Interactions between a plant growth-promoting rhizobacterium and smoke-derived compounds and their effect on okra growth

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Abstract

Plant growth-promoting rhizobacteria (PGPR) are used in agriculture to improve crop yield. Crude smoke–water (made by bubbling plant-derived smoke through water) stimulates germination and improves seedling growth. Some active compounds have been isolated from smoke with karrikinolide (KAR_1) stimulating plant growth and trimethylbutenolide (TMB) being inhibitory. These smoke compounds have great potential in agriculture but their interaction with PGPR is unknown. In the present study, a two-factorial pot trial with three replicates per treatment was designed to investigate the interactions between *Bacillus licheniformis* and two concentrations each of smoke–water, KAR_1, and TMB on okra (*Abelmoschus esculentus*). Growth and physiological parameters (chlorophyll, carotenoid, protein, sugar and α-amylase) of okra as well as bacterial abundance in the rhizosphere were measured after 5 weeks. Application of *B. licheniformis* and 10^{-7} M KAR_1 significantly improved the shoot biomass and 10^{-7} M KAR_1 also significantly improved leaf area of okra. However, when 10^{-7} M KAR_1 was applied in combination with *B. licheniformis*, there was an antagonistic effect on plant growth. While TMB had a negative effect on plant growth, a combination treatment of TMB and *B. licheniformis* overcame the inhibitory effect of TMB resulting in plant growth similar to the control plants. All treatments had no effect on chlorophyll, carotenoid, protein and sugar concentrations, while α-amylase activity was significantly elevated in okra root treated with 1:500 v/v smoke–water. Determining the rhizobacteria populations at harvest showed that all treatments had no significant effect on the rhizosphere microbial abundance. The modes of interaction between PGPR and smoke-derived compounds need to be further elucidated.

Key words: *Bacillus licheniformis* / karrikinolide / natural plant biostimulant / smoke–water / trimethylbutenolide

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1 Introduction

There is a global trend towards organic farming in order to increase crop productivity to meet the demands of an expanding population, while simultaneously improving water-use efficiency and mitigating environmental pollution and high energy inputs associated with synthetic fertilizers, pesticides and herbicides (*Gomiero* et al., 2011; *da Costa* et al., 2013). Areas of research for sustainable and environmentally friendly agricultural practices include the use of plant growth-promoting rhizobacteria (PGPR) and the application of natural plant biostimulants (*Kulkarni* et al., 2011; *da Costa* et al., 2013; *Bashan* et al., 2014).

Soil microbes play an essential role in recycling nutrients in the soil (*Miransari*, 2013). Plant growth-promoting rhizobacteria are a diverse group of rhizosphere-colonizing bacteria that have the potential to enhance plant growth, help suppress root disease, and provide some protection against environmental stresses (*Mayak* et al., 2004). There are multiple mechanisms by which these PGPR influence plant growth. These include: (1) plant-growth regulators that promote growth, *e.g.*, auxins, cytokinins, gibberellins (GAs), and nitric oxide (NO), (2) stress-related plant growth regulators, *e.g.*, abscisic acid (ABA) and jasmonic acid (JA), (3) antimicrobial compounds, (4) plant defense mechanisms, (5) N_2 fixation, (6) iron mobilization by siderophores, (7) phosphate solubilization, and (8) production of 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase (*Mayak* et al., 2004; *da Costa* et al., 2013; *Cassán* et al., 2014). Many PGPR inocula are available commercially and are applied with either an organic or inorganic carrier to improve the survival of the bacteria and to maintain a threshold PGPR population to ensure positive effects on plants. The most common genera include the symbiotic *Rhizobium* and the free-living *Azospirillum, Pseudomonas*, and *Bacillus* strains (*Bashan* et al., 2014).

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Smoke derived from the combustion of plant material stimulates germination as well as having other beneficial effects such as enhancing seedling growth and vigor, increasing root growth, improving resistance to environmental and heavy metal stress, increasing flowering and improving crop yield (reviewed in *Light* et al., 2009; *Kulkarni* et al., 2011; *Nelson* et al., 2012). This response is not limited to only species from fire-prone environments but encompasses a wide range of wild and cultivated species including weeds (*Light* et al., 2009). However, in a survey of 301 fynbos species collected from the fire-prone Western Cape region (South Africa), only 50% of the species showed positive germination when treated with smoke-derived compounds. Families that did not respond to smoke include Amaryllidaceae, Hycinthaceae, and many Iridaceae (*Brown* et al., 2003).

The most active component in smoke was isolated and identified as 3-methyl-2*H*-furo[2, 3*c*]pyran-2-one, referred to as karrikinolide (KAR_1), from burning fynbos material (*Passerina vulgaris*) and climax grass (*Themeda triandra; van Staden* et al., 2004) and combustion of cellulose paper (*Flematti* et al., 2004). Five additional active karrikin analogs with closely related structures, incorporating a butenolide moiety fused to a pyran ring with various methyl substitutions, have since been identified and synthesized, *i.e.*, KAR_2–KAR_6 (*Flematti* et al., 2007). Smoke is composed of thousands of volatile compounds and bubbling smoke through water produces crude "smoke–water" (*Light* et al., 2009). Unlike KAR_1, smoke–water has a "dual regulatory" effect in plants with low concentrations promoting germination and higher concentrations being inhibitory. This observation led to the isolation of an inhibitory butenolide compound 3,4,5-trimethylfuran-2(5*H*)-one, referred to as trimethylbutenolide (TMB; *Light* et al., 2010).

Karrikins are water-soluble, thermostable, long-lasting in solution, highly active at very low concentrations (down to 10^{-9} M; *Light* et al., 2009) and have no mutagenic and genotoxic effects (*Trinh* et al., 2010). Thus, this relatively new smoke biotechnology offers great potential for both conventional and organic agriculture (*e.g.*, weed management and land rehabilitation). However, before this smoke biotechnology can be used in agriculture, a number of questions need to be addressed including the impact it has on the soil microbial population (*Light* et al., 2009) to ensure "healthy" soil. Diverse populations of PGPR provide a better resource for improving plant growth and disease management as each strain has a different mode of action and survival in changing environmental conditions (*Negi* et al., 2011). Thus, the aim of the present study was to investigate the interactive effects of smoke-derived compounds (smoke–water, KAR_1, and TMB) and one selected PGPR (*Bacillus licheniformis*) by monitoring various aspects of plant growth.

2 Material and methods

2.1 Preparation of smoke–water, KAR_1, and TMB

Smoke–water was prepared by burning 5 kg *Themeda triandra* leaf material and bubbling the smoke through 500 mL distilled water for 45 min (*Baxter* et al., 1994). The resulting solution was diluted 1 : 250 v/v to give the stock solution. KAR_1 (purity > 95%) and TMB (purity > 98%) were synthesized according to the protocols of *Flematti* et al. (2005) and *Surmont* et al. (2010), respectively.

2.2 Bacterial inoculum

Bacillus licheniformis strain Rt4M10 was isolated from the root surface of *Vitis vinifera* L. cv. Malbec grown in a commercial vineyard in Mendoza, Argentina. It was characterized biochemically and phylogenetically and identified by 16S rRNA gene sequences (*Salomon* et al., 2014). Bacterial inoculum was prepared by growing *B. licheniformis* in 200 mL Luria Broth (LB) media on an orbital shaker at 27°C for 2 d. The optical density (Varian Cary 50 UV-Vis Spectrophotometer) was measured to achieve uniform populations of bacteria of $\approx 10^8$ colony-forming units (CFU) mL^{-1}. The inoculum was centrifuged at 8,000 *g* for 10 min (Beckman Coulter Avanti J-E Centrifuge) and the pellet rinsed with distilled water to remove traces of LB medium. A bacterial suspension was made using distilled water so that the absorbance value was 1.0 when measured at 660 nm.

2.3 Pot trial

Okra cv. Clemson spineless seeds (*Abelmoschus esculentus*) were purchased from McDonald's Seed Company, Pietermaritzburg, South Africa. New pots (10 cm diameter) were filled with 242 g autoclaved garden soil which comprised 80% compost, 19% bark (chipped and decomposed), 0.5% LAN (limestone ammonium nitrate) and 0.5% of a 5 : 7 : 4 NPK fertilizer (soil pH 5.8). Three seeds were sown per pot with nine pots per treatment. Pots were arranged on a metal bench in a greenhouse with a daily maximum and minimum temperature of 22 ± 3°C and 15 ± 2°C, respectively, and midday light intensity of 500–600 μmol m^{-2} s^{-1}.

A two-factorial pot trial was carried out in which the interactions between *B. licheniformis* and two concentrations each of smoke–water, KAR_1, and TMB were investigated and compared to a control treatment with/without *B. licheniformis* application. Eleven days after sowing, the seedlings were treated with either 15 mL bacterial inoculum per pot or 15 mL smoke-derived compounds per pot applied to the soil around the plants. For the combination treatments, the smoke-derived compounds were incorporated into the 15 mL bacterial inoculum. The treatments were as follows: control (distilled water), distilled water + bacterial inoculums, smoke–water (1 : 500 and 1 : 1000 v/v); smoke–water (1 : 500 and 1 : 1000 v/v) + bacterial inoculum, KAR_1 (10^{-7} M and 10^{-8} M), KAR_1 (10^{-7} M and 10^{-8} M) + bacterial inoculum; TMB (10^{-3} M and 10^{-4} M), TMB (10^{-3} M and 10^{-4} M) + bacterial inoculum. A second application of these solutions was done 2 weeks later. Pots were watered twice weekly with tap water for the duration of the pot trial.

Plants were harvested 5 weeks after sowing over a period of 3 d with one replicate per treatment harvested each day. Five plants, randomly harvested from the three pots belonging to one replicate, were combined to make a sample. During

harvesting, the soil adhering to the roots (defined as "rhizosphere soil") was gently shaken into a plastic bag to determine bacterial abundance (section 2.5). The roots were then immediately washed by dipping into a beaker of water and gently shaken until the remaining potting medium was removed. The roots were placed on paper towel and air-dried for 30 min to remove the adherent water. Fresh weights of roots and shoots (combined leaf and stem material) and leaf area (measured with a leaf area meter LI-31000, LI-COR Inc) were recorded as a measure of growth. Fresh material was taken for the various biochemical analyses. The remaining plant material was immersed in liquid nitrogen and ground by hand using a mortar and pestle and then further lyophilized.

2.4 Biochemical analysis

Chlorophyll (chl *a*+*b*) and carotenoids in the leaves (250 mg FW) were determined by a double sequential extraction in acetone with mortar and pestle with a pinch of acid-washed sand to aid homogenization. Following centrifugation (1,400 *g* for 5 min), absorbance of the combined supernatants was measured at 470 nm, 645 nm, and 662 nm (Cary 50 UV-Vis spectrophotometer). Pigment concentration ($\mu g\ g^{-1}$ FW) was calculated as (*Lichtenhaler*, 1987):

$$\text{Chl } a = 11.23A_{662} - 2.04A_{645}, \quad (1)$$

$$\text{Chl } b = 20.13A_{645} - 4.19A_{662}, \quad (2)$$

$$\text{Chl } a{+}b = 7.05A_{662} + 18.09A_{645}, \quad (3)$$

$$\text{Total carotenoids} = (1000A_{470} - 1.90\text{Chl } a - 63.14\text{Chl } b) / 214. \quad (4)$$

Protein in the shoots and roots was determined following the method of *Lowry* et al. (1951). Plant material (500 mg FW) was homogenized using an Ultra-Turrax and extracted in 10 mL 20% trichloroacetic acid and following centrifugation (55 *g* for 15 min), 5 mL 0.1 N NaOH added to the pellet and centrifuged. The supernatant was mixed with 5 mL alkaline copper reagent (50 mL 2% Na_2CO_3 in 0.1 N NaOH mixed with 1 mL 0.5% $CuSO_4 \cdot 5\ H_2O$ in 1% $C_4H_4KNaO_6 \cdot 4\ H_2O$) and kept in the dark for 10 min, after which 0.5 mL Folin phenol reagent (diluted 1 : 1 with distilled water) was added and the sample was kept in the dark for a further 30 min. Absorbance was read at 660 nm. A standard graph of protein prepared with Bovine's Serum Albumin was used to calculate protein concentration (mg g^{-1} FW).

Sugar in the shoots was estimated in duplicate using anthrone reagent (*Jermyn*, 1975). Powdered material (25 mg DW) was extracted in 10 mL 80% ethanol at 95°C for 60 min. Following centrifugation (600 *g* for 15 min), the supernatant was made up to 10 mL with distilled water. The extract (500 μL) and 3 mL anthrone reagent were heated at 100°C for 10 min after which the reaction was stopped by placing on ice. Absorbance was read at 620 nm. A standard curve prepared with glucose was used to calculate sugar concentration.

Activity of α-amylase in shoot and root samples was assayed following the method of *Sadasivam* and *Manickam* (1996). Plant material (1 g FW) was homogenized with an Ultra-Turrax and extracted with 10 mL 10 mM calcium chloride. Following centrifugation (30,000 *g* at 4°C for 15 min), 1 mL supernatant used as the enzyme source was added to 1 mL soluble starch (1%) prepared in acetate buffer. The extract was incubated at room temperature for 5 min and the reaction then stopped with 2 mL 3,5-dinitrosalicylic acid reagent. Sodium potassium tartrate (1 mL) was added to the warm tubes (50°C) and, after cooling, the extract was made up to 10 mL with distilled water. Absorbance was read at 560 nm. Activity was expressed as μmol maltose released mg^{-1} protein min^{-1}.

2.5 Estimating soil bacterial abundance

Bacterial abundance in the rhizosphere sample was estimated using the method of *Alam* et al. (2013) slightly modified. Briefly, 500 mg of the soil were suspended in sterile 50 mL LB medium and shaken on an orbital shaker for 20 min at 27°C. Before sampling, the flasks were gently shaken to resuspend the soil and then 1 mL suspension was transferred to a bottle containing 99 mL LB media. This diluted suspension was gently shaken and 1 mL of the homogenous suspension was spread on a Petri dish of LB medium under sterile conditions. The petri dishes were incubated at 27°C in the dark. The bacterial colonies were counted after 24 h using a colony counter (Colony Anderman Counter) and the number of bacterial CFU g^{-1} soil was calculated.

2.6 Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) and the means separated using Duncan's multiple range test at 5% level of significance ($P < 5\%$). In addition, two-way ANOVA was conducted to determine the significant differences between the treatment-and-bacteria interaction ($P < 5\%$; GenStat® release 14 statistical package).

3 Results and discussion

3.1 Growth of okra

Single application of *B. licheniformis* promoted growth of okra with a significant increase in shoot biomass as well as a slight (but not significant) increase in root biomass and leaf area (Fig. 1). This positive growth response may possibly be due to the hormones produced by *B. licheniformis*. Previously, ABA, indole-3-acetic acid (IAA), and GAs (GA_1 and GA_3) were identified in the *B. licheniformis* strain used in the present study. It had non-pathogenic characteristics and was able to improve shoot and root length and leaf area in *in vitro Vitis vinifera* cv. Malbec and increased ABA in the shoots and IAA in the roots (*Salomon* et al., 2014). PGPR have been used to enhance growth and yield of a number of crops such as tomato and pepper (*Mayak* et al., 2004). Other examples of hormone-producing PGPR include *Azospirillum* sp. (which synthesizes ABA, auxins, cytokinins, ethylene, GAs, NO, and polyamines; *Cassán* et al., 2014) and *Bacillus pumilus* and *Achromobacter xylosoxidans* (which synthesize ABA, JA, and salicylic acid; *Castillo* et al., 2013).

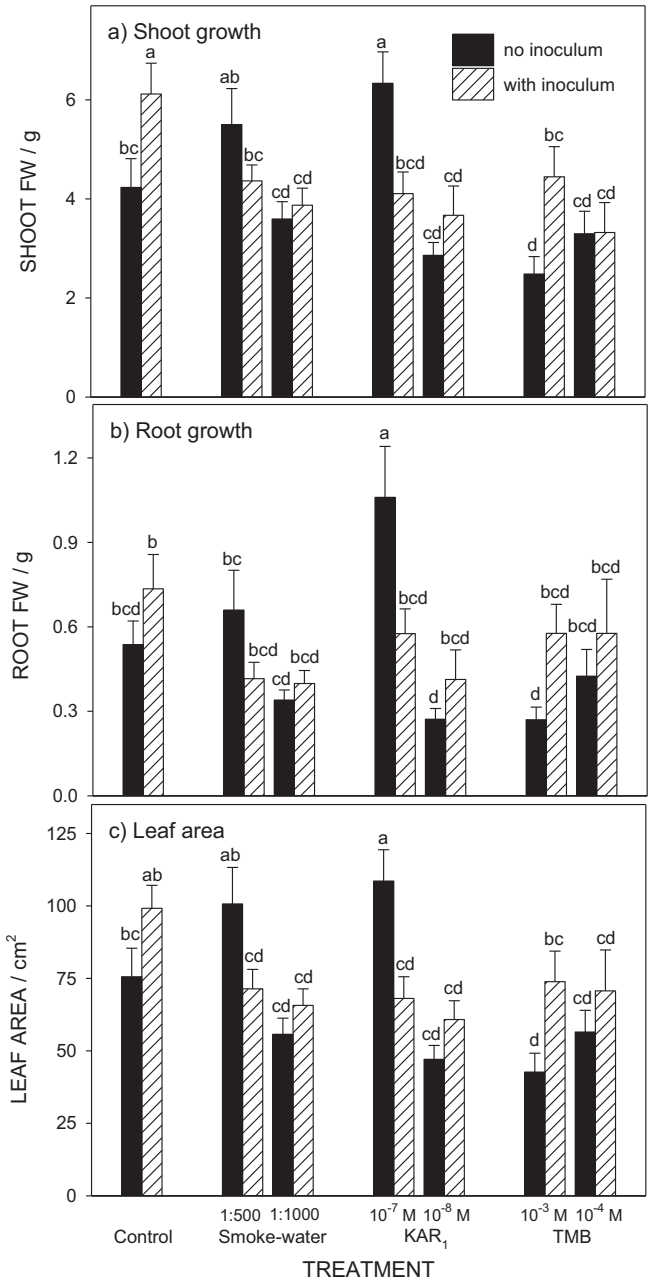

Figure 1: Growth parameters showing (a) shoot biomass, (b) root biomass, and (c) leaf area of okra harvested after 5 weeks when treated with combinations of smoke-derived compounds and *Bacillus licheniformis* inoculum. Results are presented as means ± SE (n = 3) with significant differences indicated by different letters (P > 5%). KAR_1 = karrikinolide; TMB = trimethylbutenolide.

It is necessary to maintain a threshold number of bacterial cells in the rhizosphere to achieve a positive effect on plant growth. The bacterial inoculum was applied twice at a two-week interval and this ensured a sufficient rhizobacteria population to achieve a positive effect as seen by the improved growth of okra treated with bacterial inoculum (Fig. 1).

Single application of 10^{-7} M KAR_1 significantly increased shoot and root biomass and leaf area and 1 : 500 v/v smoke–water slightly improved growth of okra although not significantly compared to the control. Lower concentrations of KAR_1 (10^{-8} M) and smoke–water (1 : 1000 v/v) had no significant effect on okra growth (Fig. 1). This positive growth of okra in response to KAR_1 may be due to interactions between the smoke-derived compound and endogenous plant hormones. Previous studies have shown that smoke compounds interact with GAs and affect endogenous GA and ABA concentrations in various species and can substitute for auxins in somatic embryogenesis (reviewed in *Light* et al., 2009). KAR_1 stimulated cell division in the soybean callus bioassay which is used to measure cytokinin-like activity and stimulated rooting in the mungbean bioassay which is used to measure auxin-like activity (*Jain* et al., 2008). The different growth responses of okra obtained with KAR_1 and smoke–water application may be explained by these compound(s) having different modes of action, with smoke–water comprising thousands of volatile compounds (*Light* et al., 2009). The mode(s) of action by which the various smoke-derived compounds influence plant growth have not been fully elucidated although gene expression and protein ubiquitination patterns are different with smoke–water and KAR_1 application (*Soós* et al., 2012).

Although single application of *B. licheniformis* and 10^{-7} M KAR_1 significantly enhanced growth of okra, an antagonistic effect was observed when smoke–water or KAR_1 were applied in combination with *B. licheniformis* with no improvement in the growth compared to the control (Fig. 1). A possible explanation for this antagonistic effect may be that *B. licheniformis*, smoke–water, and KAR_1 have overlapping modes of action, all being involved in hormone crosstalk with the associated plant with *B. licheniformis* producing hormones (*Salomon* et al., 2014) and KAR_1 having synergistic effects (*Jain* et al., 2008). Thus, combined applications could potentially disrupt hormone homeostasis in okra and thus inhibit growth. A synergistic effect between smoke-derived compounds and plant hormones was previously reported when KAR_1 was applied in combination with a kinetin and indole-3-butyric acid in the soybean callus and mungbean bioassays, respectively (*Jain* et al., 2008). This interaction between PGPR and smoke compounds requires further investigation with lower concentrations of the smoke-derived compounds being tested.

The inhibitor TMB had a negative effect on okra growth with the highest TMB concentration (10^{-3} M) significantly decreasing shoot biomass and leaf area. However, application of *B. licheniformis* inoculum in combination with TMB overcame the inhibitory effects of TMB so that growth was similar to the control plants (Fig. 1). This improved growth may have been caused by *B. licheniformis* favorably influencing the hormone profile although this interaction requires further investigation. Although not elucidated, the mechanisms of action of TMB are different to those of KAR_1 with TMB reducing the stimulatory effect of KAR_1 in a concentration-dependent manner but not competing for the same binding sites (*Soós* et al., 2012).

Two-way ANOVA showed that bacteria alone did not have a significant effect on okra growth but its interaction with the different treatments showed significant differences for shoot growth (FW) and leaf area but not for root growth (Table 1).

Table 1: Two-way ANOVA with treatment-and-bacteria interactions on the growth parameters of okra.

	Shoot FW / g		Root FW / g		Leaf area / cm^2	
Source of variation	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**
Treatment (T)	4.51	< 0.004	1.81	0.148	3.91	< 0.025
Bacteria (B)	0.45	0.502	0.02	0.886	0.19	0.664
T × B	3.56	< 0.015	2.58	0.055	3.20	< 0.018

3.2 Biochemical quantification

Bacillus licheniformis inoculum and the various smoke-derived compounds, in single application and in combination, had no significant effects on the total chlorophyll and carotenoid concentrations in okra. The one exception was the combined 1 : 1000 v/v smoke–water and *B. licheniformis* treatment in which there was a significant decrease in the photosynthetic pigment concentration compared to the other treatments (data not shown). These results are in contrast to previous reports of PGPR and smoke treatments altering the chlorophyll and macromolecule composition of plants. For example, mango trees (*Mangifera indica* cv. Ataulfo) inoculated with the ACC-deaminase producing *Burkholderia caribensis* and a hormone producing *Rhizobium* sp. initially had higher nitrogen and carbohydrate concentrations in the leaves (*de los Santos-Villalobos* et al., 2013). Similarly, smoke–water and KAR_1 increased photosynthetic pigments (chl *a*, chl *b*, and carotenoids) and secondary metabolites (phenolics, flavonoids, and proanthocyanidins) in micropropagated "Williams" bananas (*Aremu* et al., 2012).

The bacterial and smoke treatments also had no effect on the α-amylase activity in the shoots of okra (data not shown). However, application of 1 : 500 v/v smoke–water significantly increased α-amylase activity in the roots and 10^{-8} M KAR_1 and *B. licheniformis* inoculum also increased the activity, although not significantly (Fig. 2). Lower concentrations of smoke–water (1 : 1000 v/v), KAR_1 (10^{-8} M), and TMB (10^{-3} M and 10^{-4} M) had no measureable effects on the α-amylase activity in the okra roots. None of the treatments had a significant effect on the protein and sugar concentrations of okra (data not shown). These results indicate that the effects of *B. licheniformis* and the smoke-derived compounds on okra growth are not due to changes in physiologically relevant molecules of primary metabolism. Future experiments should focus on changes in the composition of secondary metabolites to gain further insight into the interactions between PGPR and natural smoke-derived biostimulants. For example, PGPR alleviate abiotic stress in plants by altering reactive oxygen species (ROS) scavenging enzymes and osmolytes (glycine betaine, proline; *Gururani* et al., 2013). Smoke treatments also modified the secondary metabolite composition with significantly higher flavonoid and phenolic concentrations of smoke-treated *Aloe arborescens* (*Kulkarni* et al., 2014) with molecular evidence suggesting that smoke–water can up-regulate the phenylpropanoid pathway and flavonoid-related genes (*Soós* et al., 2010).

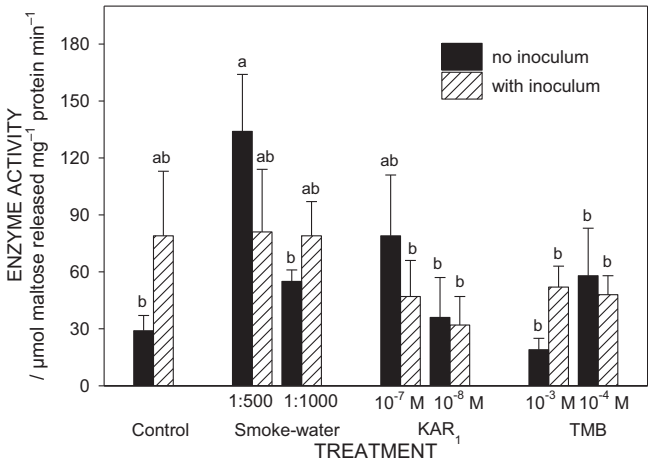

Figure 2: α-Amylase activity in roots of okra harvested after 5 weeks when treated with combinations of smoke-derived compounds and *Bacillus licheniformis* inoculum. Results are presented as means ± SE (n = 3) with significant differences indicated by different letters (P > 5%). KAR_1 = karrikinolide; TMB = trimethylbutenolide.

3.3 Bacterial abundance

Bacterial population abundance in the rhizosphere was quantified at the end of the pot trial. Although there was little difference in the bacterial abundance between treatments, of note is the significantly lower bacterial population in the 1 : 500 v/v smoke–water treatment compared to the *B. licheniformis* treatment (Fig. 3). This may be due to the antimicrobial properties of smoke–water. Some traditional agricultural methods expose seeds to smoke to reduce microbial contamination during seed storage. There are also examples of smoke compounds applied to crops being able to reduce harmful phytopathogenic bacteria (reviewed by *Kulkarni* et al., 2011).

Root exudates consisting of organic compounds such as sugars, polysaccharides, amino acids, peptides, proteins, vitamins, and phenolics as well as rhizodeposits (sloughed cells and decaying roots) provide a substrate for the microbial population. The quantity and composition of the exudate influences the rhizobacterial community composition (*Gregory*, 2006; *Miransari*, 2013). Thus, treatments such as 10^{-7} M KAR_1, that significantly alter the root biomass, may indirectly affect the microbial population in the rhizosphere by altering the amount and composition of the root exudate. This requires further investigation.

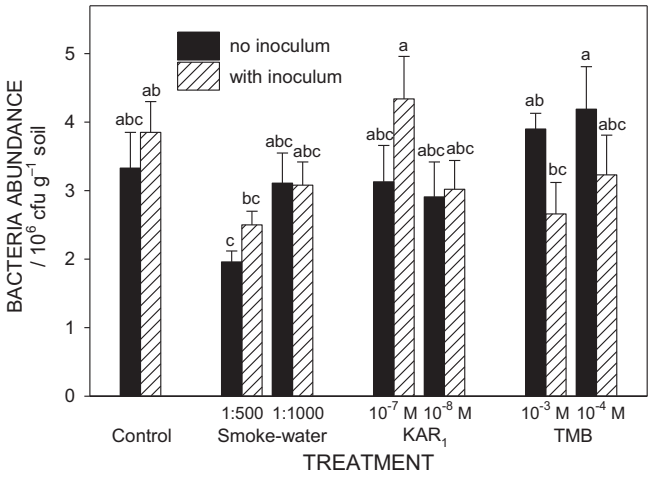

Figure 3: Bacterial population abundance in the rhizosphere of okra when treated with combinations of smoke-derived compounds and *Bacillus licheniformis*. Results are presented as means ± SE (n = 3) with significant differences indicated by different letters (P > 5%). KAR_1 = karrikinolide; TMB = trimethylbutenolide.

4 Conclusions

The present study is the first to demonstrate interactions between a PGPR strain and various smoke-derived compounds on plant growth. Although both *B. licheniformis* inoculum and 10^{-7} M KAR_1 had positive effects on okra growth, there were antagonistic effects when *B. licheniformis* was applied in combination with smoke–water or KAR_1. In addition, the bacterial inoculum alleviated the inhibitory effects of TMB on okra growth. We speculate that the possible explanation for this antagonistic effect may be due to overlapping modes of action of the hormone-producing *B. licheniformis* (*Salomon* et al., 2014) and smoke–water or KAR_1 which have synergistic effects with plant hormones (*Jain* et al., 2008). Thus, the combination treatments may have disrupted hormone homeostasis in okra. However, alternative modes of action cannot be ruled out as other *Bacillus* strains have been characterized as having enhanced ACC deaminase activity, phosphate solubilization, and siderophore production (*Gururani* et al., 2013). Smoke treatment also affected the rhizosphere microbial populations with crude smoke–water displaying mild antimicrobial activity. Application of these smoke-derived compounds could also potentially have an indirect effect on the rhizosphere population by affecting root biomass and root exudate composition. This aspect also requires further investigation.

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