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Integrated Effects of Potassium Humate and Planting Density on Growth, Physiological Traits and Yield of *Vicia faba* L. Grown in Newly Reclaimed Soil

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Abstract: Several agronomic factors, including planting density, affect plant growth and final yield. New soil suffers from severe fertility shortage and crop productivity. Potassium humate (KH) application improves soil fertility and plant performance under new soil conditions. Therefore, this investigation was performed in two seasons of 2018/2019 and 2019/2020 to study the impact of KH application at the rate of 50 kg hectare^{-1} (ha^{-1}) on growth, yield, physio-biochemical attributes, plant water status and nutrients in faba bean plants grown in newly reclaimed soil under three planting densities, i.e., D_1 = 222.222 plants ha^{-1} (15 × 60 cm), D_2 = 166.666 plants ha^{-1} (20 × 60 cm) and D_3 = 133.333 plants ha^{-1} (25 × 60 cm). The results showed that KH application enhanced tissue water status by increasing the membrane stability index (MSI%) and relative water content (RWC%), while electric leakage (EL%) was reduced, alongside increased growth attributes physio-biochemical properties and nutrients. These results were positively reflected by the improved yield and its components (i.e., number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed yield ha^{-1}) in favor of the medium planting density (166.666 plants ha^{-1}). The results of the current study showed that the application of KH with the medium planting density (20 × 60 cm) was the best treatment combination to enhance the performance and productivity (2.97 ton ha^{-1}**)** of faba bean plants grown under newly reclaimed soil conditions.

Keywords: faba bean; planting density; KH; yield; physio-biochemical attributes

1. Introduction

Faba bean (*Vicia faba* L.) is a widely consumed public food in all countries of the world, and is a good source of protein in human and animal food. Its seeds are also rich in carbohydrates, minerals and fibers [1–3]. The bean has a beneficial role in crop succession, due to the fixation ability of atmospheric nitrogen, which improves the soil through an increase in the content of both nitrogen and organic matter [4]. Faba bean plants often change the shape and size of their canopy through different agronomic factors. This feature should take into account obtaining information regarding the response of faba bean plants to the differences in planting density and their impact on faba bean productivity [5].

Intra- and interplant competition is a key stress impacting plant performance, crop productivity and its economic return. Increasing planting density per unit area increased the two types of competition on production requirements, i.e., the light, water and nutrients. In this respect, the results collected from different regions, which explain plant performance under the different plant densities, are very variable [5]. In Egypt, faba bean breeding for different stress tolerances is restricted, due to the lack of good characters for selection. Thus, it is necessary to evaluate the variations in tolerance techniques for faba bean plants based on their growth and yield characteristics [1].

Planting densities are among the most important agronomic factors that play a prominent role in faba bean production by its effect on the ability of nutrient translocation inside the plant, the performance of plant growth and the utilization of environmental conditions. Moreover, planting density significantly impacts yield and its components due to competition for its limited requirements, i.e., light, water and nutrients. The obtained results, which describe the effect of plant density on yield in different cultivation systems, are highly variable [6]. Planting density is directly related to intra- and interrow spacing, which not only impacts crop quantity but also its quality. Plant spacing is the limiting factor in achieving acceptable plant density for the improvement of crop productivity [7].

The expansion of planting distances may cause a decreased yield; weed growth is harmful and reduces light-use efficiency. On the other hand, the narrowing of planting distances can cause lodging, poor light permeation between plants, reduced photosynthetic efficiency and a decrease in yield [8]. Optimum planting density, which can be obtained through the number of plants per unit area, and the adequate distribution of plants in the field, is a prerequisite to increased faba bean yield. In Egypt, there is currently a focus on the utilization of some crops in newly reclaimed agricultural lands; nevertheless, most of these agricultural lands are stressed environments (i.e., faced with salinity, drought and nutrient deficiency) [9]. Therefore, planting density may be an applicable alternate to improving faba bean yield under changing agricultural factors [6].

Egyptian soils are suffering from a lack of nutrients, such as those lacking in recently reclaimed soil. The exogenous application of potassium humate can improve soil properties and increase their fertility through increasing the amounts of potassium (K) in the soil, which boosts plant performance. K is a crucial element that could increase plant dry matter and enhance productivity [10]. Humic substances generally impact the bioavailability of nutrients through their ability to form compounds with metallic ions, which promote micronutrient availability and macronutrients, especially when these nutrients in the soil are rare [11].

Potassium humate (KH) is a necessary natural material that can be used to enhance the physio-biochemical properties of soils, its performance and the productivity of plants [12]. Humic materials result from the chemical and biological humification of animal and plant matter through the biological processes of microorganisms. These substances impact plant performance and soil fertility by an increase in the soil microbial numbers, which stimulates the soil properties, and the increase in cation exchange capacity [13].

KH is an efficient fertilizer that positively impacts the growth and productivity of plants. KH increases the rate of nutrient uptake, improves plant biomass and decreases soil compaction [14]. Humic substances improve membrane permeability, enzyme activities and hormonal activity and increase water-holding ability [15]. Humates are used as a foliar spray on plants or an addition to soil because they contain about 30% to 60% humic acid, which can be easily taken up by the roots [12].

KH application alleviated the stress by enhancing plant performance, soil properties and nutrient dynamics [15]. KH improves soil health and environmental quality during crop cultivation [16]. Humic substances have been proven to increase membrane permeability, photosynthesis, the efficiency of absorption and the utilization of some important elements. Additionally, the application of humic substances has positive effects on cytokinin and auxin substances, in addition to the indirect impact on plant metabolism [17].

The major aim of this work was to assess the efficiency of KH application with optimum planting density to improve the performance of faba bean plants and maximize seed use efficiency to increase yield.

2. Materials and Methods

2.1. Plant Material, Growing Condition, Experimental Design and Treatments

In Egypt, Southeast Fayoum—specifically, the farm of the Faculty of Agriculture, Fayoum University (29°17′ N; 30°53′ E), Egypt—2 trials were carried out during the 2018/2019 and 2019/2020 seasons to examine the impact of potassium humate (KH) as a soil application on faba bean plants grown under different planting densities. Faba bean seeds of the Giza 429 variety were attained from the Field Crop Research Institute, which is part of the Agricultural Research Centre located in Giza, Egypt. Healthy seeds were chosen and sown on the 21 and 23 October in 2018 and 2019, respectively. The daily temperatures averaged 23.3 ± 3.1 °C and 23.9 ± 3.7 °C, and the daily relative humidity averaged 57.4 ± 4.9% and 59.1 ± 4.3% in both seasons, respectively. Each trial unit involved 5 ridges, 60 cm apart and 3.5 m long, comprising an area of 10.5 m^2. Sowing was achieved at both sides of the ridge, before the first irrigation; thinning was performed on one plant per hill. During soil preparation and plant growth, the soil was supplemented with a full dose of NPK fertilizer, according to the recommendations of the Ministry of Agriculture and Land Reclamation (i.e., 450 kg ha^{-1} calcium superphosphate (15.5% P_2O_5), 250 kg ha^{-1} ammonium sulfate (20.5% N), and 120 kg ha^{-1} potassium sulfate (48% K_2O)). Irrigation water was added to 100% of the reference crop evapotranspiration (ETo), values from the Fayoum Meteo Station. In total, 7 irrigations were applied in each season, with total water rates of about 2975 m^3 ha^{-1} in each growing season. All other cultivation applications were accomplished as recommended for faba bean yield trial packages. A split-plot in a randomized complete block design with three replications was used. The main plots were assigned to treatments of planting density, while rates of potassium humate were arranged in the subplots. The physical and chemical characteristics of the soil at a depth of 50 cm during the two seasons of the study are presented in Table 1, following the methods described in [18,19].

Table 1. Physical and chemical properties of the experimental soil in two seasons *.

Properties	2018/2019	2019/2020
Physical analysis		
Clay (%)	32.5	31.4
Sand (%)	48.3	51.1
Silt (%)	19.2	17.5
Texture class	Loamy sand	Loamy sand
Chemical analysis		
Organic matter (%)	0.73	0.75
$CaCO_3$ (%)	3.97	3.89
PH	7.83	7.92
ECe (dS m^{-1})	5.89	5.75
Total N (%)	0.059	0.058
P (mg kg^{-1} soil)	3.41	3.56
K (mg kg^{-1} soil)	43.53	47.25

* All analyses were performed at the Central Laboratory of Soil, Water and Plant Analysis (Iso17025), Faculty of Agriculture, Fayoum University, Fayoum, Egypt.

Plants were grown under three planting densities, i.e., D_1 = 222.222 plants ha^{-1} (15 × 60 cm), D_2 = 166.666 plants ha^{-1} (20 × 60 cm) and D_3 = 133.333 plants ha^{-1} (25 × 60 cm). Potassium humate (KH; 65% humic acid+25% fulvic acid+10% K_2O), at a rate of 50 kg $hectare^{-1}$ (ha^{-1}), was applied as a soil amendment at soil preparation. The amount of KH (50 kg ha^{-1}) was thoroughly mixed with 250 kg fine sand, spread over the surface of the soil, and then mixed into the top 20 cm of the soil surface layer.

2.2. Measurement of Growth Parameters

Sixty days after sowing (DAS), 10 plants were removed at random from the quarter ridge in each experimental plot. Using a meter scale, the shoot lengths were measured, from the cotyledonary node to the apical bud of the main stem. Leaf and branch numbers were recorded per plant. Plants shoots were weighed to count their fresh weights, while the dry weights were estimated by placing the shoots in an oven at 65 ± 5 °C until stable dry weights.

2.3. Determination of Photosynthetic Pigments

As stated in the method of [20], the chlorophylls (a,b) and carotenoid contents were estimated. Leaf discs of 2 mg were homogenized in acetone (80%, 50 mL). The mixture was placed in a centrifugation machine for ten minutes to centrifuge at 10,000× g. Using a spectrophotometer, the readings were recorded at 665, 649, and 440 nm.

2.4. Estimation of Osmolytes and Total Soluble Phenols

Total soluble sugar content was estimated by using the phenol-sulphuric method according to [21]. Using the method in [22], the ninhydrin reagent was used to determine total free amino acid content. As followed in the colorimetric method of Folin–Ciocalteau [23] total soluble phenol content was measured. Through the procedure followed by the authors in [24], the rapid colorimetric was used to estimate free proline content.

2.5. Determination of Membrane Stability Index (MSI%), Relative Water Content (RWC%) and Electrolyte Leakage (EL%)

The membrane stability index (MSI) was measured via the method of [25], and calculated by the formula: MSI (%) = [1 − (C1/C2)] × 100 (C1; electrical conductivity at 40 °C, C2; electrical conductivity at 100 °C). The relative water content (RWC) was determined as stated in the procedure of [26]; the RWC was counted by the equation of RWC (%) = [(FM − DM)/(TM − DM)] × 100 (FM; fresh mass, DM; dry mass, TM; turgid mass). Electrolyte leakage (EL) was measured by the procedure of [27], and calculated through using the following equation: EL (%) = [(EC2 − EC1)/EC3] × 100 (EC1; electrical conductivity at boiling degree, EC2; electrical conductivity at 45 °C, EC3; electrical conductivity at 100 °C).

2.6. Estimation of Inorganic Cations Content

After 50 days of sowing, the nitrogen content of leaves (N; mg/g DW) was estimated by using the method of [28]. According to the method of [29], the leaf contents of potassium (K; mg/g DW), phosphorus (P; mg/g DW), sodium (Na; mg/g DW) and calcium (Ca; mg/g DW) were measured by using a Perkin-Elmer Atomic Absorption and Flame Photometer.

2.7. Measurement of Yield and Its Components

At harvest (5 April 2019 and 8 April 2020), all pods plant^{-1} of each experimental unit were harvested and left to air dry and then counted. The dry seeds of faba bean were then extracted from the pods and weighed to calculate the average 100-seed weight, and dry seed yield plant^{-1} and hectare^{-1} (ha^{-1}).

2.8. Statistical Analysis

Treatments arranged in a split-plot in randomized complete blocks design were subjected to analysis of variance (ANOVA), after performing a homogeneity test of errors, according to [30]. Combined analysis was performed for the results of the two seasons using Duncan's multiple range test; the significant variations between treatments were compared at $p \leq 0.05$.

3. Results

3.1. Growth Parameters

KH applied ameliorated the growth of faba bean plants by significantly increasing all examined growth parameters (i.e., shoot fresh length, leaf number, branch number, shoot fresh weight and shoot dry weight) compared to their controls (Figure 1). The increases in the abovementioned growth traits were 65.4%, 27.2%, 37.7%, 36.8% and 49.3%, respectively, for D_1; 73.1%, 31.5%, 43.4%, 42.9% and 54.5%, respectively, for D_2; 69.1%, 29.1%, 39.4%, 38.1% and 51.1%, respectively, for D_3, compared to the controls. In respect to planting density, there were significant increases in all growth parameters, excluding shoot length for D_2 compared to D_1 and D_3.

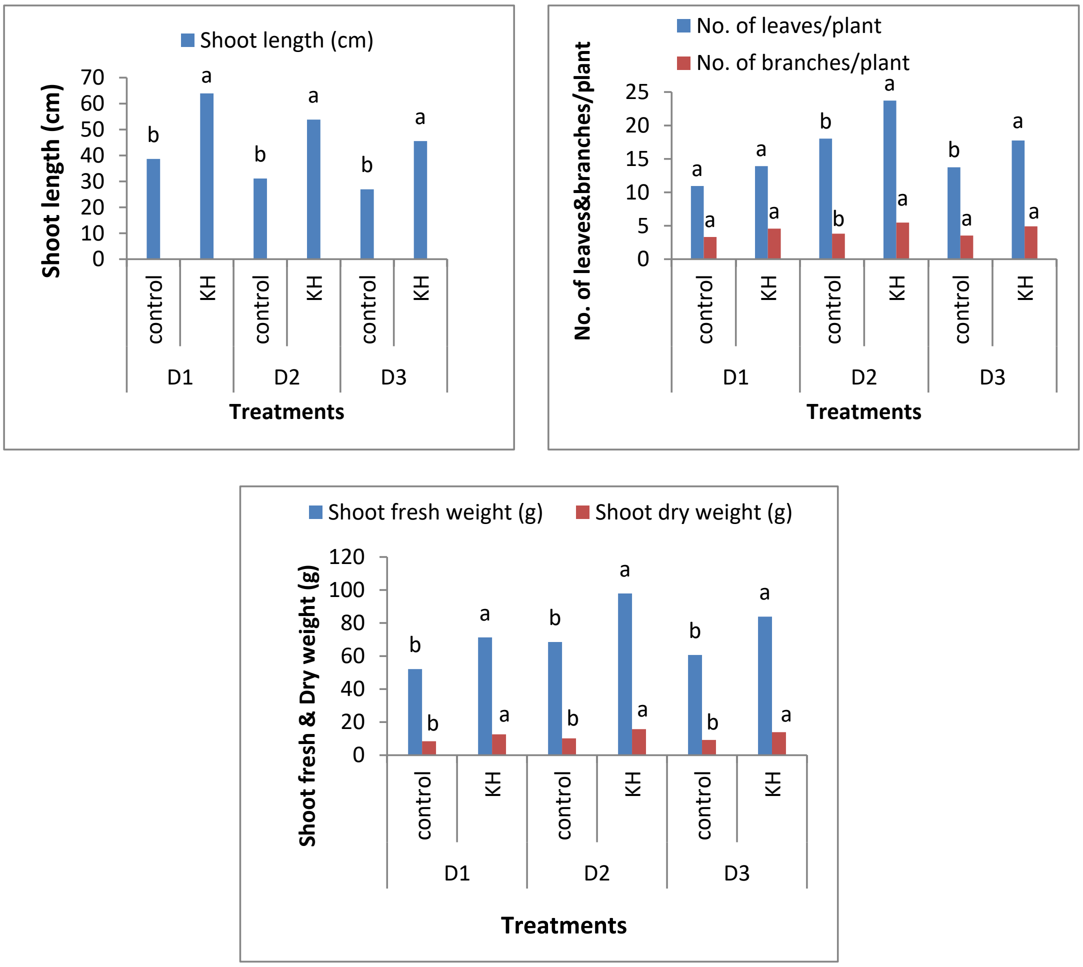

Figure 1. Effect of potassium humate (KH) application and planting density treatment on shoot length (cm), no. of leaves/plant, no. of branches/plant, shoot fresh weight (g) and shoot dry weight (g) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

3.2. Photosynthetic Pigments

KH application enhanced faba bean growth in the field; subsequently, it significantly increased the contents of chlorophylls a, b and carotenoids in faba bean leaves compared to no KH application (Figure 2). These increases in the contents of chlorophylls a, b and carotenoids were 42.6%, 30.6% and 25.7%, respectively, for D_1; 57.6%, 34.8% and 29.8%, respectively, for D_2; and 47.9%, 32.8% and 26.8%, respectively, for D_3, compared to their controls. Regarding planting density, there were significant increases in the contents of chlorophylls a, b and carotenoids for D_2 compared to those in the other planting densities.

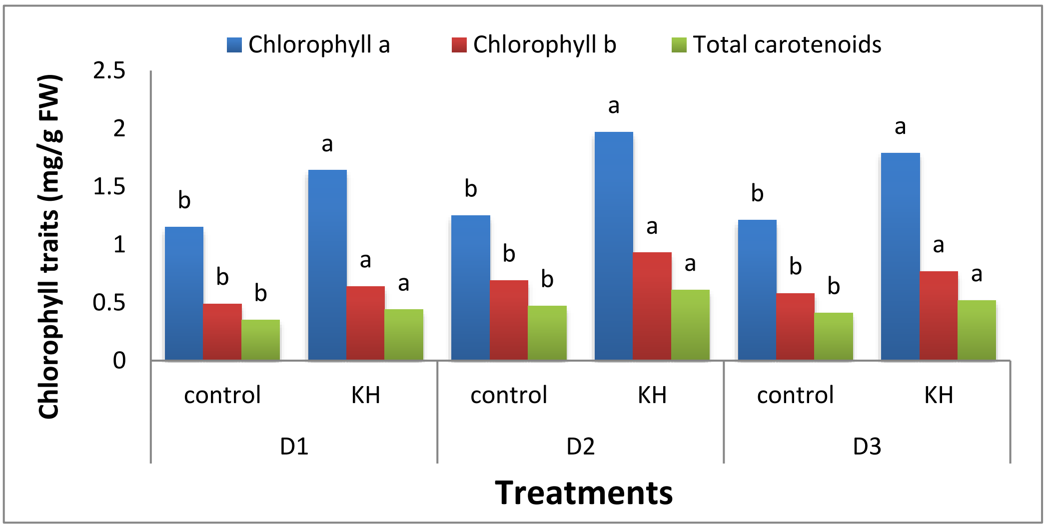

Figure 2. Effect of potassium humate (KH) application and planting density treatment on leaf pigments (mg/g FW) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

3.3. Organic Solutes and Total Soluble Phenols

The total soluble sugars, total free amino acids, total soluble phenols and free proline significantly increased in faba bean plants grown in soil treated with KH compared to those plants grown in KH-free soil (Figure 3). The increases in the abovementioned traits were 15.7%, 31.2%, 32.9% and 24.4%, respectively, for D_1; 21.4%, 37.8%, 36.4% and 39.6%, respectively, for D_2; and 17.8%, 33.3%, 34.7% and 30.0%, respectively, for D_3, compared to their controls. Concerning planting density, there were significant increases in the abovementioned physiological attributes for D_2 compared to D_1 or D_3.

3.4. MSI, RWC and EL

Plants grown under the application of KH exhibited an improvement in plant water relations by obtaining significant increases in MSI% and RWC%, while they showed a significant reduction in EL% compared to no KH application (Figure 4). The increases in the MSI% and RWC% were 15.2% and 14.3%, respectively, for D_1; 17.9% and 19.0%, respectively, for D_2; and 16.4% and 16.3%, respectively, for D_3, while the decreases in EL% were 15.4%, 17.6% and 16.5% for D_1, D_2 and D_3, respectively, compared to the controls. Regarding planting density, there were significant increases in RWC% and MSI%, but reduced EL% for D_2 compared to D_1 and D_3.

3.5. Inorganic Cations Content

KH application enhances the nutrient status of faba bean plants by achieving significant increases in the contents of N, K, P and Ca, while causing a decline in the content of Na compared to their controls (Figure 5). The increases in the contents of N, K, P and Ca were 15.6%, 19.0%, 21.2% and 30.9%, respectively, for D_1; 20.9%, 20.8%, 25.6% and 33.5%, respectively, for D_2; and 17.3%, 19.5%, 24.2% and 31.5%, respectively, for D_3, while the decreases in the content of Na were 19.8%, 23.2% and 21.8%, respectively, for D_1, D_2 and D_3, compared to their controls. For planting density, an increase in D_2 was observed for N, P, K and Ca nutrient concentrations, and a decrease in Na compared to the other planting densities.

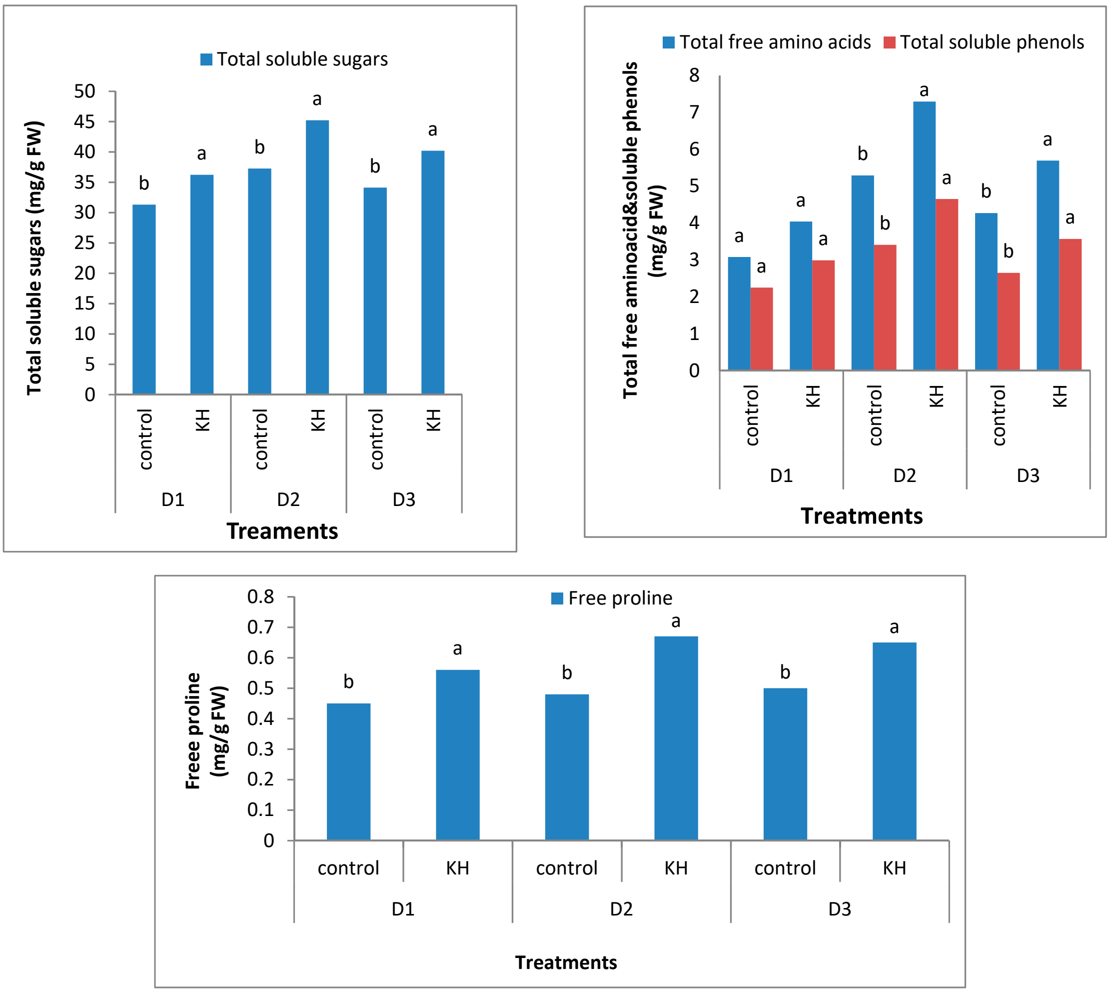

Figure 3. Effect of potassium humate (KH) application and planting density treatment on total soluble sugars, free amino acids, soluble phenols and proline content (mg/g FW) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

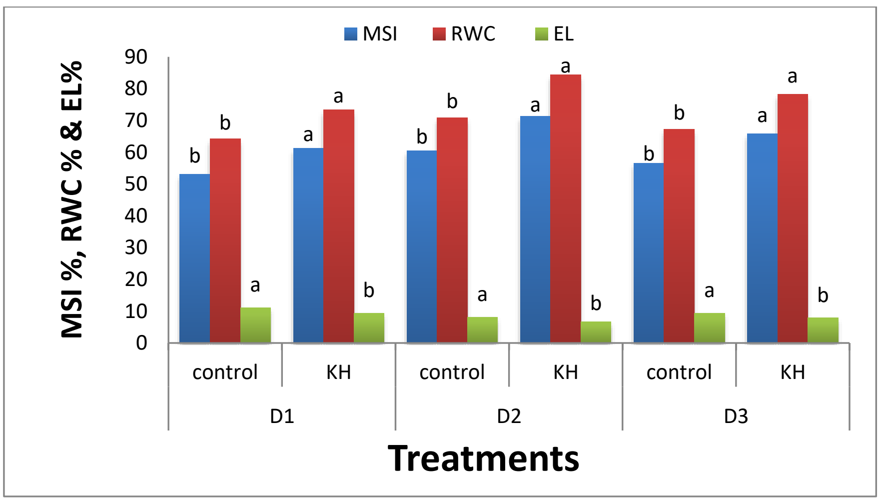

Figure 4. Effect of potassium humate (KH) application and planting density treatment on membrane stability index (MSI%), relative water content (RWC%) and electrolyte leakage (EL%) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

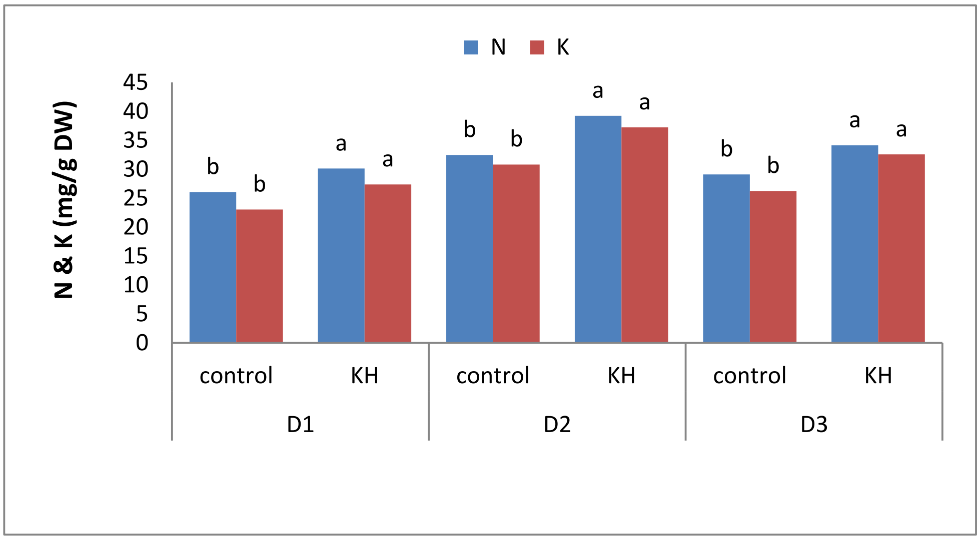

Figure 5. Effect of potassium humate (KH) application and planting density treatment on content of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca) and sodium content (Na) (mg/g DW) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

3.6. Yield and Its Components

The application of KH improved the productivity of faba bean plants by causing significant increases in the yield and its components, e.g., number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed yield ha^{-1} compared to no KH application (Figure 6). The increases in the abovementioned traits were 33.4%, 18.5%, 17.4% and 27.1%, respectively, for D_1; 38.6%, 22.3%, 24.9% and 34.4%, respectively, for D_2; and 36.6%, 19.8%, 21.3% and 31.4%, respectively, for $D_{3,}$ compared to the controls. Concerning planting density, there was a significant increase in the seed yield of faba bean plant and its components for D_2 compared to D_1 or D_3.

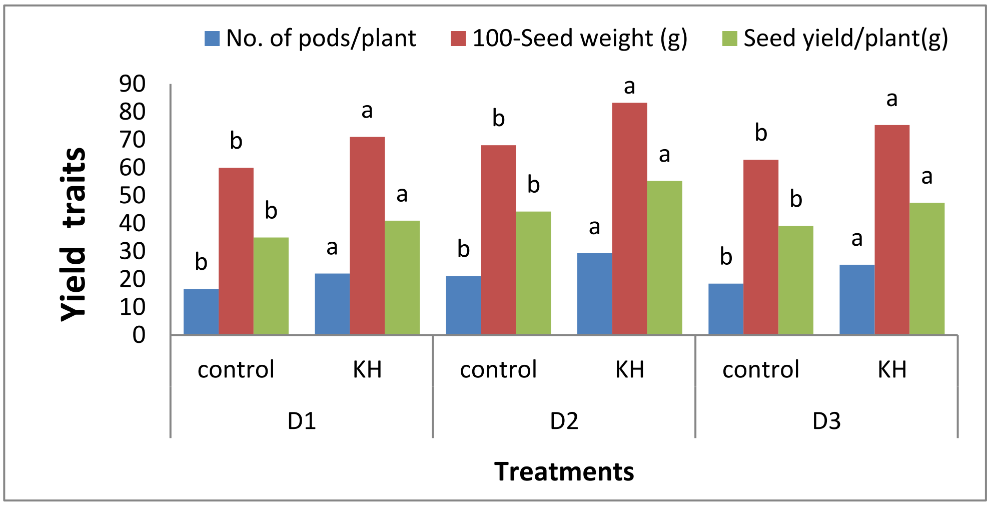

Figure 6. Effect of potassium humate (KH) application and planting density treatment on number of pods/plants, 100-seed weight (g), seed yield/plant (g) and seed yield/ha (ton) of *Vicia faba* L. plants. For each parameter, treatments followed by the same letter are not significantly different at $p \leq 0.05$.

4. Discussion

In this study, increasing planting distance from 15 to 20 cm caused significant increases in all growth traits excluding shoot length, which significantly increased with the narrow planting distance (Figure 1). These increases may be due to expanding planting distance, leading to a better chance for plants to produce more metabolite contents [7]. On the other hand, the narrow planting distance leads to a decrease in light intensity, which stimulates IAA production in stem tissues. The increase in IAA may reflect cell elongation and, hence, the increment in shoot length [6]. In this context, KH application significantly improved all growth traits of faba bean plants compared to untreated KH plants (Figure 1). This result may be due to the fact that KH includes many elements required to improve plant life, stimulate possible growth, enhance photosynthesis and accelerate cell division. The obtained results agree with those obtained by the authors of [14,31].

The lack of interest in farming operations could result in a disordered photosynthesis process. Chlorophyll fluorescence has been reported as a credible method to evaluate the changes in the emission of pigment–protein complexes (proteins of PSII) which are synthesized in the chloroplasts [32]. Carotenoids are considered non-enzymatic antioxidants that reduce the accumulation of reactive oxygen species (ROS) under adverse conditions [14]. Our results show that plants grown at a distance of 20 cm in soil treated with KH exhibited improve photosynthesis efficiency by increasing the contents of chlorophylls a, b and carotenoids compared to no KH application (Figure 2). These results revealed the high efficiency of KH to ameliorate photosynthetic efficiency by increasing the levels of chlorophylls a, b and carotenoids. This amelioration may be attributed to the high-efficiency KH, which stimulates the enzymes related to photosynthesis and increases the Mn and Fe uptake required for the biosynthesis of chlorophyll [33,34]. Free proline is considered a good source of some elements that are necessary to overcome different stresses and a protective factor for cellular organelles and cytosolic enzymes [14,35]. In this study, faba bean plants grown at a planting density of 20 × 60 cm, with the application of KH, showed significant increases in the contents of total soluble sugars, total soluble phenols, total free amino acids and free proline, compared to plants grown in KH-free soil (Figure 3). These compatible solutes stimulate the tolerance of plants and increase their adaptation responses to acute stresses. KH application improved the biosynthesis of organic compounds and this might be directly related to its role in increasing the efficiency of plant resistance [36]. This is due to the increase in metabolism by improving photosynthesis and the composition of starch [14].

In this context, planting faba bean seeds in soil treated with KH with a 20 cm hill spacing and a 60 cm ridge width led to significant increases in MSI% and RWC%, while this showed a significant decrease in EL%, compared to soil without KH application (Figure 4). This positive impact may be attributed to the entrance of KH into the cells, carrying both micronutrients and water. It also increases the water permeability of plant membranes and water holding capacity [37]. Similar results have been previously reported by the authors in [14].

Humic substances play a prominent role in the uptake of nutrients [15]. KH application can, directly and indirectly, affect the nutrient status of faba bean plants. Our results reveal that plants grown with a plant density of 166.666 plants ha^{-1} (20 × 60 cm), under the application of KH, showed significant increases in the contents of N, P, K and Ca, while they showed a significant reduction in the content of Na compared to soil without any KH (Figure 5). This is related to the existence of hydrophilic and hydrophobic sites that promote surface activity. Hence, the humic substances react with the cell membrane structures and interact as a carrier of nutrients [11]. These outcomes are in accordance with the authors in [38], who indicated that humic substances may improve nutrient uptake. Additionally, the authors in [39] found that humic acid, as a soil application, led to significant decreases in Na content.

Optimum planting distance maximizes yield because it reduces plant-to-plant competition for the production input [5]. In this study, decreasing planting density from 222.222

to 166.666 plants ha^{-1} significantly increased the yield and its components (i.e., number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed yield ha^{-1}) (Figure 6). This increase in the yield and its components may be due to the increased leaf area, which allows for better light and air penetration, and thus increases the number of metabolites in the plant tissues. The low plant density reduced the competition between plants for light, water and nutrients. This finding agrees with that of the authors in [7,40]. In this respect, applying KH with the second planting density (20 × 60 cm) increases faba bean yield and its components (i.e., number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed yield ha^{-1}), compared to no KH application (Figure 6). The application of KH improved plant performance in newly reclaimed soil conditions. These effects were positively reflected in faba bean growth and yield [41]. The higher yield may be because of the stimulating impact of the humic acid, improving plant performance. Moreover, it indirectly provides the soil with micronutrients [31]. Additionally, using humic acid enhances photosynthetic efficiency, nutrient uptake and its translocation inside the plant and its metabolism, thus increasing the yield in general [13].

5. Conclusions

It is concluded that the application of potassium humate (KH) at 50 kg hectare^{-1} (ha^{-1}) on faba bean plants grown in three different planting densities improved tissue water status by enhancing the membrane stability index (MSI%) and relative water content (RWC%) while reducing electric leakage (EL%). Additionally, it markedly increased the contents of total soluble sugars, total free amino acids, total soluble phenols, free proline, N, K, P and Ca, but reduced Na content. These increases were affirmatively reflected in the ameliorating of the growth and boosting the yield representing in number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed yield hectare^{-1} (ha^{-1}). The application of KH with medium planting density (20 × 60 cm) was shown to be the best treatment combination to ameliorate the performance and productivity (2.97 ton ha^{-1}**)** of faba bean plants under new reclaimed soil conditions.

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