

INOCULATION OF POTASSIUM SOLUBILIZING BACTERIA WITH DIFFERENT POTASSIUM FERTILIZATION SOURCES MEDIATES MAIZE GROWTH AND PRODUCTIVITY

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Potassium (K) deficiency is becoming a major productivity constraint in semi-arid agriculture. It is an essential mineral nutrient that has key role in various plant physiological and biochemical functions. Balanced nutrient application always tends to ensure better plant growth and development. Therefore, in the present study, efficacy of different K fertilizers for mediating maize plant growth and productivity in the presence and absence of KSB inoculant was assessed. A potassium solubilizing rhizobacterial strain, *Klebsiella oxytoca* KSB-17, was used in a 5 × 2 factorial pot experiment involving maize plant. The treatments comprised of control, waste mica (WM), K chemical fertilizer (KCF), WM-enriched compost (WMEC) and KCF-enriched compost (KCFEC). Compared to control treatment, KCF fertilization increased all plant physiological parameters both in presence and absence of KSB inoculation. From the results obtained, KCFEC followed by CF alone with KSB, demonstrated the most pronounced effect with significant increase in plant height (50%), shoot biomass (59%), root biomass (73%), root length (92%), plant K uptake (154%), grain starch (16%), protein (31%), oil content (22%) as well as photosynthetic rate (104%), transpiration rate (30%) and water use efficiency (54%) compared to control. Potassium solubilizing bacteria (KSB) prompted down-regulation of plant antioxidant enzyme activities against all K fertilizer treatments. Compared to control treatment, catalase (CAT) activity decreased significantly on application of KCF as well as with KCFEC in the presence of KSB inoculation. Regardless of KSB inoculation, plant growth, physiological, antioxidant and K nutritional traits showed slight improvement with either of WM fertilizers compared to control. Our findings concluded that K enriched compost with efficient KSB inoculant can be used to enhance maize growth and productivity in semi-arid agroecosystems.

Keywords: Compost, bio-fertilizer; waste mica; plant nutrition, grain quality, maize.

INTRODUCTION

Maize (*Zea mays* L.) is third most widely cultivated crop, consumed not only for the fulfillment of energy and protein needs, but also for industrial and biofuel production (Cruz-Cárdenas *et al.*, 2019). Given the projected increase in world population by 2050, maize has been foreseen as an economically the most important crop to tackle future food security issue (Jones, 2009). Global dryland ecosystems, arid and semi-arid, cover ~42% of world terrestrial land surface (Bastin *et al.*, 2017), and contribute largely to ensure both subsistence and livelihood benefits in these regions (Sujakhu *et al.*, 2018). Of various soil challenges, impoverished agricultural productivity is one of the major issues of dry land ecosystems, mainly due to historically low organic matter content, reduced soil fertility and exacerbated agriculture intensification (Shahzad *et al.*, 2017).

Being an important constituent of plant cell structure, K not only activates protein synthesis, carbohydrate metabolism and key enzyme reactions (Chérel, 2004), but also triggers variety

of plant adaptive responses under stressful conditions (Anschütz *et al.*, 2014). Many soils of arid and semi-arid regions are increasingly being depleted in soil K fertility, primarily due to the coupled effect of intensive agriculture practices with little or no K application (Ameen *et al.*, 2018). The K availability fluctuates accordingly to the soil types and is mainly governed by nature of clay minerals and organic matter status (Yadav *et al.*, 2016). Cereals are characteristically more efficient K absorber from soil compared to other crop plants, and resultantly cereal based agroecosystems are more likely to experience K deficiency because of negative K balance (Rao *et al.*, 2014). In semi-arid agroecosystem, farmers are paradoxically inclined to the application of N and P fertilizers in large quantities for higher productivity, and neglected altogether the use of K fertilizer in the past three decades (Samra *et al.*, 2008). Because of this incessant imbalanced fertilizer use in semi-arid ecosystem, K deficiency in crop plants has now become widespread and often inflicted crop productivity constraints in high K demanding crops i.e. maize, sugarcane, potato (Mian and

Ahmad, 2007). Moreover, the recent global estimate on potassium use efficiency of cereals crops reported a considerable decline in the last 50 years (Dhillon *et al.*, 2019). Therefore, a credible fertilization approach aiming to improve K utilization efficiency as well as farm productivity would be of great importance to conserve this non-renewable natural resource (Sui *et al.*, 2017).

Among various ecological approaches, use of compost amendments has frequently been advocated as a mean of promoting soil ecological functions for a resilient agroecosystems (Arif *et al.*, 2018). Often addition of compost can enhance agricultural productivity by its conditioning properties as well as recycling nutrients to the soil system (Conti *et al.*, 2014). The ancillary benefits of compost application may not be fully recognized with sole application largely because of insufficient nutrients supply that could reduce yield potential and farm productivity (Tian *et al.*, 2016). Integrating fertilizer nutrients such as K with organic materials could be a useful management option to counteract potassium deficiency in soils. It is well documented now that organic materials enriched fertilizer are environmentally safer and economically more feasible than chemical fertilizers alone (Zorb *et al.*, 2014). Numerous studies on the effects of nutrient enriched compost have highlighted the significance for being taken as an appealing soil management option in nutrient poor dryland ecosystems (Khalid *et al.*, 2014; Yadav *et al.*, 2017).

A hyphenating zone between plant roots and soil, known as rhizosphere, typically characterized for intense biochemical activities by releasing array of metabolites as well as recruiting variety of microbes (Hinsinger *et al.*, 2005). Potassium ions are usually taken up by the plants from root zone through facilitated process of diffusion and mass flow. In most soils, ~90% of potassium exist in unavailable mineral form i.e. feldspar, mica and orthoclase. Owing to their inherent resistivity against decomposition, only minor fraction of these mineral makes up into the pool of plant available K. Certain root associated bacteria called as potassium solubilizing bacteria (KSB) have shown exceptional affinity to solubilize the insoluble K minerals (Basak *et al.*, 2009; Meena *et al.*, 2018). The KSB can improve plant K acquisition while maintaining sufficient supply of K in soil solution via accelerated bacterial K solubilization and/or mobilization. The bacterial solubilization of insoluble K is usually driven by the secretions of various polysaccharides and carboxylic acids (Meena *et al.*, 2014). Application of KSB to increase available K in soil has some substantial evidences for their practical application in crop plants. However, the nature and extent of K dissolution by KSB can vary ranging from different soils and plants.

Although rate of K fertilizer application in cereal crops has seen a worldwide increase in last few decades, utilization efficiency of the added K has decreased considerably, raising

concerns of sustainable yield and farm profitability. Moreover, global K reserves are expected to last for next 100 years at current rate of consumption. The projected K fertilizer scenario establishes the needs to look for sustainable K fertilization approach in agroecosystems. The prospect of integrating K with organic materials such as compost in the presence of suitable KSB holds a great promise for semi-arid agriculture. Addition of compost not only could provide necessary substrate support to introduced KSB, but it could also safeguard K fertilizer from being fixed in these soils. The objectives of the present study were to evaluate the effect of two potassium fertilization material, WM and CF either alone or enriched compost on plant growth, physiological and biochemical attributes, plant K nutrition and grain quality of semi-arid grown maize. In addition, plant growth and productivity were also assessed, both in the presence and absence of KSB strain (*Klebsiella oxytoca* KSB-17).

MATERIALS AND METHODS

Bacterial strain: The KSB strain, *Klebsiella oxytoca* KSB-17, chosen for this study was originally isolated from the root zone of maize. Pure culture of KSB-17 was maintained in tryptic soy broth (TBS) supplemented with 20% glycerol (w/v) at -40°C. The selected bacteria exhibited numerous plant growth promoting attributes (Table 1). 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an ethylene regulating enzyme activity, was quantified by monitoring ACC cleavage into α -ketobutyrate (Penrose *et al.*, 2003). Auxin biosynthesis by KSB-17 was assayed according to Sarwar *et al.* (1992). Bacterial ability to produce siderophores was determined in Luria-Bertani (LB) using Chrome Azurol Sulphonate (CAS) according to the modified method of Hu and Xu (2011). Briefly, 15 μ L of freshly grown bacterial culture was inoculated into LB broth at 28 \pm 1 °C. After 48 h, 0.5 mL of aliquot was mixed with an equal volume of CAS reagent followed by incubation for 3hr in dark. Bacterial siderophores was expressed in percent siderophores unit (PSU) by measuring absorbance at 630 nm. Chitinase activity of KSB-17 was executed following the method of Cattelan *et al.* (1999). Phosphate solubilization capacity of KSB-17 was quantified by using rock phosphate as sole P source of least solubility. Bacterial root colonization assay was performed as described by Simons *et al.* (1996).

Table 1. Characterization of K-solubilizing rhizobacterial strain used for inoculation of maize seeds.

| Plant growth promoting (PGP) traits | Bacterial strain <i>Klebsiella oxytoca</i> - KSB17 |
|-------------------------------------|--|
| ACC-deaminase | 370.41 \pm 17.3 |

| | |
|---|---|
| (α -KB $\mu\text{mol g}^{-1}$ protein h^{-1}) | |
| Indole-acetic acid production (mg L^{-1}) | |
| -L-TRP | 34.80 \pm 3.76 |
| +L-TRP | 64.43 \pm 5.23 |
| Chitinase | 42.42 \pm 2.86 |
| ($\mu\text{mol of Glc NAc min}^{-1}$ mg^{-1} protein) | |
| Exopolysaccharide production | 84.64 \pm 4.65 |
| ($\mu\text{g mL}^{-1}$) | |
| K-solubilization [0.5% WMEM] | 116.83 \pm 5.62 |
| (mg L^{-1}) | |
| P-solubilization [0.5% RPEM] | 47.31 \pm 1.92 |
| (mg L^{-1}) | |
| Siderophores production (SU %) | 54.32 \pm 3.84 |
| Root colonization (CFU mL^{-1}) | 6.58 $\times 10^7 \pm 2.31 \times 10^6$ |

ACC: 1-aminocyclopropane-1-carboxylate, α -KB: α -ketobutyrate, L-TRP, L-tryptophan, GlcNAc: N-acetyl D-glucosamine, K-solubilization: Potassium solubilization, P-solubilization: Phosphate solubilization, WMEM: Waste mica enriched medium, RPEM: Rock phosphate enriched medium, CFU: Colony forming unit, FRB: Fresh root biomass

Potassium (K) solubilization assay: Fresh bacterial culture of KSB-17 was grown in waste mica powder (K source) spiked Aleksandrov's medium broth. KSB culture was kept in shaking incubator at 28 \pm 2 $^{\circ}\text{C}$ for 7 days and centrifuged (7,000 \times g) for 10 min at 4 $^{\circ}\text{C}$. After cell harvesting, filtrate volume was extended up to 50 mL with distilled water in a volumetric flask. The solubilized K was quantified in the solution using Flame photometer (Sugumaran and Janarthanam, 2007). The analysis was performed in triplicate using KCl standard.

Compost materials: Potassium enriched composts i.e. potassium sulfate/chemical fertilizer enriched compost (CFEC) and waste mica enriched compost (WMEC) were prepared from fruit and vegetable wastes collected from main fruit and vegetable market of Faisalabad. The compost feedstock material was left under ambient temperature for 3-4 days and then exposed to oven drying for 24 hr at 65 $^{\circ}\text{C}$ before fine crushing of \sim 2.0 mm. Whole, crushed material was shifted into a locally manufactured compost reactor. Moisture content was adjusted to 40%, a pre-requisite for an efficient microbial activity, for an efficient compost process. A 9 weeks long composting process was carried out as described by Arif *et al.* (2018). The compost process was monitored as follow, a mesophilic phase at 30-40 $^{\circ}\text{C}$ for 1 week; a thermophilic phase at 40-60 $^{\circ}\text{C}$ for 5 weeks; and concluded with 3 week's mesophilic phase at 28 $^{\circ}\text{C}$ for a desired maturation. After this, compost was exposed to air drying and cut into a uniform size fraction by passing through 2 mm sieve. The CF and WM at 225 and 500 g kg^{-1} compost were used for enrichment as 100% recommended source of K, respectively. At the end of composting, sub-samples were taken out from the bulk compost for physio-chemical analysis of both compost products (Table 2).

Table 2. Physio-chemical and biochemical properties of different types of compost.

| Compost properties | Types of compost | |
|---|--------------------|--------------------|
| | CF-EC | WM-EC |
| Physical properties | | |
| Moisture (%) | 46.00 \pm 3.11 | 45.63 \pm 2.13 |
| Chemical properties | | |
| pH | 7.17 \pm 0.04 | 7.19 \pm 0.03 |
| EC ($\mu\text{S cm}^{-1}$) | 3267.00 \pm 33.4 | 3156.00 \pm 27.2 |
| Organic matter (g kg^{-1}) | 390.54 \pm 2.78 | 391.10 \pm 2.13 |
| Total organic-C (g kg^{-1}) | 225.40 \pm 2.37 | 226.80 \pm 1.93 |
| Total nitrogen (g kg^{-1}) | 19.53 \pm 1.23 | 19.56 \pm 1.29 |
| Total phosphorus (g kg^{-1}) | 7.42 \pm 0.13 | 7.34 \pm 0.11 |
| Total potassium (g kg^{-1}) | 63.17 \pm 2.47 | 62.48 \pm 2.29 |
| C: N ratio | 11.54 \pm 0.83 | 11.60 \pm 0.78 |
| C: P ratio | 30.37 \pm 1.06 | 30.89 \pm 1.13 |
| C: K ratio | 3.57 \pm 0.08 | 3.63 \pm 0.07 |
| Biochemical properties | | |
| Dehydrogenase activity | 44.78 \pm 2.89 | 43.19 \pm 3.12 |
| ($\mu\text{g TPF g}^{-1}$ EC h^{-1}) | | |
| β -glucosidase activity | 67.90 \pm 3.60 | 64.63 \pm 3.90 |
| ($\mu\text{g PNP g}^{-1}$ EC h^{-1}) | | |
| Microbial biomass-C | 192.56 \pm 8.12 | 184.41 \pm 7.34 |
| (mg kg^{-1} EC) | | |
| Microbial biomass-N | 22.43 \pm 1.10 | 21.13 \pm 1.07 |
| (mg kg^{-1} EC) | | |

Seed inoculation: Fresh seed inoculum was prepared by streaking previously isolated KSB onto tryptic soy agar (TSA) and incubated at 28 \pm 2 $^{\circ}\text{C}$ for 48 hr. A distinct colony of KSB-17 was further used to prepare bacterial culture by inoculating into the TSA broth at 28 \pm 2 $^{\circ}\text{C}$ under 120 rev min^{-1} shaking conditions. After 48 hr, KSB culture was centrifuged (6,000 rpm; 6 minutes) and the pellet was mixed in sterilized water. Bacterial cell suspension (10 8 -10 9 CFU mL^{-1}) of selected KSB-17 obtained at 600 nm was used as seed inoculum. Hybrid maize seeds [P7414] were used in this study. Surface sterilization of seeds was performed by soaking seeds into 5% NaClO solution for 3-5 min, three washing with 95% ethanol and final rinsing with sterilized water. For seed inoculation with KSB-17 inoculum mixture, 150 mL inoculant; 10% sucrose solution; 1, 4 peat to seed ratio w/w, was executed 24 hr before sowing. A homogenous mixture of sucrose and peat without KSB-17 inoculum was used as a control treatment.

Experimental conditions: A sandy clay loam soil, classified as Haplic Yermosols, was collected from agricultural fields of the University of Agriculture Faisalabad. Soil samples were taken from 0-15 cm depth, hand sorted in the field, and uniformly mixed for a field representative sample. Sub-samples were separated to perform initial physico-chemical and biochemical analysis (Table 3). An earthen pot (15 kg soil pot^{-1}) experiment was set up at agricultural research farm,

University of Agriculture Faisalabad. In this experiment, a 5×2 factorial treatment orientation was followed i.e. 5 level of K fertilizer (Control, WM, CF, CFEC and WMEC) and 2 inoculation level (Without KSB, With KSB). Nitrogen, phosphorus and potassium fertilizers were applied at a standard rate i.e. 296-167-125 kg ha⁻¹, as urea, diammonium phosphate (DAP) and K from two different sources (sulfate of potash and waste mica powder), respectively. Basal dose of phosphorus and potassium fertilizers were supplied at the time of sowing. In case of nitrogen, dose rate was splitted into three applications and applied at the time of sowing, knee height and flowering stage, respectively. Potassium fertilization, either WM or CF, was executed through compost application at 2000 kg ha⁻¹ at the time of seed bed preparation. The pots were surface irrigated with canal water to maintain optimum moisture condition (~70% of WHC) for plant growth. Five maize seeds were sown and only one seedling per pot was retained. All pots were subjected to routine agronomic practices throughout the experiment.

Table 3. Physio-chemical and biochemical properties of the soil used for experiments.

| Soil properties | Value |
|--|-----------------|
| Physical properties | |
| Textural class | Sandy clay loam |
| Sand (g kg ⁻¹ of soil) | 570.0 |
| Silt (g kg ⁻¹ of soil) | 200.0 |
| Clay (g kg ⁻¹ of soil) | 230.0 |
| Chemical properties | |
| pH | 7.93 ± 0.04 |
| Electrical conductivity (µS cm ⁻¹) | 1130.00 ± 12.6 |
| Total organic-C (g kg ⁻¹) | 5.82 ± 0.12 |
| Dissolved organic C (mg kg ⁻¹) | 45.54 ± 4.63 |
| Total nitrogen (mg kg ⁻¹) | 262.92 ± 9.23 |
| Available P (mg kg ⁻¹) | 6.93 ± 0.65 |
| Available K (mg kg ⁻¹) | 184.90 ± 9.23 |
| Biochemical properties | |
| Microbial biomass C (mg kg ⁻¹) | 286.43 ± 8.35 |
| Microbial biomass N (mg kg ⁻¹) | 12.48 ± 1.08 |
| Dehydrogenase (µg TPF g ⁻¹ soil h ⁻¹) | 15.13 ± 2.19 |
| β-glucosidase (µg PNP g ⁻¹ soil h ⁻¹) | 2.53 ± 0.83 |

Plant sampling and analyses: At flowering stage, plant physiological attributes and antioxidant enzyme activities of maize were determined. Plant height was measured at physiological maturity. Both above and belowground biomass was determined at harvesting. After harvesting, grain and associated biomass were separated, dried and analyzed for protein content, oil content, starch content and potassium concentration. Potassium (K) content in shoot, root and grain was determined by Flame Photometer (Perkin-Elmer 2340) using standard methods (Chapman and Pratt, 1961). For this, samples were washed twice with distilled water, air-dried at 70°C for 48h and ground in a mill to pass through a thirty-

mesh screen. Tissue extraction was made by dry ashing of a 0.5 g sample in a muffle furnace at 550°C for 6hr. Subsequently, the ash was dissolved in 3 mL of 6 N HCl and the solution was diluted with deionized water to 50 mL final volume. Photosynthetic, transpiration rate and stomatal conductance (at flag leaf stage) were measured by Infrared Gas Analyzer (IRGA, LCA-4, ADC, Hoddesdon UK) as suggested by Long and Bernacchi (Long *et al.*, 2003). Water use efficiency (WUE) was calculated by dividing net photosynthetic rate by transpiration rate. The chlorophyll contents were measured by using chlorophyll meter.

Catalase (CAT) and peroxidase (POD) were measured by using the method of Chapman and Pratt (1961). Superoxide dismutase (SOD) was assayed using the procedure outlined by Gong *et al.* (2005). Shoot, root and grain K content were determined by using Flame Photometer (Perkin-Elmer 2340) according to the method of Chapman and Pratt (1961). Grain protein contents were quantified through Micro-Kjeldahl's procedure and starch contents were determined as described by McCleary *et al.* (1994). Oil contents were extracted and determined by Soxhlet system according to the American Oil Chemists' Society method (1993).

Statistical analysis: The whole data set was statistically analyzed by using SPSS, version 21. Two-way analysis of variance (ANOVA) was used to assess the effects of different K fertilization and KSB inoculation levels on selected physiological, biochemical, and qualitative variables of maize plant. Differences within K fertilizers and KSB inoculation were tested by using Tukey's post-hoc test. All figures and tables represent means of four replicates followed by standard error of means. All tests were reported as significant at a $p \leq 0.05$.

RESULTS

Shoot growth parameters: In the present study, data indicated that application of WM had non-significant effect on plant height, fresh and dry biomass of maize compared to both KSB inoculated and uninoculated control (Table 4). Without KSB inoculation, KCF application significantly ($p \leq 0.05$) increased plant height up to 48% compared to uninoculated control, while fresh and dry shoot biomass was increased up to 54% higher, respectively. Compared to both untreated and treated control, WMEC fertilization had significant effect on plant height, fresh and dry shoot biomass. Similarly, KCFEC application significantly improved plant height and shoot biomass compared to all fertilization treatments under non-KSB conditions. Whilst, the effect of KCFEC application was

Table 4. Effects of different K fertilizers on plant height, fresh and dry shoot biomass of pot grown maize under KSB inoculated and uninoculated conditions.

| Treatments | Plant height (cm) | | Fresh shoot biomass (g pot ⁻¹) | | Dry shoot biomass (g pot ⁻¹) | |
|------------|-------------------|--------------|--|--------------|--|--------------|
| | -KSB | +KSB | - KSB | + KSB | - KSB | + KSB |
| Control | 109.3±8.7g | 120.5±9.4f | 189.3±14.3g | 213.3±15.3f | 150.5±11.7h | 167.2±13.9fg |
| WM | 113.2±7.9fg | 122.3±10.7f | 195.8±15.2g | 216.5±14.2f | 156.7±12.3gh | 172.4±14.1f |
| KCF | 162.3±13.4c | 170.7±12.7b | 292.2±19.7c | 313.4±21.5bc | 232.5±17.6c | 239.5±18.8bc |
| WMEC | 133.3±10.4e | 156.8±11.4cd | 230.7±12.2e | 261.6±15.3d | 190.3±10.8de | 212.7±11.9d |
| KCFEC | 174.5±13.7ab | 180.7±14.3a | 323.2±22.6b | 340.4±23.2a | 246.9±16.5b | 262.9±19.4a |

KSB= Potassium solubilizing bacteria, WM = Waste mica, KCF = K chemical fertilizer, WMEC = WM-enriched compost, KCFEC = KCF-enriched compost. Values represent means ± standard error of four replicates (n=4), while values with different case letters, in each column, indicate level of significance at $p \leq 0.05$ among treatments

Table 5. Effects of different K fertilizers on root length, fresh -dry root biomass, rhizosphere KSB population of pot grown maize under KSB inoculated and uninoculated conditions.

| Treatments | Root length (cm) | | Fresh root biomass (g pot ⁻¹) | | Dry root biomass (g pot ⁻¹) | | KSB population (CFU g ⁻¹ dry soil) | |
|------------|------------------|-----------|---|-----------|---|-------------|---|--|
| | - KSB | + KSB | - KSB | + KSB | - KSB | + KSB | - KSB | + KSB |
| Control | 27.3±2.3e | 30.3±2.8d | 48.1±4.2f | 54.5±5.2e | 8.21±0.52f | 9.12±0.81de | 4.23x10 ⁴ ±2.69x10 ³ e | 6.78x10 ⁴ ±2.45x10 ³ d |
| WM | 28.5±2.4de | 31.5±2.7d | 49.3±4.1f | 55.7±4.9e | 8.74±0.48f | 9.26±0.83de | 4.40x10 ⁴ ±2.63x10 ³ e | 7.23x10 ⁴ ±2.57x10 ³ d |
| KCF | 39.1±2.6c | 42.4±4.0b | 70.4±5.4bc | 74.4±6.4b | 10.60±0.69d | 12.46±1.21c | 4.30x10 ⁵ ±3.31x10 ⁴ c | 6.32x10 ⁵ ±3.09x10 ⁴ c |
| WMEC | 32.9±2.2d | 41.3±2.7b | 61.2±4.4d | 76.4±6.2b | 11.7±0.72cd | 14.30±1.13b | 3.29x10 ⁶ ±3.89x10 ⁵ b | 4.69x10 ⁶ ±3.71x10 ⁵ a |
| KCFEC | 45.6±3.4b | 52.5±4.1a | 78.5±6.2ab | 83.3±7.1a | 14.67±1.24b | 16.87±1.32a | 3.36x10 ⁶ ±4.07x10 ⁵ b | 4.72x10 ⁶ ±4.26x10 ⁵ a |

KSB= Potassium solubilizing bacteria, WM = Waste mica, KCF = K chemical fertilizer, WMEC = WM-enriched compost, KCFEC = KCF-enriched compost. Values represent means ± standard error of four replicates (n=4), while values with different case letters, in each column, indicate level of significance at $p \leq 0.05$ among treatments

Table 6. Effects of different K fertilizers on net-photosynthesis, transpiration rate, water use efficiency, stomatal conductance and total chlorophyll contents of pot grown maize under KSB inoculated and uninoculated conditions.

| Treatments | Net photosynthesis (µmol m ⁻² s ⁻¹) | | Transpiration rate (mmol H ₂ O m ⁻² s ⁻¹) | | Water use efficiency (µmol CO ₂ mmol ⁻¹ H ₂ O) | | Stomatal conductance (mmol m ⁻² s ⁻¹) | | Total chlorophyll content(mg g ⁻¹ FB) | |
|------------|--|-------------|---|------------|---|------------|--|------------|--|-------------|
| | -KSB | + KSB | -KSB | + KSB | -KSB | + KSB | -KSB | + KSB | -KSB | + KSB |
| Control | 9.74±0.91ef | 10.82±0.88e | 3.18±0.09d | 3.36±0.11c | 3.12±0.10d | 3.22±0.08d | 0.21±0.01d | 0.23±0.02d | 2.90±0.09d | 3.07±0.10cd |
| WM | 10.03±0.87e | 10.88±0.93e | 3.15±0.07d | 3.38±0.13c | 3.15±0.09d | 3.24±0.09d | 0.21±0.01d | 0.23±0.02d | 2.94±0.11d | 3.12±0.10c |
| KCF | 16.03±1.33b | 18.82±1.47a | 3.47±0.15c | 3.96±0.21b | 4.12±0.19b | 4.75±0.13a | 0.35±0.02ab | 0.38±0.03a | 3.80±0.15ab | 3.89±0.15a |
| WMEC | 12.52±1.13d | 15.72±1.17b | 3.27±0.19cd | 3.85±0.12b | 3.62±0.14c | 4.08±0.11b | 0.26±0.02cd | 0.32±0.03b | 3.19±0.13c | 3.74±0.12b |
| KCFEC | 15.48±1.32c | 19.83±1.63a | 3.76±0.22b | 4.14±0.22a | 4.61±0.18a | 4.79±0.13a | 0.38±0.03a | 0.41±0.03a | 3.86±0.14a | 4.03±0.15a |

KSB= Potassium solubilizing bacteria, WM = Waste mica, KCF = K chemical fertilizer, WMEC = WM-enriched compost, KCFEC = KCF-enriched compost. Values represent means ± standard error of four replicates (n=4), while values with different case letters, in each column, indicate level of significance at $p \leq 0.05$ among treatments

even more pronounced with KSB inoculation and increased plant height, fresh and dry shoot biomass up to 50, 59 and 57% respectively, than control treatment.

Root growth parameters: Compared to control treatment, addition of WM had non-significant effect on root length, root biomass and root associated KSB population at both inoculation levels (Table 5). However, a marked increase in maize root traits and KSB population was recorded upon WMEC application. Similarly, KCF application significantly ($p \leq 0.05$) improved root traits and KSB population than control treatment at both inoculation levels. In the absence of KSB inoculation, compost enrichment of KCF significantly affected the root length, root biomass and KSB root population compared to control treatment. Addition of

KCFEC with KSB inoculation always resulted in the highest root length (52.5 cm), fresh root biomass (83.3 g pot⁻¹), dry root biomass (16.87 g pot⁻¹) and root adhered KSB population (4.72×10^6 CFU g⁻¹ dry soil), respectively, when compared to the rest of treatments.

Physiological parameters: At both inoculation levels, WM addition did not influence the plant physiological parameters compared to both control treatments (Table 6). Whereas WMEC application significantly ($p \leq 0.05$) increased plant physiological parameters at both inoculation levels, except for transpiration rate and stomatal conductance, wherein no significant difference was recorded in the absence of KSB inoculation. Similarly, KCFEC application resulted in significantly higher net photosynthesis, transpiration rate,

water use efficiency, stomatal conductance and total chlorophyll contents by 59, 18, 48, 81 and 33%, respectively, compared to control treatment under no KSB inoculation condition. The interactive effect of KCFEC with KSB inoculation was even stronger that led to the highest increase in all tested plant physiological parameters when compared to the rest of the treatments.

Antioxidant enzymes activities: Results regarding the activities of antioxidant enzymes (CAT, POD, SOD) were examined in maize plant (Fig.1).

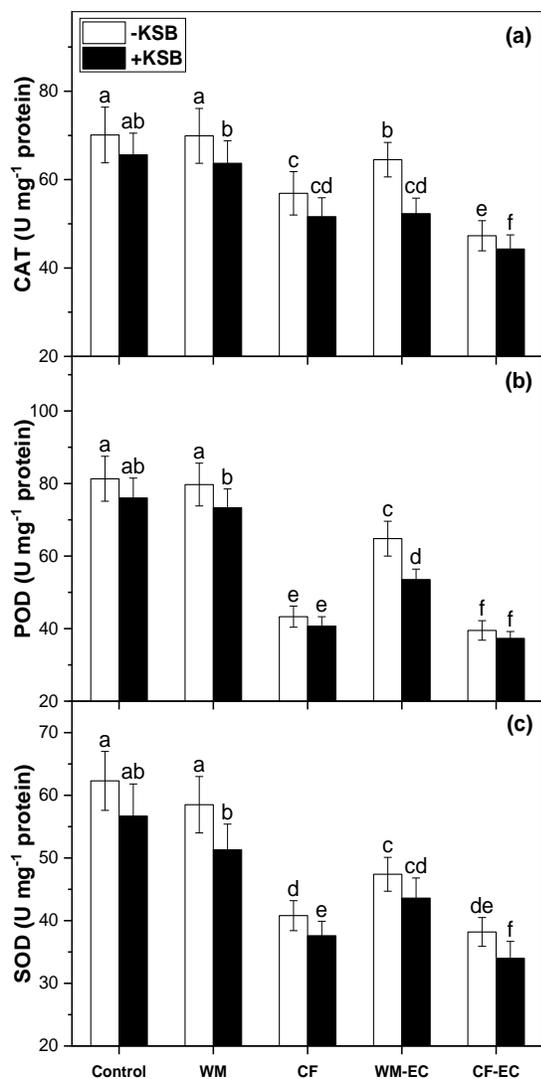


Figure 1. Efficacy of different K fertilizers on antioxidant enzymes activity of (a) CAT (b) POD, and (c) SOD in maize plant under KSB inoculated and uninoculated conditions. Values represent means ± standard error of four replicates (n=4), while values with different case letters indicate level of significance at $p \leq 0.05$ among treatments.

Under no KSB inoculation, all treatments, except for the WM decreased significantly the activity of CAT when compared with control treatment. With KSB inoculation, CAT activity of WM treated plants was comparable with that in control treatment (Fig.1a), and was significantly higher than rest of the treatments. Overall, KSB inoculation with KCFEC had the lowest CAT activity (44.30 U mg⁻¹ protein) compared to the rest of treatments.

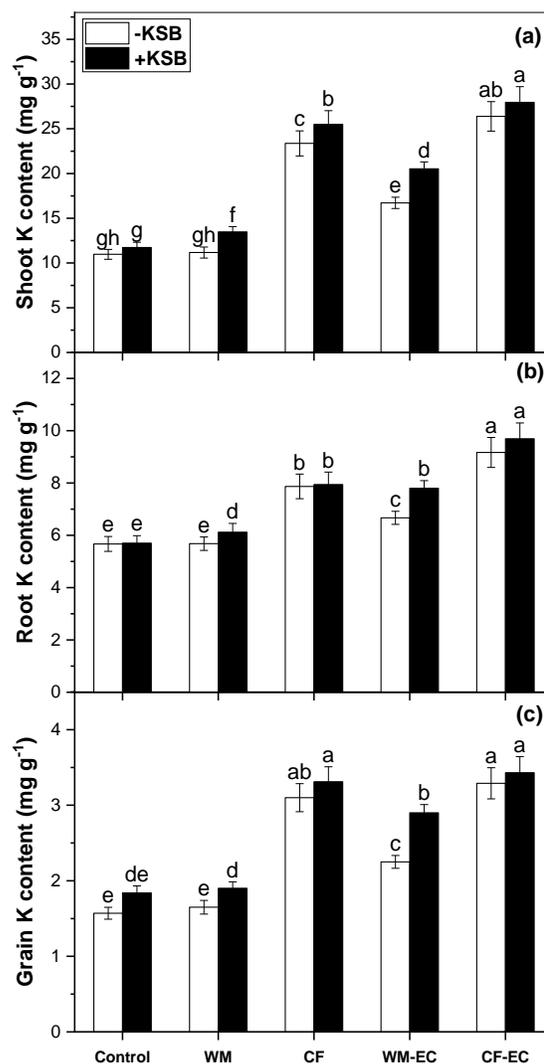


Figure 2. Efficacy of different K fertilizers on K contents of (a) shoot (b) root, and (c) grain in maize plant under KSB inoculated and uninoculated conditions. Values represent means ± standard error of four replicates (n=4), while values with different case letters indicate level of significance at $p \leq 0.05$ among treatments

POD activity was significantly reduced by the addition of WMEC compared to control treatment, but no effect was

noted when WM was applied without compost at both inoculation levels (Fig.1b). Application of KCF and KCFEC had significant effect on POD activity at both inoculation levels. A significant decrease in POD activity by 47 and 51% than control was recorded with KCF and KCFEC, respectively, in the absence of KSB inoculation. However, reduction in POD activity was more pronounced with KCFEC followed by KCF under KSB inoculation.

Application of WM showed insignificant changes in SOD activity compared to control treatment at both inoculation levels (Fig.1c). However, WMEC addition led to significant decline in SOD activity compared to control under both inoculation conditions. Similarly, KCF also resulted in significant reduction of SOD activity, whilst KCFEC application along with KSB inoculation had the highest reduction of SOD activity by 40% in relation to control treatment.

Plant potassium uptake: Without KSB inoculation, significant increase in shoot K contents was observed after KCF application, especially under compost fertilization, contributing the largest K contents of all treatments (Fig.2a). In the presence of KSB inoculation, application of KCF and KCFEC increased shoot K contents compared to control. Although to lesser extent, a similar trend was also observed for WM and WMEC treatment.

Without KSB inoculation, WM had non-significant effect on root K contents compared to control but showed significantly higher K content with WM under KSB inoculation (Fig.2b). At both inoculation level, root K content increased significantly compared to respective control treatments. In order of magnitude, the effects were highest for KCFEC followed by KCF and WMEC, respectively.

No significant difference in grain K uptake was detected when compared with control after WM application at both inoculation levels (Fig.2c). However, WMEC resulted in significantly higher grain K contents than control at both inoculation levels. Whilst statistically comparable but highest grain K contents were recorded in KCF and KCFEC fertilization when compared with the rests of treatments in KSB inoculated and uninoculated conditions.

Grain quality: Without KSB inoculation, significant increase in grain protein content was recorded among treatments except for WM that revealed no significant difference when compared to control treatment (Fig.3a). The grain protein contents were highest in KCFEC treatment and lowest in control treatment under no KSB inoculation. Grain protein contents showed gradual but significant increase in response to all treatments under KSB inoculation.

WM addition had no significant effect on oil and starch contents compared to control treatments at both inoculation levels (Fig.3b,c). However, WMEC, KCF and KCFEC fertilization resulted in significantly higher oil and starch contents as compared to the respective control treatments under inoculated as well non-inoculated conditions.

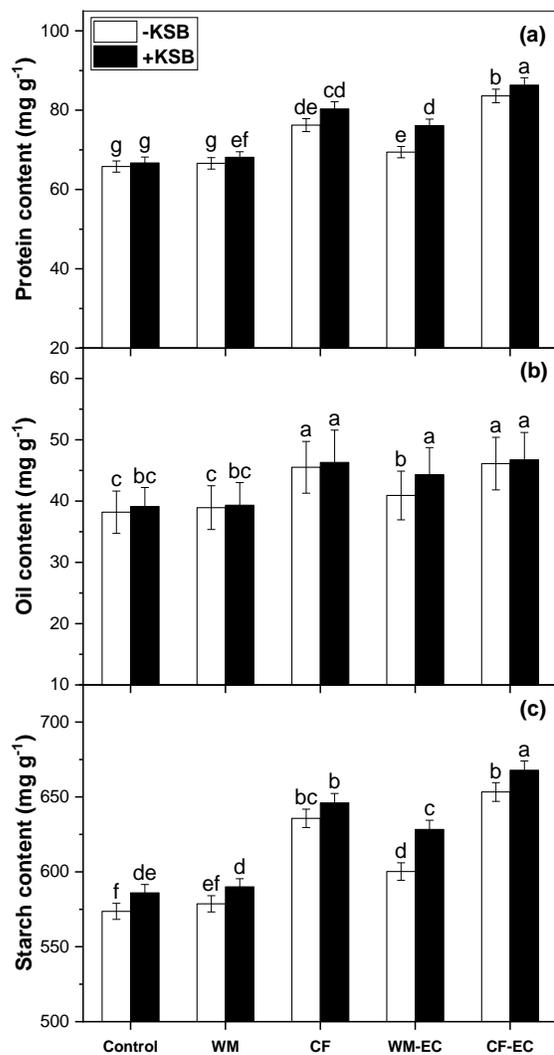


Figure 3. Efficacy of different K fertilizers on (a) protein (b) oil, and (c) starch contents of maize grain under KSB inoculated and uninoculated conditions. Values represent means \pm standard error of four replicates ($n=4$), while values with different case letters indicate level of significance at $p \leq 0.05$ among treatments.

DISCUSSION

In the current study, selected potassium fertilizer sources exhibited varied effects on plant growth parameters of maize (Table 4). Application of KCFEC had a highly significant effect on plant height and biomass. Overwhelming evidence of nutrient enriched compost application and its positive impact on plant growth promotion can be found in literature (Iqbal *et al.*, 2012; Arif *et al.*, 2016). These positive effects were often attributed to the steady supply of plant nutrients and enhanced soil conditions following these amendments

application (Agegehu *et al.*, 2015). The effect on plant growth by compost enriched K sources was more pronounced with KSB inoculation than individual K fertilization sources; perhaps due to elevated functional response of inoculated KSB strain in the presence of compost, i.e. KSB can increase plant growth by a variety of direct and indirect plant growth promotion mechanisms (Table 1). Previous studies have also indicated a close linkage of balanced mineral nutrition and increased in plant growth inoculated with KSB in different crop plants (Zhang *et al.*, 2014). Our results also showed prolific effects of KCFEC application on root growth related parameters (Table 5). These results corroborate the findings of Postma *et al.* (2014), who demonstrated that root proliferation can be stimulated by the establishment of nutrient rich patches at root-soil interface. Application of suitable microbial inoculant can also improve plant root growth. Past reports showed that some species of root colonizing bacteria can promote maize root growth and modify root architecture by regulating ethylene concentration via the expression of ACC-deaminase activity (Arshad *et al.*, 2008). Overall, slower K releasing source i.e. waste mica enriched with compost, improved plant shoot and root growth, although to a lesser extent both in the presence and absence of KSB inoculant. This confirms that K dissolution potential of KSB can facilitate a slow release K pattern from compost. Higher population of root associated KSB also indicated the improvement in KSB rhizosphere competence. In some other studies, persistence and proliferation of introduced bacterial inoculum with various types of organic amendments has been reported (Fox *et al.*, 2014).

An improvement in plant photosynthetic rate, transpiration rate, water use efficiency, stomatal conductance and total chlorophyll content was recorded in KCF and KCFEC fertilization. Elevation in plant physiological responses and gaseous exchange has previously been noticed in soil treated with KSB (Supanjani *et al.*, 2006). Similarly, Han and Lee (2005) also observed increase in photosynthetic rate of eggplant up to 12% because of *B. mucilaginosus* inoculation, which led to higher K uptake from KSB solubilized minerals. A strong relationship between improved plant nutrition and resultantly higher chlorophyll contents, physiological and gaseous exchange parameters has been noticed in variety of crops viz. wheat (Sheng *et al.*, 2006), maize (Parmar *et al.*, 2013), sorghum (Basak *et al.*, 2009), and tomato (Lin *et al.*, 2001). Plants show antioxidant enzymes (CAT, SOD and POD) activity to combat the detrimental upshots of reactive oxygen species (ROS) produced because of K deficiency stress, like other biotic and abiotic stresses (Mittler, 2002). In this study, decrease in antioxidant enzyme activities following K fertilizer and compost application indicates a downregulation pattern of these stress indicators to counteract the harmful effects of K deficiency stress. Microbial inoculant led downregulation of antioxidants enzyme activities with higher plant growth as well as improved plant nutrition has

recently been reported by Armada *et al.* (2015) and Fukami *et al.* (2018). Regulation of stomatal passage for gaseous exchange with adequate supply of K could avoid the deleterious effects of ROS by hindering their production under K deficiency (Kant *et al.*, 2002; Egilla *et al.*, 2005). Transport of K to the guard cells ensures gaseous regulation and normal photosynthetic activity which eventually attenuates the destructive effects of stress (Kant *et al.*, 2002).

In the present study, significantly higher uptake of K by shoot, root and grain in KCFEC treatment could highlight the shielding effect of additional compost substrate causing slower K release pattern of compost enriched K source compared its sole application (Fig.2). Slow release pattern of nutrient enriched organic amendments and their positive impacts on soil plant system are earlier reported by Ryals *et al.* (2016) and Gwenzi *et al.* (2018). The most significant finding from this study was that the KSB inoculation in combination with different K sources significantly improved plant K nutrition (Fig.2). In particular, combination of KSB and KCFEC that led to the highest accumulation of K in maize plant biomass. These results indicate that KSB inoculation was very efficient in exploiting compost enriched potassium by improving its solubility and/or mobility and thereby making it more available to crop plants. Compost, a rich carbon source, is known to induce positive effects on plant nutrition, and also stimulate microbial growth and activity (Arif *et al.*, 2016), such as to KSB that used in this study. Studies to date also suggest that nutrients solubilization activity of root associated microbes increase manifold when receiving suitable substrate material in the form of organic amendments (Shahzad *et al.*, 2017). Overall, KSB inoculation remained less effective in non-composted treatments, suggesting the functional loss of introduced KSB into the competitive root environment in the absence of suitable substrate material. In order to establish a successful plant-microbe interaction, sufficient supply of nutrients as well as access to compatible substrate medium is an essential component for an effective microbial inoculation conferring plant growth promotion (Song *et al.*, 2015). Differences in maize grain quality i.e. protein, starch and oil contents are generally attributed to the genetic potential of grown crop variety, rather than soil amendment or microbial inoculants. Our results demonstrate that addition of KCFEC with KSB was the most effective treatment combination and improved all the selected grain quality parameters. The extent of improvement in maize grain quality following these amendments may be explained by the improved plant K nutrition in this experiment. Similarly, Usherwood (1985) also reported higher carbohydrate, protein and oil contents of grain with increasing availability of K to maize. Moreover, Yang *et al.* (2004) also reported an increase of protein and amino acids in grain of well K fertilized plants as compared to control. Application of rock K supplemented with suitable

microbial inoculant increased maize grain quality (Sheng *et al.*, 2006).

Conclusions: Conclusively, it may be inferred that application of potassium fertilizer enriched compost with potassium solubilizing bacteria improved the plant shoot and root growth, also led to the enhancement of various physiological and biochemical traits. Noticeable improvement in plant potassium nutrition as well as grain quality were also found following the addition of these amendments. Integrated use of waste mica along with suitable potassium-solubilizing bacteria could offer some promise with regard to improving potassium-nutrition of maize, but it requires some extensive investigation across varied crop plants and soil conditions. Overall, use of potassium-solubilizing bacteria with different sources of potassium fertilizer could offer an environment friendly and economical option, which not only can counteract potassium deficiency in crop plants but also overall improvement in their growth and development.

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