#### **ORIGINAL PAPER**



# Effect of Potassium Solubilizing Bacteria and Humic Acid on Faba Bean (*Vicia faba* L.) Plants Grown on Sandy Loam Soils

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#### Abstract

Potassium use efficiency (KUE) in faba bean production is often low, and the luxury of fertilization has negative environmental impacts. The current study aims to reduce the recommended dose of potassium (K) for faba bean by potassium solubilizing bacteria (PSB) and humic acid (HA). The studied treatments were 50 and 100% of K recommended dose with or without PSB and 40 kg of HA ha<sup>-1</sup>. The studied treatments were applied to faba bean ((*Vicia faba* L., cv. Giza 843) plants grown in sandy loam soils for two successive seasons. In this study, the maximum KUE (40%) was obtained in the soil treated with HA and PSB while the lowest one (14%) was found in the case of the full recommended dose of mineral form. Humic acid and PSB that were applied to the plants fertilized with 50% of the recommended dose gave the maximum growth and yield. Humic acid and PSB increased the soil cation exchange capacity (CEC) by 6% and the soil organic matter (SOM) by 12%. Chlorophyll and carbohydrates in the leaves were increased by 36 and 50%, respectively, above the control, as results of HA and PSB application. Adding half of K requirements for faba bean in a mineral form with 40 kg of HA and PSB led to 14% and 19% increases in the seed and straw yield compared to the full mineral fertilization without bacterial inoculation. Humic acid and potassium solubilizing bacteria can be used to improve soil quality and increase the availability and uptake of nutrients, and thus increase the yield of faba bean plants. The experimental results from our 2-year research on faba bean grown on sandy loam soils establish a deductive scientific basis for using bio-fertilizers and organic materials to produce cleaner food and better environment conditions.

Keywords Bacillus circulars · Humic acid · Potassium sulfate · Sandy loam soil

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# **1** Introduction

Faba bean (*Vicia faba* L.) is a leguminous plant, and its seeds are the edible part which contains high levels of protein, mineral nutrients, and vitamins (Neme et al. 2015). The cultivated area is about 2.6 million ha and produces about 4.6 million tonnes of dry grains (FAO 2006).

Humic acid is rich in several organic compounds and contains different active chemical groups, e.g., carboxyl, carbohydrate, hydroxyl, phenol, and methoxyl (Peuravuori et al. 2006; Bulgari et al. 2019; de Jesus Souza et al. 2019). Organic matter structure in humic acid enhances the availability and uptake of essential plant nutrients (Dinçsoy and Sönmez 2019; Almaroai and Eissa 2020; Ding et al. 2020; Rekaby et al. 2020). The application of humic acid to coarse texture soils has several advantages through increasing root growth, enhancing the growth of soil microorganisms, increasing soil water holding capacity, and increasing soil aggregate size (Canellas et al. 2015; Dinçsoy and Sönmez 2019; Torun and Toprak 2020; Aalipour et al. 2020). Humic acid enhances the availability and uptake of plant nutrients in comparison with mineral fertilizers (Selladurai and Purakayastha 2016; Dincsoy and Sönmez 2019). Humic substances play an important role in plant growth and increasing productivity under stress or normal conditions (Bulgari et al. 2019; de Jesus Souza et al. 2019). The root growth of plants treated with humic acid was higher than the non-treated plants; moreover, the above-ground parts were also increased as a result of HA application (Zhang et al. 2014; Aalipour et al. 2020; Bulgari et al. 2019; de Jesus Souza et al. 2019). The humic substances have another direct effect in increasing the plant growth by increasing the nutrient uptake and availability (Zhang et al. 2014). The increase in root growth enables the plants to absorb more nutrients and water, thus increasing the plant productivity and obtained yield (Canellas et al. 2015; Torun and Toprak 2020; Aalipour et al. 2020).

The aim of bio-fertilizer application to vegetable and field crops is to decrease the adverse effect of chemical fertilizers on our environment (Meena et al. 2016; El Naim et al. 2017). Excessive and continuous use of chemical fertilizers causes health and environmental hazards, deterioration in soil properties, and consequently crop shortages. Therefore, the application of different microbial strains, as bio-fertilizers, plays essential roles in reducing the amount of mineral fertilizers and enhances food quality (Youssef and Eissa 2017). The application of bio-fertilizers individually or in combination with organic and inorganic amendments has been found to improve plant growth (Singh et al. 2017). In the last decades, the use of bio-fertilizers in increasing crop productivity has been elevated increasingly (Youssef and Eissa 2017). Biofertilizer can supply plants with essential nutrients and stimulate plant growth and productivity through several mechanisms: hormone production, reduce the activity of plant pathogens, and increase soil quality (Meena et al. 2016). The ability of potassium solubilizing bacteria (PSB) in solubilizing some silicate minerals is well known, so it has been used as a bio-fertilizer to enhance K availability in soils (Narula et al. 2005; Xiao et al. 2017). Several studies confirmed the roles of PSB in improving uptake of nutrients by plants, stimulating plant enzymes, and enhancing yield and quality of crops. Moreover, PSB has essential roles in increasing resistance against stress conditions (Meena et al. 2014). The bacteria help in increasing the soil fertility, crop production, and reducing the amount of chemical fertilizers (Zhang et al. 2013). The use of PSB supports the conditions of nutrient availability, increase the plant growth, and finally improve the plant performance (El-Azab and El-Dewiny 2018).

Most of the applied K fertilizers remain in soil due to the low potassium use efficiency (KUE). Hence, it is imperative to find novel strategies to improve KUE (Dhillon et al. 2019). Although the use of potassium solubilizing bacteria in enhancing growth and productivity is well known for several crops, there are not enough research findings and experimental studies that can justify its application and role in faba bean production. Inspired by the need to increase the ration of biofertilizer and other organic matter applications in faba bean production, this study endeavors to explore the role of potassium solubilizing bacteria with or without humic acid in reducing the potassium requirements of faba bean plants grown on sandy loam soils.

# 2 Materials and Methods

### 2.1 Field Experiment

Field experiments were conducted during 2018 and 2019 to examine the effects of humic acid and potassium solubilizing bacteria on reducing K requirements of faba bean plants. The trials were established in a private farm which is located in Sohage Governorate, Egypt, and the soil of the experimental site was classified as Arenosols, according to FAO (2006). The data in Suppl. Table 1 show the principal features of the experimental site. Suppl. Table 2 shows the weather condition of the experimental site during the two growing seasons. The treatments in Suppl. Table 3 were mixed with the soil during the preparation of the field and before cultivation. Seeds of faba bean (Vicia faba L., cv. Giza 843) were sown on 20 October in 2018 and 2019 growing seasons. The distance between rows was 40 cm, while it was 20 cm between plants in the same row, with an average density of 125,000 plant ha<sup>-1</sup>. Humic acid is a commercial product (Pro Humic) and contains 80% humic acid, 1.5% N, and 10% K<sub>2</sub>O. A biofertilizer contains potassium solubilizing bacteria obtained from the National Research Center, Giza, Egypt. The biofertilizer contains Bacillus circulars and this was confirmed by the 16S rDNA sequence analysis. These bacteria were added to the plants after emergence and 1 week later to guarantee their function. The plants were irrigated with groundwater (EC =  $1.40 \text{ dS m}^{-1}$ ) to near field capacity during the experiment period. Seeds of faba bean were inculcated with a commercial bio-fertilizer that contains rhizobium bacteria before cultivation. The plants were fertilized with a basal dose of 50 kg of urea (46% N) and 100 kg of P<sub>2</sub>O<sub>5</sub> from superphosphate (15% P<sub>2</sub>O<sub>5</sub>). Nitrogen fertilizer was added as an activation dose to provide the plants with N requirement during the development of rhizobium bacteria. Plant samples, each consist of 10 plants, were collected at the beginning of the flowering stage to determine the nutrient uptake and the growth parameters. The root samples were collected by washing and sieving the top surface soil layer (0 to 20 cm) through a 0.5 mesh. The root biomass in the surface (0 to 100 cm) of soil depth was calculated based on that roots in the 0- to 20-cm layer accounted for 60% of the root biomass (Jackson et al. 1996). Faba bean plants were collected after 160 days from cultivation, and straw and seed yield were recorded.

Potassium use efficiency (KUE) was calculated based on the equation given by Dhillon et al. (2019). The K uptake (kg) by seed and straw at harvest were used in the calculation of KUE.

$$KUE = \frac{K \text{ uptake in treatment}-K \text{ uptake in control}}{\text{amount of K applied}}$$

## 2.2 Soil and Plant Analysis

Soil samples (0-30 cm) were collected by an auger, dried in the air, crushed, and then sieved by a 2-mm sieve. Physiochemical properties were performed based on the standard methods described by Burt (2004). The soil pH was determined in the soil saturated suspension (1:1) using the pH meter method. The total soluble salts were determined in the soil saturation paste by the electrical conductivity method. Soil organic carbon (SOC) was determined using the Walkley and Black method. The total CaCO<sub>3</sub> was measured by the calcimeter method. Cation exchange capacity (CEC) was determined using 1 M sodium acetate solution at pH 8.2 as a saturation solution, and then exchangeable Na+ was replaced by NH<sup>4+</sup> using 1 M ammonium acetate solution at pH 7.0. The replaced Na<sup>+</sup> ions were measured by a flame photometer. Total nitrogen was measured by Kjeldahl's distillation method after the digestion of soil samples with concentrated sulfuric and perchloric acids at 7:3 ratios, respectively. Sodium bicarbonate solution (0.5 M) at pH 8.5 was used to extract the available soil phosphorus. Soil samples were analyzed for water-soluble, exchangeable, and non-exchangeable K (Burt 2004). Exchangeable K was extracted by 1 N NH<sub>4</sub>OAc and non-exchangeable K with 1 N boiling HNO<sub>3</sub>. The values of potassium in the extracts were measured by flame photometer. The population of potassium solubilizing bacteria (PSB) was measured in soil samples, according to Thomas et al. (2015).

#### 2.3 Plant Analysis

Faba bean plant samples were collected from each experimental unit after the recording of the growth parameters and then transferred directly to the laboratory. Afterwards, the plant samples were washed with tap and distilled water, and the samples were separated on the laboratory benches to reduce the moisture content. After 48 h, the plant samples were dried by oven at 70 °C and then ground. Two grams of each dried sample was digested with a mixture of 350 ml  $H_2O_2 + 0.42$  g selenium powder + 14 g LiSO<sub>4</sub>. +  $H_2O$  + 420 ml of concentrated  $H_2SO_4$  (Parkinson and Allen 1975). The digested plant samples were analyzed for N, P, and K according to the standard methods described by Burt (2004) which were described in the soil analysis section. Chlorophyll in the fresh leaves of plants was measured by using SPAD 502 plus. The total soluble carbohydrates were extracted from 0.5-g fresh leaves by ethanol (80%) and then measured spectrophotometrically by anthrone reagent at 620 nm (El-Mahdy et al. 2018; Akhtar et al. 2020).

## 2.4 Statistical Analysis

A randomized complete block design (RCBD) with five replicates was used in the current field experiment. Duncan's multiple range tests and one two-ANOVA were run by SPSS 17.0 package (SPSS, Chicago, IL, USA) at a 5% level of probability.

## **3 Results**

# **3.1 Effect of Humic Acid and PSB on Some Chemical Characteristics of the Studied Sandy Loam Soil**

Faba bean plants grown in the coarse texture soil as affected by potassium treatments were examined, and Table 1 shows some soil properties. Potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) and humic acid (HA) with or without inoculation with potassium solubilizing bacteria (PSB) affected the soil organic matter (SOM), salinity, and cation exchange capacity (CEC). The highest soil pH value (8.00) was obtained in T<sub>3</sub>, while the lowest value was recorded in T2. The application of potassium sulfate alone in T<sub>1</sub> reduced the soil pH by only 1.3% compared to the control, while this application with PSB caused a reduction in the soil pH by 3.5% compared to the control soil. The value of soil pH was 8.00 in T<sub>3</sub>, but when this treatment was inoculated with PSB, the pH changed to 7.87. The tested treatments had little insignificant effects on the soil pH values, but in general, the soil pH was reduced as a result of potassium sulfate application and PSB inoculation. The ECe values ranged between 1.30 and 1.65  $dsm^{-1}$ . The tested treatments caused negligible increases in the soil salinity. The highest  $EC_e$  value was recorded in  $T_2$ , while the lowest value was found in the control soil.

The soil amended with humic acid (HA) and inoculated with potassium solubilizing bacteria (PSB) (p < 0.5) significantly improved the soil organic matter (SOM) and cation exchange capacity (CEC) of the studied sandy loam soil (Suppl. Table 4) The SOM was increased by 7% in the case of T<sub>2</sub> and T<sub>3</sub>, and by 12% in the case of T<sub>4</sub> compared with the control soil. The application of HA gave higher values of SOM than the mineral fertilization and control treatments. PSB inculcation of soil that was fertilized with HA (T<sub>4</sub>) increased the SOM by 4% above the same treatment without inoculation (T<sub>3</sub>). Humic acid (HA) and PSB increased the CEC by 6% above the control. In general, the highest significant values of SOM and CEC were found in the soil treated with humic acid (HA) and inculcated with PSB. **Table 1** Effect of treatments onsome soil chemical properties

Treatment	pH (1:1)	$EC_e (dsm^{-1})$	SOM (g kg <sup>-1</sup> )	$CEC \ (cmol \ kg^{-1})$
С	7.90±0.08a	1.30±0.04b	4.24±0.15c	17±1b
$T_1$	$7.80{\pm}0.08a$	1.50±0.05a	4.26±0.12c	17±1b
$T_2$	7.62±0.05a	1.65±0.03a	4.24±0.10c	18±1a
T <sub>3</sub>	$8.00 {\pm} 0.06a$	1.44±0.00a	$4.52{\pm}0.08b$	18±1a
$T_4$	7.87±0.04a	1.45±0.02a	4.70±0.07a	18±1a

 $EC_e$  electrical conductivity of a saturated soil extract, SOM soil organic matter, CEC cation exchange capacity Means ( $\pm$  SD, n = 10) in the same column denoted by different letters are significantly different according to Duncan's test at p < 0.05

The total count of PSB was increased by 13, 58, 20, and 85% above the control when  $T_2$ ,  $T_3$ , and  $T_4$  were applied (Fig. 1). PSB inoculation increased the total count of PSB compared to the non-inoculated treatments. Humic acid (HA) and PSB inoculation gave the highest significant value of PSB count.

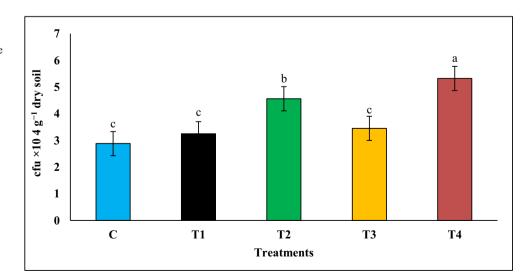
# 3.2 Effect of Humic Acid and PSB on Some Nutrient Availability in the Studied Sandy Loam Soil

The availability of nitrogen (N) and phosphorus (P) in the studied sandy loam soil was investigated as affected by the fertilization treatments and potassium solubilizing bacteria (PSB), and the data are illustrated in Fig. 2A and B. Generally, the results clearly showed that the application of the investigated treatments significantly (p < 0.05) increased the concentrations of available nutrients either with or without PSB compared to the control. The application of T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> increased the available soil N by 9%, 82%, and 91%, respectively, compared with the control (Fig. 2A). Humic acid (HA) gave higher values of available soil N than mineral fertilization and control treatments. PSB inoculation for the soil fertilized with

 $K_2SO_4$  (T<sub>2</sub>) increased the available soil N by 8% above the same treatment without inoculation (T<sub>1</sub>). PSB inculcation of the sandy loam soil that was amended with HA (T<sub>4</sub>) increased the available soil N by 5% compared to the same treatment without inoculation (T<sub>3</sub>). The application of T<sub>4</sub> to the sandy loam soil increased the available soil N by 91% above T<sub>1</sub>.

The application of T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> increased the soil available P by 6, 40, 75, and 100%, respectively, compared to the control (Fig. 2B). Humic acid (HA) gave higher values of available soil P than the mineral fertilization and control treatments. PSB inoculation for the soil fertilized with  $K_2SO_4$  (T<sub>2</sub>) increased the available soil P by 31% above the same treatment without inoculation (T<sub>1</sub>). PSB inculcation of the sandy loam soil that was amended with HA (T<sub>4</sub>) increased the available soil P by 14% compared to the same treatment without inoculation (T<sub>3</sub>). The application of T<sub>4</sub> to the sandy loam soil increased the available soil P by 88% above T<sub>1</sub>.

The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased the watersoluble K by 63, 63, 63, and 100%, respectively, compared to the control (Fig. 3A). Humic acid (HA) and PSB inoculation gave higher values of water-soluble K than mineral fertilization and control treatments. PSB inculcation for the



**Fig. 1** Potassium dissolving bacteria count (means ( $\pm$  SD, *n* = 10) denoted by different letters are significantly different according to Duncan's test at *p* < 0.05)

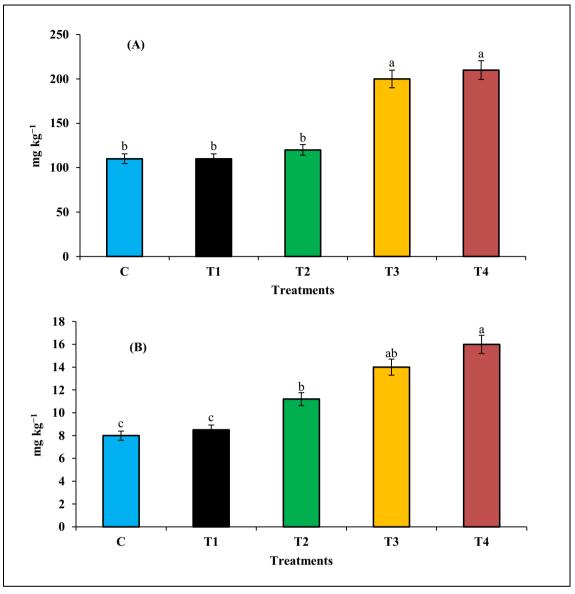
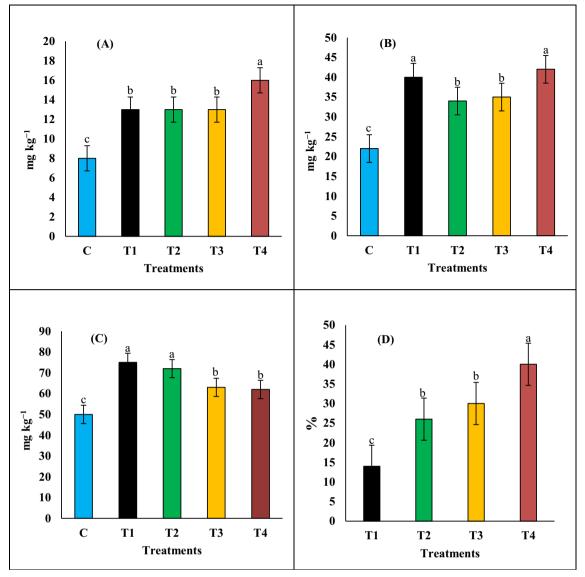


Fig. 2 Effect of treatments on nitrogen (A) and (B) phosphorus availability in the studied soil (means ( $\pm$  SD, n = 10) denoted by different letters are significantly different according to Duncan's test at p < 0.05)

sandy loam soil amended with HA ( $T_4$ ) increased the watersoluble K by 23% compared to the same treatment without inoculation ( $T_3$ ). The application of  $T_4$  to the sandy loam soil increased the water-soluble K by 23% above  $T_1$ . The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased the exchangeable K by 52, 55, 59, and 91%, respectively, compared to the control (Fig. 3B). Humic acid (HA) and PSB inoculation gave higher values of the exchangeable K than mineral fertilization and control treatments. PSB inculcation for the sandy loam soil that was amended with HA ( $T_4$ ) increased the exchangeable K by 20% compared to the same treatment without inoculation ( $T_3$ ). The application of  $T_4$  to the sandy loam soil increased the exchangeable K by 5% above  $T_1$ . Humic acid (HA) and PSB inoculation gave lower values of the non-exchangeable K than the mineral fertilization (Fig. 3C).

# 3.3 Effect of Humic Acid and PSB on Nutrient Uptake by Faba Bean Plants

The uptake of nitrogen (N), phosphorus (P), and potassium (K) by faba bean plants were investigated as affected by the fertilization treatments and PSB, and the data are illustrated in Table 2. The experimental results revealed that the application of the investigated treatments significantly (p < 0.05) increased the uptake of nutrients either with or without bio-fertilizers compared to the control. The application of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> increased N uptake



**Fig. 3** Effect of treatments on water soluble (**A**), exchangeable (**B**), non-exchangeable (**C**), and K use efficiency (**D**) in the studied soil (means ( $\pm$  SD, n = 10) denoted by different letters are significantly different according to Duncan's test at p < 0.05)

by 43, 45, 74, and 97%, respectively, compared to the control. PSB inculcation for the sandy loam soil amended with HA ( $T_4$ ) increased N uptake by 13% compared to the

same treatment without inoculation ( $T_3$ ). The application of  $T_4$  to the sandy loam soil increased the available soil N by 38% above  $T_1$ .

Table 2	Effect of treatments on
some nut	rient's concentrations,
carbohyd	rates, and chlorophyll in
faba bean	leaves

Treatments	N (g kg <sup>-1</sup> )	$P (g kg^{-1})$	K (g kg <sup>-1</sup> )	Carbohydrates (mg g <sup>-1</sup> )	Chlorophyll (SPAD)
С	15.2±1.5c	4.0±0.1e	28.0±2.1d	24±1.4d	33±2.0c
$T_1$	21.0±2.2b	5.0±0.2d	35.8±2.1c	28±1.2c	38±2.3b
$T_2$	22.0±2.0ab	5.50±0.3c	42.6±2.5b	32±1.4b	42±2.0ab
T <sub>3</sub>	26.5±2.2a	6.0±0.2b	46.2±2.6b	33±1.3b	45±2.3a
$T_4$	30.0±3.2a	6.7±0.1a	50.2±3.2a	36±1.2a	45±2.0a

Plant samples were collected at the beginning of flowering stage

Means ( $\pm$  SD, n = 10) in the same column denoted by different letters are significantly different according to Duncan's test at p < 0.05

The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased P uptake by 25, 38, 50, and 68%, respectively, compared to the control. PSB inoculation for the soil fertilized with  $K_2SO_4$  ( $T_2$ ) increased P uptake by 10% above the same treatment without inoculation ( $T_1$ ). PSB inculcation for the sandy loam soil amended with HA ( $T_4$ ) increased P uptake by 12% compared to the same treatment without inoculation ( $T_3$ ). The application of  $T_4$  to the sandy loam soil increased P uptake by 34% above  $T_1$  (100%  $K_2SO_4$ ).

The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased K uptake by 28, 52, 65, and 79%, respectively, compared to the control. PSB inoculation for the soil fertilized with  $K_2SO_4$  ( $T_2$ ) increased K uptake by 19% above the same treatment without inoculation ( $T_1$ ). PSB inculcation for the sandy loam soil amended with HA ( $T_4$ ) increased K uptake by 9% compared to the same treatment without inoculation ( $T_3$ ). The application of  $T_4$  to the sandy loam soil increased the K uptake by 40% above  $T_1$ .

# 3.4 Effect of Humic Acid and PSB on Growth of Faba Bean Plants

Some growth parameters, as affected by potassium sources and potassium solubilizing bacteria (PSB), are shown in Table 3. The maximum growth was obtained from  $T_4$ , followed by  $T_3$ ,  $T_2$ , and  $T_1$ , and the lowest growth parameters were recorded in the control. In general, humic acid (HA) gave higher values of plant growth than the mineral fertilization, and the PSB inoculation increased plant growth compared to the non-inoculated plants. The application of  $T_4$  to the sandy loam soil increased the plant highest, branch number, number of leaves, and leaf area by 20, 23, 42, and 33% above  $T_1$ .

The experimental results indicated that the application of the investigated materials significantly (p < 0.05) increased the shoot and root weights of faba bean plants either with or without bio-fertilizers compared to the control. The application of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> increased the fresh shoot weight by 38, 50, 63, and 88%, respectively, when compared with the control, while the increases in the case of root were 15, 18, 25, and 38%. Humic acid (HA) gave higher values of roots and shoot matter than mineral fertilization and control treatments. PSB inoculation of the soil fertilized with  $K_2SO_4$  (T<sub>2</sub>) increased the root and the shoot biomass by 2 and 9% above the same treatment without inoculation (T<sub>1</sub>). PSB inculcation of the soil that was amended with HA (T<sub>4</sub>) increased the root and the fresh shoot weights by 10 and 15% compared to the same treatment without inoculation (T<sub>3</sub>). The application of T<sub>4</sub> to the sandy loam soil increased root and shoot fresh weight by 19 and 36% above T<sub>1</sub>.

The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased the chlorophyll by 15, 27, and 36%, respectively, compared with the control. Meanwhile, increases in the case of carbohydrates were 17, 33, 38, and 50% (Table 2). Humic acid (HA) gave higher values of chlorophyll and carbohydrates than the potassium sulfate and control treatments. PSB inoculation of the soil fertilized with  $K_2SO_4$  ( $T_2$ ) increased the chlorophyll and carbohydrates by 10 and 14% above the same treatment without inoculation ( $T_1$ ). The application of  $T_4$  to the sandy loam soil increased the chlorophyll and carbohydrates by 18% and 29% above  $T_1$ .

# 3.5 Effect of Humic Acid and PSB on Yield of Faba Bean Plants and K Use Efficiency

The response of seed and straw yield to the tested fertilization treatments is shown in Table 4. The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased the seed yield by 16%, 17%, 20%, and 30%, respectively, compared with the control in the first season and by 18%, 20%, 26%, and 36% in the second season. The application of  $T_1$ ,  $T_2$ ,  $T_3$ , and  $T_4$  increased the straw yield by 24%, 34%, 37%, and 44%, respectively, compared with the control in the first season and by 18%, 24%, 37%, and 44%, respectively, compared with the control in the first season and by 18%, 24%, 37%, and 43% in the second season. Humic acid (HA) with 50% of mineral K dose gave higher values of seed and straw yields than the full recommended dose of  $K_2SO_4$  and control treatments. PSB

Treatments	PH (cm)	BN/plant	LN/ plant	LA (cm <sup>2</sup> /plant)	SFW g/plant	RFW g/plant
С	50±3d	2.0±0.2d	100±6e	350±10d	80±3e	40±2d
$T_1$	60±2c	3.0±0.2bc	120±6d	450±14c	110±3d	$46\pm 2c$
T <sub>2</sub>	62±2b	$3.3 \pm 0.1b$	150±7c	520±12c	120±3c	47±3c
T <sub>3</sub>	70±4a	3.5±0.1ab	$160\pm8b$	550±13b	$130{\pm}4b$	$50\pm 3b$
T <sub>4</sub>	72±4a	3.7±0.2a	170±7a	600±12a	150±5a	55±3a

PH plant highest, BN branches number, LN leaves number, LA leaf area, SFW shoot fresh weight, RFW root fresh weight

The data were recorded at the beginning of flowering stage

Means ( $\pm$  SD, n = 10) in the same column denoted by different letters are significantly different according to Duncan's test at p < 0.05

**Table 3** Effect of treatments onsome growth parameter of fababean plants

 Table 4
 Effect of treatments on yield of faba bean plants during 2018

 and 2019 growing seasons
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Treatments	2018		2019		
	Seed (t ha <sup>-1</sup> )	Straw (t ha <sup>-1</sup> )	Seed (t $ha^{-1}$ )	Straw (t ha <sup>-1</sup> )	
С	5.00±0.03d	5.12±0.07d	4.85±0.05d	5.12±0.09e	
$T_1$	5.80±0.02c	6.34±0.07c	5.70±0.06c	6.04±0.05d	
$T_2$	5.84±0.06bc	6.84±0.08b	5.80±0.04c	6.34±0.06c	
T <sub>3</sub>	$6.00{\pm}0.05b$	$7.00 {\pm} 0.06b$	6.10±0.07b	$7.00{\pm}0.08b$	
$T_4$	$6.52{\pm}0.04a$	7.35±0.09a	6.60±0.05a	7.32±0.07a	

Means ( $\pm$  SD, n = 5) in the same column denoted by different letters are significantly different according to Duncan's test at p < 0.05

inoculation for the soil fertilized with  $K_2SO_4$  (T<sub>2</sub>) caused slight increases in the seed and straw yield above the same treatment without inoculation (T<sub>1</sub>).

The inoculation of sandy loam soil with potassium solubilizing bacteria (PSB) and the application of humic acid (T<sub>4</sub>) increased the seed and straw yield compared to the same treatment without inoculation (T<sub>3</sub>). The application of T<sub>4</sub> to the sandy loam soil increased seed and straw yield by 14% and 19% above T<sub>1</sub> (full mineral potassium nutrition). The potassium use efficiency (KUE) is affected significantly by the potassium treatments (Fig. 3D). KUE ranged from 14–40%. The highest significant value of KUE was T<sub>4</sub>, while the lowest one was recorded in T<sub>1</sub>.

## **4** Discussion

Faba bean plants grown in sandy loam soil are affected by humic acid (HA) and inoculation with potassium solubilizing bacteria (PSB). Vegetative growth of faba bean plants increased significantly in response to PSB and HA; this may be due to the enhancement of photosynthesis and nutrient uptake (Labib et al. 2012; Abou-Zaid and Eissa 2019; Al-Sayed et al. 2020; Ali et al. 2021). The bio-fertilization of faba bean plants with Bacillus circulars caused remarkable elevation in nitrogen (N), phosphorus (P), and potassium (K) availability in the studied sandy loam soil as well as the uptake of these nutrients by faba bean plants. Remarkable increases in the water-soluble and exchangeable potassium were found as a result of PSB inoculation. The increasing of potassium availability in the current study may be due to the potassium solubilizing from feldspar and increasing the activity of potassium solubilizing bacteria in the rhizosphere of faba bean plants (Massoud et al. 2009; Al-Sayed et al. 2020). Under the current study, the inoculation of sandy loam soil amended with 40 kg ha<sup>-1</sup> of humic acid increased the population of PSB by 84% above the control. Increasing the total count of PSB may enhance the availability and uptake of plant nutrients (Massoud et al. 2009; Al-Sayed et al. 2020). The availability of nitrogen (N) and phosphorus (P) was increased as a result of bio-fertilization with Bacillus circulars applied with humic acid. The production of organic acids associated with the activity of PSB may enhance the availability of these nutrients and may stimulate plant growth. Several studies have been reported the ability of PSB to produce organic acids, e.g., citric, tartaric, and oxalic acids (Meena et al. 2016; Al-Sayed et al. 2020). Abdel-Salam and Shams (2012) and Labib et al. (2012) found that PSB increased the uptake of nitrogen (N), phosphorus (P), and potassium (K) by faba bean. Abdel-Salam and Shams (2012) studied the response of potato grown on clay soil to PSB inoculation, and they found increases in the fresh weight of shoots, leaf area, and total chlorophyll in comparison with the untreated plants. Improving the vegetative growth may be due to the increase in PSB population that could solubilize potassium from the feldspar with a continuous supply of potassium which leads to enhance the plant growth as a result of bio-fertilization (Parmar and Sindhu 2013; Selladurai and Purakayastha 2016). The biofertilization of faba bean with *Bacillus circulars* significantly increased the growth and yield above the untreated plants. The plants inoculated with PSB and amended with humic acid were more able to produce soluble carbohydrates and chlorophyll. Increasing the ability of plants to create carbohydrates and increase the efficiency of the photosynthesis process led to a distinct increase in the growth of faba bean plants, and thus increased the seed and straw yield. The increases in the growth of faba bean may be due to the promotion of nutrient uptake and through the production of plant hormones as a result of inoculation with Bacillus circulars (Mahamud et al. 2015; Meena et al. 2016).

Potassium use efficiency of the applied fertilizers ranged from 14 to 40% of the applied potassium. Most of the applied potassium was not taken up by faba beans due to the low use efficiency of potassium fertilizers (Dhillon et al. 2019). Potassium use efficiency (KUE) for cereal crops in the world stands at 19% (Dhillon et al. 2019). The highest values of KUE were recorded in the case of faba bean plants inoculated with PSB and amended with humic acid. The superiority of that treatment, which contains humic acid at a rate of 40 kg ha<sup>-1</sup>, was clearly in the current study. Humic acid maximized the availability of N, P, and K and this may be due to improving soil quality and increasing microbe's activity of PSB (Abiven et al. 2009; Van Zwieten et al. 2010; Eissa 2014; Eissa 2016. Dincsoy and Sönmez (2019) studied the humic acid impacts on wheat plants, and they found that increasing the rate of applied humic acid increased the soil organic carbon and nutrients availability; moreover, humic acid increased the growth and yield of wheat. Humic materials contain many active groups, e.g., carboxyl, carbohydrate, hydroxyl, phenol, and methoxyl (Peuravuori et al. 2006; Bulgari

et al. 2019; de Jesus Souza et al. 2019). The structure of the organic materials in humic acid plays vital roles in the availability of essential plant nutrients (Dinçsoy and Sönmez 2019). The application of humic acid to the sandy soils has several advantages through increasing the root growth, enhancing the growth of soil microorganisms, increasing soil water holding capacity, and improving the structure of soils (Canellas et al. 2015; Dinçsoy and Sönmez 2019).

# **5** Conclusions

The use of bio-fertilization and humic acid to reduce mineral fertilization and to obtain clean food is crucial to both our health and the environment. There is sufficient information about the symbiotic fixation of nitrogen by rhizobia in the case of faba beans plants, but little is available about its response to potassium solubilizing bacteria. The response of faba bean plants to a bio-fertilizer containing Bacillus circulars and the application of 40 kg ha<sup>-1</sup> of humic acid was studied via experimentation in a 2-year field study. According to the results of field studies in this research, it is possible to use biological fertilizers that contain Bacillus circulars to produce faba bean with low rates of mineral K fertilization. The application of 40 kg ha<sup>-1</sup> of humic acid and the inoculation with potassium solubilizing bacteria can reduce the rates of mineral potassium fertilization by 50%. Usually, farmers aspire to achieve higher yields by increasing fertilization rates, which may have a negative impact on food safety and the integrity of the ecosystem. The findings of the current study achieve the aspirations of farmers and at the same time reduce the high mineral fertilization. More research studies are required to investigate the biological behavior and agricultural interactions between potassium solubilizing bacteria and nitrogen-fixing bacteria in faba bean plants.

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#### **Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflicts of interest.

### References

Aalipour H, Nikbakht A, Ghasemi M, Amiri R (2020) Morphophysiological and biochemical responses of two turfgrass species to arbuscular mycorrhizal fungi and humic acid under water stress condition. J Soil Sci Plant Nutr 20:566–576. https://doi.org/10.1007/ s42729-019-00146-4

- Abdel-Salam MA, Shams AS (2012) Feldspar-K fertilization of potato (Solanum tuberosum L.) augmented by biofertilizer. J Agric Environ Sci 12(6):694–699. https://doi.org/10.5829/idosi.aejaes.2012.12.06. 1802
- Abou-Zaid EAA, Eissa MA (2019) Thompson Seedless Grapevines Growth and Quality as Affected by Glutamic Acid, Vitamin B, and Algae. J Soil Sci Plant Nutr 19(4):725–733
- Abiven S, Menasseri S, Chenu C (2009) The effects of organic inputs over time on soil aggregate stability–a literature analysis. Soil Biol Biochem 41(1):1–12. https://doi.org/10.1016/j.soilbio.2008.09.015
- Akhtar A, Rizvi Z, Irfan M, Maqbool A, Bashir A, Malik KA (2020) Biochemical and morphological risk assessment of transgenic wheat with enhanced iron and zinc bioaccessibility. J Cereal Sci 91: 102881. https://doi.org/10.1016/j.jcs.2019.102881
- Ali AM, Awad MYM, Hegab SA, Abd El Gawad AM, Eissa MA (2021) Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. J Plant Nutr 44(3):411–420
- Almaroai YA, Eissa MA (2020) Effect of biochar on yield and quality of tomato grown on a metal-contaminated soil. Scientia Horticulturae 265:109210
- Al-Sayed HM, Hegab SA, Youssef MA, Khalafalla MY, Almaroai YA, Ding Z, Eissa MA (2020) Evaluation of quality and growth of roselle (Hibiscus sabdariffa L.) as affected by bio-fertilizers. J Plant Nutr 43(7):1025–1035. https://doi.org/10.1080/01904167.2020. 1711938
- Bulgari R, Franzoni G, Ferrante A (2019) Biostimulants application in horticultural crops under abiotic stress conditions. Agronomy 9(6): 306. https://doi.org/10.3390/agronomy9060306
- Burt R (2004) Soil survey laboratory methods manual. Soil survey investigations report, United States department of agriculture natural resources conservation service No: 42, Version4 ftp://ftp-fc.sc.egov. usda.gov/NSSC/Lab\_Methods\_Manual/SSIR42\_2004.view.pdf. Accessed 15 Aug 2020
- Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, Piccolo A (2015) Humic and fulvic acids as biostimulants in horticulture. Sci Hortic 196:15–27. https://doi.org/10.1016/j.scienta. 2015.09.013
- de Jesus Souza B, do Carmo DL, Santos RHS, de Oliveira TS, Fernandes RBA (2019) Residual contribution of green manure to humic fractions and soil fertility. J Soil Sci Plant Nutr 19:878–886. https://doi. org/10.1007/s42729-019-00086-z
- Dhillon JS, Eickhoff EM, Mullen RW, Raun WR (2019) World potassium use efficiency in cereal crops. Agron J 111(2):889–896. https:// doi.org/10.2134/agronj2018.07.0462
- Dinçsoy M, Sönmez F (2019) The effect of potassium and humic acid applications on yield and nutrient contents of wheat (*Triticum* aestivum L. var. Delfii) with same soil properties. J Plant Nutr 42(20):2757–2772. https://doi.org/10.1080/01904167.2019. 1658777
- Ding Z, Zhou Z, Lin X, Zhao F, Wang B, Lin F, Ge Y, Eissa MA (2020) Biochar impacts on NH3-volatilization kinetics and growth of sweet basil (*Ocimum basilicum* L.) under saline conditions. Industrial Crops and Products 157:112903
- Eissa MA (2014) Performance of river saltbush (*Atriplex amnicola*) grown on contaminated soils as affected by organic fertilization. World Appl Sci J 30(12):1877–1881. https://doi.org/10.5829/idosi. wasj.2014.30.12.19
- Eissa MA (2016) Nutrition of drip irrigated com by phosphorus under sandy calcareous soils. J Plant Nutr 39(11):1620–1626
- El Naim AM, Ahmed AI, Ibrahim KA, Suliman AM, Babikir ES (2017) Effects of nitrogen and bio-fertilizers on growth and yield of roselle (*Hibiscus sabdariffa* L. var. sabdariffa). Int J Agric For 7(6):145– 150. https://doi.org/10.5923/j.ijaf.20170706.05

- El-Azab ME, El-Dewiny CY (2018) Effect of bio and mineral nitrogen fertilizer with different levels on growth, yield and quality of maize plants. J I P B S 5(2):2349–2759
- El-Mahdy MT, Youssef M, Eissa MA (2018) Impact of in vitro cold stress on two banana genotypes based on physio-biochemical Evaluation. South African Journal of Botany 119:219–225
- FAO (2006) Guidelines for soil description, 4th edn. Viale delle Terme di Caracalla, Rome
- Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108(3):389–411
- Labib BF, Ghabour TK, Rahim IS, Wahba MM (2012) Effect of potassium bearing rock on the growth and quality of potato crop (*Solanum tuberosum*). J Agric Biotechnol Sustain Dev 4(1):7–15. https://doi.org/10.5897/JABSD11.033
- Mahamud MA, Chowdhury MAH, Rahim MA, Sheel PR (2015) Performance of some potato accessions of USA and Bangladesh in relation to dry matter yield and biochemical constituent. J Bangladesh Agril Univ 13(2):215–220
- Massoud ON, Morsy EM, El-Batanony NH (2009) Field response of snap bean (*Phaseolus vulgaris* L.) to N<sub>2</sub>-fixers *Bacillus circulans* and arbuscular mycorrhizal fungi inoculation through accelerating rock phosphate and feldspar weathering. Aust J Basic Appl Sci 3:844– 852
- Meena VS, Maurya BR, Bahadur I (2014) Potassium solubilization by bacterial strain in waste mica. Bangladesh J Bot 43(2):235–237. https://doi.org/10.3329/bjb.v43i2.21680
- Meena VS, Maurya BR, Verma JP, Meena RS (2016) Potassium solubilizing microorganisms for sustainable agriculture. Springer, New Delhi
- Narula N, Saharan BS, Kumar V, Bhatia R, Bishnoi LK, Lather BPS, Lakshminarayana K (2005) Impact of the use of biofertilizers on cotton (*Gossypium hirsutum*) crop under irrigated agro-ecosystem. Arch Agron Soil Sci 51(1):69–77. https://doi.org/10.1080/ 03650340400029275
- Neme K, Bultosa G, Bussa N (2015) Nutrient and functional properties of composite flours processed from pregelatinised barley, sprouted faba bean and carrot flours. Int J Food Sci Technol 50:2375–2382. https://doi.org/10.1111/ijfs.12903
- Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun Soil Sci Plant Anal 6(1):1–11. https://doi.org/10. 1080/00103627509366539
- Parmar P, Sindhu SS (2013) Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. J Microbiol Res 3(1):25–31. https://doi.org/10.5923/j.microbiology. 20130301.04

- Peuravuori J, Žbánková P, Pihlaja K (2006) Aspects of structural features in lignite and lignite humic acids. Fuel Process Technol 87(9):829– 839. https://doi.org/10.1016/j.fuproc.2006.05.003
- Rajendiran Selladurai, Tapan Jyoti Purakayastha, (2016) Effect of humic acid multinutrient fertilizers on yield and nutrient use efficiency of potato. J Plant Nutr 39(7):949–956
- Rekaby SA, Awad MYM, Hegab SA, Eissa MA (2020) Effect of some organic amendments on barley plants under saline condition. J Plant Nutr 43(12):1840–1851
- Singh M, Biswas SK, Nagar D, Lal K, Singh J (2017) Impact of biofertilizer on growth parameters and yield of potato. Int J Curr Microbiol App Sci 6(5):1717–1724. https://doi.org/10.20546/ ijcmas.2017.605.186
- Thomas P, Sekhar AC, Upreti R, Mujawar MM, Pasha SS (2015) Optimization of single plate-serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast cfu enumeration and single colony isolation from diverse samples. Biotechnol Rep 8:45–55. https://doi.org/10.1016/j.btre.2015.08.003
- Torun H, Toprak B (2020) Arbuscular mycorrhizal fungi and K-humate combined as biostimulants: changes in antioxidant defense system and radical scavenging capacity in *Elaeagnus angustifolia*. J Soil Sci Plant Nutr 20:2379–2393. https://doi.org/10.1007/s42729-020-00304-z
- Van Zwieten L, Kimber S, Downie A, Morris S, Petty S, Rust J, Chan KY (2010) A glasshouse study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. Soil Res 48(7):569–576. https://doi.org/10.1071/SR10003
- Xiao Y, Wang X, Chen W, Huang Q (2017) Isolation and identification of three potassium-solubilizing bacteria from rape rhizospheric soil and their effects on ryegrass. Geomicrobiol J 34(10):873–880. https://doi.org/10.1080/01490451.2017.1286416
- Youssef MA, Eissa MA (2017) Comparison between organic and inorganic nutrition for tomato. J Plant Nutr 40(13):1900–1907. https:// doi.org/10.1080/01904167.2016.1270309
- Zhang AM, Zhao GY, Gao TG, Wang W, Li J, Zhang SF, Zhu BC (2013) Solubilization of insoluble potassium and phosphate by *Paenibacillus kribensis* CX-7: a soil microorganism with biological control potential. Afr J Microbiol Res 7(1):41–47. https://doi.org/10. 5897/AJMR12.1485
- Zhang L, Sun XY, Tian Y, Gong XQ (2014) Biochar and humic acid amendments improve the quality of composted green waste as a growth medium for the ornamental plant *Calathea insignis*. Sci Hortic 176:70–78. https://doi.org/10.1016/j.scienta.2014.06.021

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