in the volatile oil components with a ratio higher than 1% according to the PGPR applications. In the first analysis, a polygon was created by performing PGPR x Trait Component analysis to determine which PGPR application positively or negatively affected which trait (Figure 2). The second biplot analysis was prepared to determine how and in what direction the macro components of volatile oil changed due to BF1 and NPK fertilizer applications (Figure 3).

The GGEbiplot Software Version 8 was used for biplot analyses based on the mathematical model shown below (Yan, 2014):

where $$\frac{\hat{Y}_{ij} - \mu - \beta_j}{dj} = a_{i1}e_{1j} + a_{i2}e_{2j} + \varepsilon_{i1}$$

$\hat{Y}_{ij}$= the expected value of application i in trait j,
μ = the total mean of all observations,
β_j= the main effect of application j, and
where dj is the phenotypic standard deviation in traits j, and is calculated by:

$$dj = \sqrt{\frac{1}{m-1}\sum_{i=1}^{m}(Y_{IJ} - Y_J)^2}$$

m is the number of applications tested.

where $ai1$ and $e1j$ are called the primary scores for application i and trait j, respectively; $ai2$ and $e2j$, the secondary scores for application i and trait j, respectively; εi1is the residue not explained by the primary and secondary effects. PGPR x Trait-biplot is constructed by plotting $ai1$ against $ai2$, and $e1j$ against $e2j$ in a single scatter plot (Yan, 2014).

3. RESULTS AND DISCUSSION

3.1. Plant characteristics

In the field trials of the study, differences among six different applications were found to be statistically significant ($p < 0.01$) based on the F-test in terms of vetiver root's fresh weight, dry weight, plant height, and above-ground dry weight (Table 2). The maximum plant height for vetiver was measured in NPK fertilizer followed by BF3 and BF2 inoculations, while the lowest plant height was obtained for the control. Of the bacterial treatments, a consortium consisting of four indigenous *bacterial strains (*BF3) inoculation produced the highest root fresh and dry weight and dry biomass, followed by NPK fertilizer application and BF1 inoculations, while control plots gave the lowest root fresh and dry weight and dry biomass.

In the study, both commercial biofertilizer and newly tested indigenous multi-strain consortia of BF3, BF1, and BF2 showed significant positive effects on the growth of root fresh and dry weight of vetiver under field conditions compared to the control. Although vetiver is one of the rare plants that carries essential oils and aromatic compounds in its roots and has been extensively researched, no research, to our knowledge, deals with the effects of multi-functional PGPB consortia on root yield and its essential oil and composition. Plants with root exudates containing sugars, amino acids, organic acids, and aromatic compounds are reported to attract a higher number of soil microorganisms to the root rhizosphere (Zhang *et al.*, 2021). Dry biomass is not a commonly studied trait in vetiver. When compared to the control, newly tested bacterial formulations increased dry biomass by 32 to 54 %. Indeed, various plant characteristics have been reported to be positively influenced by bacteria from genera such as *Bacillus sp., Pseudomonas sp., Azospirillum sp.,* and *Enterobacter spp*. in different crops (Akçura and Çakmakçı, 2023).

The inoculation of plants with consortia BF3 was shown to be the most successful treatment in terms of biomass yield and root fresh and dry weights when compared to both the control and chemical fertilization. In particular, inoculation with a high IAA-producing bacterial consortium produced the highest root weight and total number of roots in vetiver and promoted adventitious root formation. Co-inoculations of bacteria containing IAA-producing (indole acetic acid), N2-fixing, and P-solubilizing *B. subtilis* and *B. megaterium* strains were found to positively affect the root and shoot growth and development of vetiver. While our study did not employ single-formula treatments, some previous studies has reported that bacterial combinations are more effective than single bacterial applications (Akçura and Çakmakçı, 2023). Hence, a noteworthy trend in recently reported studies has been towards the use of microbial mixtures. These mixtures highlight the use of multiple bacterial species in suspension, often consisting of a variety of species, or in conjunction with beneficial fungal species like mycorrhizae (Reed and Glick, 2023). For instance, microbial strain mixtures have been found to enhance plant growth in various studies conducted on different crops (Şahin *et al.,* 2004; Bechtaoui *et al.*, 2019; Akçura and Çakmakçı, 2023; Ramly *et al.*, 2024; Ahbar AI *et al.*, 2024; Syaziana *et al.*, 2024; Binti Othman *et al.*, 2024; Sadeq *et al.*, 2024) when compared to single-strain inoculation.

TABLE 2. Averages of vetiver grown with different applications, plant characteristics, essential oil ratio and LSD test groups

Treatments	Fresh Root Weight (g·plant^{-1})	Dry Root Weight (g·plant^{-1})	Plant Height (cm)	Dry Biomass (g·plant^{-1})	Essential Oil Content (%)
Control*	729±110 c	199±27 c	206±7e	2572±440 d	1.22±0.12 ab
NPK	1447± 41 a	284±7 ab	233±6 a	3773±225 ab	1.10±0.10 b
CLBF	1035±94 bc	263±5 b	221±4 cd	3105±290 c	1.20±0.11 ab
BF1	1126±42 b	278±15 b	218±12d	3582±212 ab	1.41±0.08 a
BF2	971±162 bc	261±14 b	226±9 bc	3384±250 bc	1.34± 0.06 a
BF3	1589±101 a	322±23 a	229±11 ab	3939±95 a	1.10±0.09 b
Mean	1149	268	222	3393	1.23
F test	**	**	**	**	**
LSD (0.01)	319	44	6	488	0.23
R^2	0.91	0.89	0.96	0.92	0.76
CV (%)	10.74	6.33	5.70	5.56	7.09

Results are expressed as mean ±SD (n=3) *Control: Without bacteria and fertilizer; NPK fertilizer (100 kg·ha-1 N, 50 kg·ha-1 P2O5, and 50 kg·ha-1 K2O); CLBF: commercial liquid bio-fertilizer; BF1: Pseudomonas fluorescens RC09 + Bacillus subtilis RC11 + Bacillus megaterium RC33 + Pantoea agglomerans RC36; BF2: Pseudomonas fluorescens RC77 + Pseudomonas putida biotype B RC90 + Bacillus subtilis RC631 + Bacillus pumilus RC22; BF3: Pseudomonas fluorescens RC84 + Bacillus subtilis RC67 + Bacillus megaterium RC34 + Pantoea agglomerans RC16; **Statistically significant differences were determined by One Way ANOVA and Least Significant Difference Test .(p < 0.01)

3.2. Volatile oil content and components

Inoculation with BF1 and BF2 increased volatile oil content compared to NPK fertilizer and BF3 consortium, but other treatments gave the same result with the control (Table 2). One of the main reasons for this notable effect can be attributed to the presence of volatile oils and aromatic compounds in its roots. It has been reported that aroma compounds in vetiver roots and volatile oil are highly influenced by microorganisms present in the root rhizosphere (Del Giudice *et al.*, 2008).

The majority of the aroma components of vetiver grown under different treatments exhibited statistically significant changes at a significance level of $P < 0.01$ depending on the applied treatments. A total of 70 aroma components were identified, and some components were either not detected or were found at very low levels in some treatments (Table 3). The GC-MS analysis revealed 32 compounds constituting about 76% of total volatiles present in the roots of the control plant. In total, 36, 35, 35, 37, and 35 compounds, representing nearly 84.7, 76.3, 85.8, 82.0, and 70.7% of total volatiles, were detected in the essential oil under NPK, CLBF, BF1, BF2, and BF3 treatments, respectively (Table 3). The contents of pharmacologically important compounds such as β-vetivone, α-vetivone, α-gurjunene, and ɣ-muurolene increased, while aromadendrene decreased with bacterial formulations. The compound longifolene, (-)-spathulenol, δ-amorphene, and α-cedrene were not detected in the oil of the control plant but were found under other treatments. On the contrary, zonarene and calamenene were found in control, NPK, and CLBF treatments but were not detected under BF1, BF2, and BF3 treatments. The BF1 inoculation outperformed the others in terms of khusimol content. In the case of chemical fertilizer application, the ratios of α-gurjunene, β-vetivenene, ɣ-muurolene, cycloisolongifolene, and valerenal were higher than in other treatments. The control and BF2 applications stood out in zonarene, α-vetivol, and junenol components, while the commercial microbial fertilizer was superior in terms of δ-amorphene and khusimene content compared to other applications. Fifteen components (cycloisolongifolene, longifolene, α-gurjunene, khusimene, ɣ-muurolene, δ-amorphene, zonarene, β-vetivenene, khusimone, junenol, valerenal, khusimol, α-vetivol, β-vetivone, vetiverone) had a content of 1% or more. Whereas Efe *et al.* (2021) detected 30 compounds using GC/MS analysis, where significant compounds such as isobutyl-angelate, α-muurolene, α-cedrene, α-patchoulene, ethylene brassylate, 2-methyl-undecanal, α-bulnesene, spathulenol, and α-gurjunene were present. David *et al.* (2019) detected 22 components, of which valerenol, valerenal, and β-cadinene accounted for the major compounds.

The biplot analysis which assessed the variations in fifteen components across treatments explained 61.3% of the total variation (Figure 2). Among the most important quality criteria in vetiver volatile oil, the ratios of khusimol, β-vetivone, vetiverone, and khusimone were superior in the BF1. Interestingly, chemical fertilizer application led to a decrease in the ratios of khusimol, β-vetivone, vetiverone, and khusimone (Figure 2). *Bacillus spp.* (Chauhan *et al.,* 2017) and *P. fluorescens* (Kumar *et al.*, 2016) inoculation has been reported to have a positive effect on plant growth, the fresh rhizome biomass, morphological yield, turmeric (*Curcuma longa*) production, and its major bioactive component curcumin. Although there is increasing evidence of the impact of PGPR on the growth and essential oils and their constituents of medicinal aromatic plants, much less is known about the biosynthesis, regulation, and localization of terpenes synthesized in the roots and whole plant of economically important medicinal aromatic plants (Çakmakçı *et al.*, 2020). Combined inoculation of N2-fixing, P solubilizing, ammonia, indole acetic acid (IAA), and siderophore-producing *Pseudomonas protegens* and *Bacillus paramycoides* increased biomass, oil content, and yields in four perennial aromatic grasses (Maddhesiya *et al.*, 2022). In addition, the use of PGPR has been shown to increase yield, essential oil components, production, and accumulation in some medicinal aromatic plants, as well as affect and increase the biosynthesis of secondary metabolites such as phenolics, flavonoids, alkaloids, and terpenoids (Çakmakçı *et al.*, 2020).

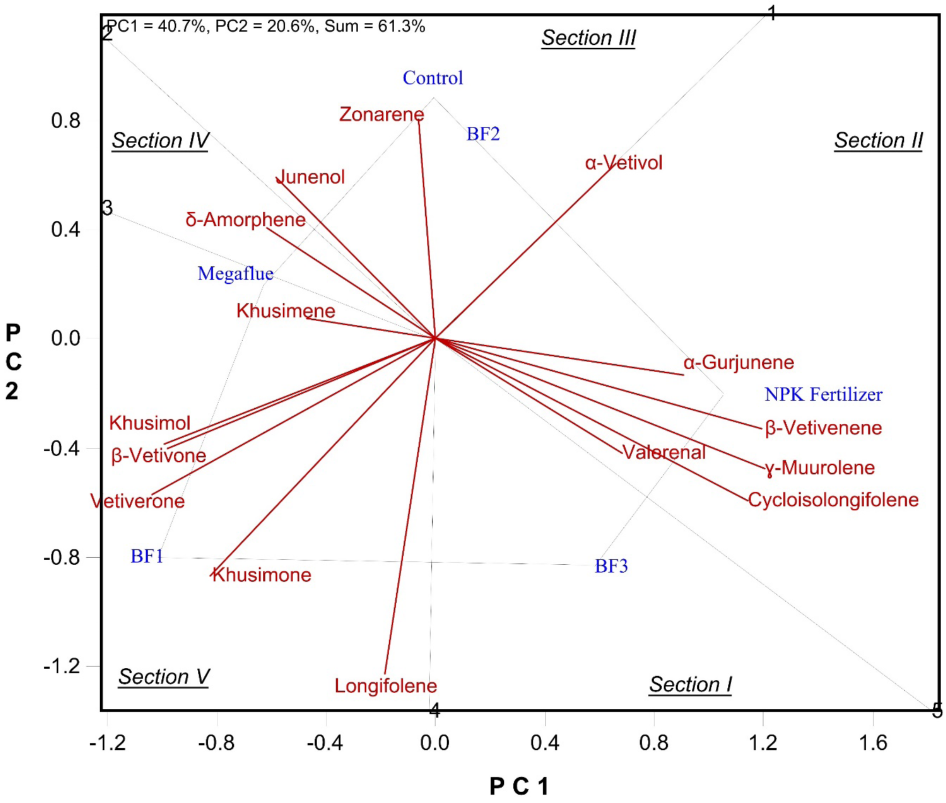

FIGURE 2. Application x componente biplot of aroma compounds with an average (n=3) of more than 1% according to applications.

In order to compare the bacterial formulation BF1 (*P. fluorescens* RC09 + *B. subtilis* RC11 + *B. megaterium* RC33 + *P. agglomerans* RC36), which had the highest volatile oil content among the new bacterial formulations used in the study with chemical fertilizer in terms of aroma components, a biplot was constructed and is presented in Figure 3. As observed in the figure, concerning the content of important components of essential oil such as khusimol, β-vetivone, vetiverone, khusimone, and longifolene, the BF1 inoculation outperformed the chemical fertilizer application (Figure 3). This result indicates that when vetiver, a versatile plant in sustainable agricultural systems, is cultivated with biologically-derived microbial fertilizers, a high-quality volatile oil can be obtained. In some studies, it has been reported that in addition to terpenes and phenolics, low-molecular weight alcohols, aldehydes, and acids are present in the volatile oil structure of roots or rhizomes (Del Giudice *et al.*, 2008).

The chemical components of the volatile oil obtained from the roots of *C. zizanioides* are very complex. So far, more than 300 compounds have been identified, and most of them are sesquiterpenes, although khusimol, khusimene, β-eudesmol, isovalencenol, β-vetivenene, β-vetivone, α-vetivone, β-vetispirene, δ-amorphene, and α-muurolene are the major compounds (Oliveira *et al.*, 2022). The major economically-important active compounds of the essential oil obtained from vetiver roots consist of sesquiterpenoids such as khusimol, β-vetivone, and α-vetivone, which are main odor contributors and show antioxidant activities (Chahal *et al.*, 2015). Among the active compounds in this study, the three components with the highest average content were identified as khusimol, β-vetivone, and α-vetivone (vetiverone), and these components were assessed in detail.

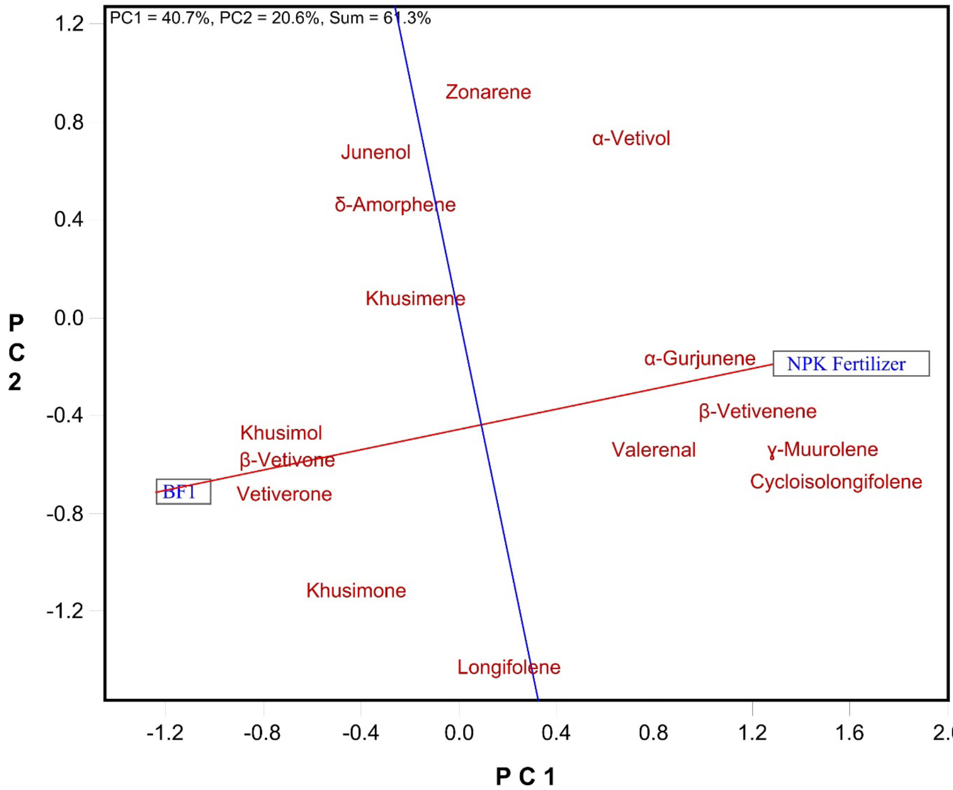

FIGURE 3. Biplot of the comparison of BF1 and NPK fertilizers according to aroma components with an average (n= 3) higher than 1 %..

Khusimol, which is the primary aroma-contributing compound in vetiver essential oil and holds economic significance in the perfume industry and aromatherapy as well as medicinal importance, is a derivative of sesquiterpene alcohol. The results showed that the root with the highest khusimol content (35.4 %) in vetiver was obtained in inoculated with BF1, followed by control (30.3 %); while the lowest khusimol content (21.3 and 23.3 %) was noted in applied NPK and BF2 (Table 3). In terms of this aroma component, the BF3 and CLBF inoculations exhibited similar proportions, with values of 26.8 and 26.5 %, respectively. The khusimol content determined in this study was higher than what some researchers have reported (Kirici *et al.*, 2011), while it was similar to values reported by others (Oliveira *et al.*, 2022).

Among the treatments, CLBF inoculation produced the highest β-vetivone content (12.93%), followed by 10.35% in the BF1 inoculation, while the control and BF3 inoculations produced the lowest β-vetivone contents (0.38 and 1.77 %). The β-vetivone content determined in this study was comparable to the values reported by Mallavarapu *et al.* (2012). The highest vetiverone (α-vetivone) content (8.08 %) was achieved with BF1 inoculation, followed CLBF (5.02 %) and BF2 (4.88 %) inoculations; while the lowest vetiverone content was obtained in the control (3.45 %) and mineral fertilizer (3.73 %) (Table 3). The α-vetivone ratios determined in this study were higher than the values reported by some researchers (Singh *et al.*, 2019). The biomass production, oil content, β-vetivenene, khusimol, and iso-valencinol contents in vetiver increased under field conditions with the addition of organic to inorganic fertilizer (Priyadarshani *et al.*, 2013).

TABLE 3. Aroma components (%) of essential oil obtained from vetiver roots grown with different applications and LSD test groups*

Components	RI	Control	NPKFertilizer	Megaflue	BF1	BF2	BF3
2-Carene	1001					0.37±0.02	
Terpinolene	1086	0.27±0.02	0.37±0.02	0.26±0.02			0.16±0.02
Azulene	1298		1.76±0.04		0.834±0.01	1.79±0.05	0.64±0.06
Cycloisolongifolene	1317		7.47±0.05 b			2.19±0.05 c	8.11±0.04 a
α-Longipinene	1350	1.43±0.03		0.86±0.02			0.66±0.01
(+)-Longicyclene	1371	0.35±0.03					
α-Ylangene	1373	0.21±0.05	0.04±0.01	0.05±0.01	0.103±0.02	0.04±0.00	0.08±0.01
Isoledene	1374	1.48±0.03		0.21±0.02	0.06±0.00		
β-Patchoulene	1379	1.01±0.02					
Longifolene	1407		1.13±0.03 c	1.39±0.05 b	1.71±0.20 a	0.28±0.01 d	1.70±0.13 a
α-Gurjunene	1409	0.02±0.00 f	9.68±0.05 a	1.65±0.01 c	0.33±0.03 e	1.74±0.01 b	1.03±0.07 d
α-Cedrene	1410		0.15±0.01	0.42±0.53	0.27±0.04	0.17±0.00	0.12±0.00
β-Copaene	1430	0.17±0.01		0.22±0.02	0.35±0.01	0.23±0.01	
β-Gurjunene	1431		0.24±0.01	0.22±0.01		1.95±0.05	
α-Guaiene	1437		1.19±0.00		1.23±0.00	1.17±0.01	
α-Himachalene	1449				0.19±0.01	0.61±0.01	0.51±0.00
α- Neo Clovene	1452				0.78±0.03	3.44±0.01	
Khusimene	1453	1.55±0.16 b	1.78±0.05 a	1.45±0.09 bc	1.95±0.02 a	1.36±0.13 c	0.81±0.02 d
Alloaromadendrene	1458		0.38±0.01	1.66±0.02		1.06±0.06	
ɣ-Gurjunene	1475			0.35±0.03			1.09±0.07
ɣ-Muurolene	1478	0.60±0.01 e	7.85±0.04 a	0.68±0.01 d	0.06±0.00 f	2.05±0.01 c	6.13±0.05 b
Curcumene<Ar->	1479		0.78±0.02			0.49±0.02	1.57±0.02
ɣ-Himachalene	1481	4.08±0.043	0.37±0.02				
Germacrene B	1484			1.33±0.03		1.59±0.01	0.85±0.03
Germacrene D	1485	3.72±0.037					
β-Selinene	1489		1.29±0.05			4.54±0.02	
Calamenene<Cis->	1491	0.67±0.05	0.17±0.01	0.70±0.05			
Cis β-Guaiene	1492	1.04±0.01			0.72±0.03	1.08±0.02	
Valencene	1496	0.82±0.03	0.35±0.03	0.27±0.01		2.23±0.01	0.66±0.09
α-Selinene	1498	1±0.025					1.02±0.04
α-Muurolene	1500	1.26±0.02			0.34±0.01	1.57±0.10	1.00±0.06
Epizonarene	1501		1.38±0.04		0.35±0.01		
Cis β-Guaiene	1502			0.60±0.05	0.20±0.03		
δ-Amorphene	1511		1.67±0.01 d	8.05±0.16 a	2.58±0.02 c	6.38±0.03 b	0.34±0.03 e
ɣ-Cadinene	1513		0.23±0.03		0.33±0.02	0.17±0.00	
δ-Cadinene	1522			0.54±0.02	1.02±0.02		
Zonarene	1528	4.45±0.02 a	0.45±0.01 c	1.21±0.03 b			
ɣ-Himachalene	1530			0.36±0.01	1.58±0.01		0.28±0.01
β-Vetivenene	1531	1.93±0.03 c	3.41±0.04 a	0.60±0.02 e	0.91±0.04 d	0.98±0.01 d	2.58±0.14 b
α-Calacorene	1544	0.47±0.01	0.56±0.01	0.26±0.01	0.44±0.01	0.64±0.02	0.33±0.00
Elemol	1548		0.43±0.01				
(-)-Spathulenol	1577		0.91±0.03	0.88±0.01	0.33±0.01	0.60±0.03	0.53±0.01
β-Elemenone	1601				0.38±0.02		

Components	RI	Control	NPKFertilizer	Megaflue	BF1	BF2	BF3
Khusimone	1604			0.73±0.005 c	8.98±0.07 a		2.02±0.05 b
Oplopenone	1607	1.14±0.02					
Junenol	1618	1.24±0.02 b	1.24±0.04 b	1.15±0.02 c	1.47±0.07 a	1.30±0.02 b	0.52±0.04 d
Isospathulenol	1625						0,08±0,039
ɣ-Eudesmol	1630			0.86±0.05	1.12±0.03		
Valerenol	1635						0.47±0.02
Aromadendrene	1639	1.36±0.01	0.46±0.05	1.20±0.02	0.20±0.00	0.93±0.02	0.32±0.03
Cubenol	1645	1.48±0.04	1.05±0.01			1.30±0.02	
Cedr-8(15)-En-10-Ol	1650			1.31±0.03			
β-Bisabolol	1674					0.77±0.01	
Cadalene	1675		0.44±0.02				
Helifolenol B	1677				0.62±0.04		1.23±0.03
(-)-Allokhusiol	1679	1.64±0.01					
Cedrenol	1687					1.25±0.03	
Valerenal	1688	1.54±0.03 c	3.16±0.05 a	0.58±0.02 f	1.96±0.63 b	0.86±0.03 e	1.28±0.04 d
Cedren-13-Ol. 8-	1689			0.08±0.02			
Acorenone B	1692			1.74±0.04			1.22±0.01
Khusimol	1741	30.29±3.75 b	21.26±0.02 e	26.46±1.05 c	35.36±2.51 a	23.26±0.55 d	26.76±0.21 c
Cyclocolorenone	1759	5.01±0.09					
Valencene	1767		2.35±0.03			1.83±0.03	
Trans-Valerenyl Acetate	1793	0.60±0.02					0.42±0.03
α-Vetivol	1811	1.30±0.02 c	4.01±0.04 b		0.32±0.02 d	4.62±0.03 a	
β-Vetivone	1822	0.38±0.03 f	2.45±0.05 c	12.93±0.05 a	10.35±0.02 b	2.30±0.07 d	1.77±0.06 e
Khusinol Acetate	1823				0.24±0.01		
α-Vetivone (Vetiverone)	1842	3.45±0.03 e	3.73±0.04 d	5.02±0.03 b	8.08±0.03 a	4.88±0.02 c	3.96±0.02 d
Vulgarol B	2350		0.46±0.01				0.44±0.00

Results are expressed as mean ±SD (n=3) RI: Retention index relative to n-alkanes (C8–C24) on the Rtx-MS column. RI: Programmed temperature retention indices determined on Rtx-MS column (40-240 °C; 5 °C·min-1). *The differences between the active ingredients shown with the same letter in aroma components with a general average of more than 1.0 % among the applications are not statistically significant compared to the Least Significant Difference Test at the probability level of $P < 0.01$.

4. CONCLUSIONS

The highest values for dry plant weight, root fresh and dry weight were observed for the consortium BF3 (*P. fluorescens* RC84 + *B. subtilis* RC67 + *B. megaterium* RC34 + *P. agglomerans* RC16) and NPK applications; while the highest values in terms of essential oil content and the important components Khusimol, β-Vetivone, α-Vetivone, Khusimone, Khusimene, and Longifolene were found for the BF1 (*P. fluorescens* RC09 + *B. subtilis* RC11 + *B. megaterium* RC33 + *P. agglomerans* RC36) inoculation. Interestingly, the applications that ranked highest in plant characteristics performed poorly in terms of volatile oil content. The contents of pharmacologically important compounds such as β-vetivone, α-vetivone, α-gurjunene, and ɣ-muurolene increased with the inoculations of multi-strain bacterial consortia, which contain compounds such as longifolene, (-)-spathulenol, δ-amorphene, and α-sedrene, which are not found in the essential oils of the control plants. The plant inoculated with the bacterial consortium BF1, which had the highest proportion of the dominant aroma component, khusimol, outperformed the others in this regard. Inoculation with a multi-strain bacterial consortium significantly increased vetiver plant growth, shoot and root weights, and content of important dominant aroma compounds used to evaluate the quality characteristics of essential oil, although responses were microbial-consortia specific. Based on these results, it can be concluded that the use of biologically-based fertilizers containing bacteria in the cultivation of vetiver, a plant with significant potential for the restoration of soil, erosion control, and valuable volatile oil production in problematic agricultural areas, offers significant advantages over chemical fertilizers.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

FUNDING SOURCES

This research was supported by the Scientific Research Projects Coordination Unit of Çanakkale Onsekiz Mart University under the grant number FHD-2022-4199. We extend our gratitude for their support.

AUTHORSHIP CONTRIBUTION STATEMENT

R Cakmakci: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing original draft, Writing review and editing.

S Akcura: Investigation, Formal analysis, Methodology, Writing – review and editing.

U Binbir: Conceptualization, Investigation, Methodology.

REFERENCES

Adams RP. 2007. *Identification of volatile oil components by gas chromatography/ mass spectrometry*. fourth ed. Allured Publishing, Carol Stream, IL.

Adams RP, Nguyen S, Johnston DA, Park S, Provin TL, Habte M. 2008. Comparison of vetiver root essential oils from cleansed (bacteria-and fungus-free) vs. non-cleansed (normal) vetiver plants. *Biochem. Syst. Ecol.* **36** (3), 177-182. https://doi.org/10.1016/j.bse.2007.10.004.

Ahbar AI, Othman NMI, Shamsuddin AS, Sari NA. 2024. Pelletized plant growth-promoting bacteria derived from fermented rice water and its influence in pakchoy growth enhancement. *J. Teknol.* **86** (6), 69-76. https://doi.org/10.11113/jurnalteknologi.v86.21087

Akçura S, Çakmakçı R, 2023. The effect of plant growth promoting bacteria on some plant traits in black cumin (*Nigella damascena* L.) *ISPEC J. Agric. Sci.* **7** (3), 472-488. https://doi.org/10.18016/10.5281/zenodo.8303783.

BMD. 2022. Balıkesir Meteorology Directorate, https://www.mgm.gov.tr/eng/radar.aspx?rG=anm&rR=10&rU=ppi Date: 25.03.2022)

Bechtaoui N, Raklami A, Tahiri A I, Benidire L, El Alaoui A, Meddich A, Oufdou K. 2019. Characterization of plant growth promoting rhizobacteria and their benefits on growth and phosphate nutrition of faba bean and wheat. *Biol. Open* **8** (7), bio043968. https://doi.org/10.1242/bio.043968.

Binti Othman NMI, Binti Hajad SS, Shamsuddin AS, Kee Zuan AT, Saiman MF, Hamid NA. 2024. Effectiveness of encapsulated plant growth-promoting bacteria, Acinetobacter sp. and organic supplements on the growth and nutrient contents enhancement in chilli plant. *Malays. J. Microb.* **20** (4), 501-506. http://dx.doi.org/10.21161/mjm.230501

Çakmakçı R, Dönmez F, Aydın A, Şahin F. 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem.* **38** (6), 1482-1487. https://doi.org/10.1016/j.soilbio.2005.09.019.

Çakmakçı R, Mosber G, Milton AH, Alatürk F, Ali B. 2020. The effect of auxin and auxin-producing bacteria on the growth, essential oil yield, and composition in medicinal and aromatic plants. *Cur. Microb.* **77** (4), 564–577. https://doi.org/10.1007/s00284-020-01917-4.

Chahal KK, Bhardwaj U, Kaushal S, Sandhu AK. 2015. Chemical composition and biological properties of *Chrysopogon zizanioides* (L.) Roberty syn. *Vetiveria zizanioides* (L.) Nash- A review. *Indian J. Nat. Prod. Resour.* **6** (4), 251-260.

Champagnat P, Figueredo G, Chalchat JC, Carnat AP, Bessiere JM. 2006. A study on the composition of commercial *Vetiveria zizanioides* oils from different geographical origins. *J. Essent. Oil Res.* **18**, 416-422. https://doi.org/10.1080/10412905.2006.9699129.

Chauhan AK, Maheshwari DK, Dheeman S, Bajpai VK. 2017. Termitarium-inhabiting *Bacillus* spp. enhanced plant growth and bioactive component in turmeric (*Curcuma longa* L.). *Cur. Microb.* 74, 184-192. https://doi.org/10.1007/s00284-016-1172-z.

David A, Wang F, Sun X, Li H, Lin J, Li P, Deng G. 2019. Chemical composition, antioxidant, and antimicrobial activities of *Vetiveria zizanioides* (L.) Nash essential oil extracted by carbon dioxide expanded ethanol. *Molecules* **24**, 1897. https://doi.org/10.3390/molecules24101897.

Del Giudice L, Massardo DR, Pontieri P, Bertea CM, Mombello D, Carata E, Alifano P. 2008. The microbial community of Vetiver root and its involvement into essential oil biogenesis. *Environ. Microbiol.* **10** (10), 2824-2841. https://doi.org/10.1111/j.1462-2920.2008.01703.x.

Hartwig S, Frister T, Alemdar S, Li Z, Scheper T, Beutel S. 2015. SUMO-fusion, purification, and characterization of a (,)-zizaene synthase from *Chrysopogon zizanioides*. *Biochem. Biophys. Res. Commun.* **458** (4), 883-889. https://doi.org/10.1016/j.bbrc.2015.02.053.

Karagöz H, Çakmakçı R, Hosseinpour A, Kodaz S. 2018. Alleviation of water stress and promotion of the growth of sugar beet (*Beta vulgaris* L.) plants by multi-traits rhizobacteria. *Appl. Ecol. Environ. Res.* **16** (5), 6801-6813. http://dx.doi.org/10.15666/aeer/1605_68016813.

Kirici S, Inan M, Turk M, Giray ES. 2011. To study of essential oil and agricultural properties of vetiver (*Vetiveria zizanioides*) in the southeastern of Mediterranean. *Adv. Environ. Biol.* **5** (2), 447-452.

Kumar A, Vandana S.M, Singh P.P, Singh S.K, Singh P.K, Pandey KD. 2016. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocat. Agric. Biotech.* **8**, 1-7. https://doi.org/10.1016/B978-0-12-818095-2.00007-2.

Maddhesiya PK, Singh K, Kumar D, Singh RP. 2022. Enhancing productivity of perennial aromatic grasses on marginal lands through plant growth promoting rhizobacteria. *Land Degra. & Develop.* **33**, 2972–2982. https://doi.org/10.1002/ldr.4244.

Mallavarapu, G.R, Syamasundar, K.V, Ramesh, S, Rao, BRR. 2012. Constituents of south Indian vetiver oils. *Nat. Prod. Commun.* **7** (2), 223-227.

Oliveira TAS, Vieira TM, Esperandim VR, Martins CHG, Magalhães LG, Miranda MLD, Crotti AEM. 2022. Antibacterial, antiparasitic, and cytotoxic activities of chemical characterized essential oil of *Chrysopogon zizanioides* roots. *Pharmaceuticals* **15**, 967. https://doi.org/10.3390/ph15080967.

Orozco-Mosqueda MC, Fadiji EA, Babalola OO, Glick BR, Santoyo G. 2022. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. *Microb. Res.* **263**, 127137. https://doi.org/10.1016/j.micres.2022.127137.

Pandur E, Balatinácz A, Micalizzi G, Mondello L, Horváth A, Sipos K, Horváth G. 2021. Anti-inflammatory effect of lavender (Lavandula angustifolia Mill.) essential oil prepared during different plant phenophases on THP-1 macrophages. *BMC Comp. Medi. and Therap.* **21**, 1-17. https://doi.org/10.1186/s12906-021-03461-5

Priyadarshani NDN, Amarasinghe MKTK, Subasinghe S, Palihakkara IR, Kumarasinghe HK. 2013. Effect of organic and inorganic fertilizers on biomass production, oil yield and quality of vetiver (*Vetiveria zizanioides* L.). *J. Agric. Sci.* **8**, 28-35.

Ramly IS, Othman NMI, Shamsuddin AS, Zuan ATK, Zakaria NH, Majid F AA. 2024. Enhanced Rice (*Oryza sativa* L.) Plant Growth and Nutrient Contents During The Vegetative Stage Through Zinc Solubilizing Bacterial Bead Inoculation. *Malays. Appl. Biol.* **53** (5), 87-97. https://doi.org/10.55230/mabjournal.v53i5.3113

Reed L, Glick BR. 2023. The recent use of plant-growth-promoting bacteria to promote the growth of agricultural food crops. *Agriculture* **13** (5), 1089-1113. https://doi.org/10.3390/agriculture13051089.

Şahin F, Çakmakçı R, Kantar F. 2004. Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant Soil* **265**, 123-129.

SAS Institute. 2017. JMP Statistics and Graphics Guide, Version 17.0 (trial). SAS Institute Cary NC USA.

Sadeq BM, Zuan ATK, Kasim S, Alkooranee JT, Yun WM, Othman NMI, Rahman E. 2024. Tripartite Introductions of PGPR, Humic Acid, and N-Fertilizer Improve the Growth and Yield of Sweet Potato under Glasshouse Conditions. *Sains Malays*. **53** (1), 23-37.

Singh N, Singh VR, Lal RK, Verma RS, Mishra A, Yadav R. 2019. Quantification of genotypic and chemotypic diversity for elite clone selection with high-quality essential oil traits in vetiver [*Chrysopogon zizanioides* (L.) Roberty]. *J. Essent. Oil-Bear. Plants* **22** (4), 1150-1162. https://doi.org/10.1080/0972060X.2019.1661795.

Syaziana NSA, Othman NMI, Soraya SA, Ali TKZ, Musliyana M. 2024. Isolation and characterization of encapsulated plant growth-promoting Enterobacter sp. SA10 for enhancing chili growth. *J. King Saud Univ. Sci.* **36** (6), 103197. https://doi.org/10.1016/j.jksus.2024.103197

Vollú RE, Blank AF, Seldin L, Coelho MRR. 2012. Molecular diversity of nitrogen-fixing bacteria associated with *Chrysopogon zizanioides* (L.) Roberty (vetiver), an essential oil producer plant. *Plant Soil* **356**, 101-111. https://doi.org/10.1007/s11104-011-0801-3.

Yan W. 2014. *Crop variety trials: Data management and analysis*. John Wiley & Sons.

Zhang J, Cook J, Nearing JT, Zhang J, Raudonis R, Glick BR, Cheng Z. 2021. Harnessing the plant microbiome to promote the growth of agricultural crops. *Microbiol. Res.* **245**, 126690. https://doi.org/10.1016/j.micres.2020.126690.