



Evaluation of Rock Phosphate and Potassium Feldspar with Biological and Organic Amendments and Its Effect on Soil Phosphorus and Potassium Availability and Uptake, Growth and Yield of Canola

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study, wrote the protocol. Author ABAEABB managed the experimental process, performed the chemical analysis, and wrote the first draft of the manuscript. Author MSH performed the statistical analysis and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/31347

Editor(s):

(1) Marco Trevisan, Institute of Agricultural Chemistry and Environmental Research Centre BIOMASS, Faculty of Agriculture, Catholic University of the Sacred Heart, Italy.

Reviewers:

(1) Rajesh Kumar Meena, Agronomy Section ICAR-NDRI Karnal, India.

(2) Aysen Akay, Selcuk University, Agricultural Faculty, Turkey.

Complete Peer review History: <http://www.sciencedomain.org/review-history/18142>

Original Research Article

Received 31st December 2016

Accepted 31st January 2017

Published 10th March 2017

ABSTRACT

This study was conducted to evaluate the efficient use of both rock phosphate (RP) and potassium feldspar (KF) combined with phosphate (PDB) and potassium (KDB) dissolving bacteria inoculation and/or humic material application on soil phosphorus and potassium availability and uptake, growth and yield of canola compared to the use of chemical manufactured fertilizers. Two field experiments using canola as a test crop were performed during the winter seasons of 2014/2015 and 2015/2016 on the experimental farm, Faculty of Agriculture, South Valley University, Qena, Egypt. They had a complete block randomized design with three replications. The experimental treatments included single and dual inoculations of the soil with phosphate and potassium solubilizing bacteria as well as a direct applications of rock phosphate and potassium feldspar with and without humic material additions.

The results indicated that the direct addition of P and K mineral materials in a combination with P

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and K dissolving bacteria as biological amendments as well as humic materials as an organic form to the sandy loam soil improved P and K availability in the soil, their uptake and growth parameters of canola compared to the control. The most effective treatment was (RP+KF) + (PDB+KDB) + humic materials which produced the highest values of growth, seed yield and nutrient uptake by canola plants compared to the control or to their sole application. There were no significant differences in the parameters produced from these treatments and those obtained from using P and K chemical fertilizers. These results reflected the efficient use of natural sources of P and K combined with the biological additions of P and K dissolving bacteria and humic materials as an organic source instead of using P and K chemical fertilizers for growing crops. Therefore, the direct application of rock phosphate and potassium minerals to soils is considered a promising and economic alternative of natural resources.

Keywords: Canola; phosphate and potassium solubilizing bacteria; P and K uptake; rock phosphate and potassium feldspar; humic substances.

1. INTRODUCTION

Phosphorus (P) and potassium (K) are important macronutrients for plants. They are added as fertilizers to achieve a good and high yield. Potassium deficiency becomes a problem when K is lost easily from soils due to plant uptake and leaching [1]. Also, available P in arid and semi-arid soils is low because its fixation as calcium phosphate due presence of CaCO_3 and Ca^{+2} ion in high levels in these soils.

Phosphate dissolving bacteria have been used to convert insoluble phosphate compounds such rock phosphate into a soluble form which is available for plant growth. They produce organic acids which convert the unavailable P form to an available one. Also, potassium solubilizing bacteria have the ability to dissolve K containing minerals, such as orthoclase into an available form, through producing organic acids. The combined application of rock phosphate and K containing minerals with these bacteria provides the growing plants with a continuous supply of phosphorus and potassium for a best plant growth rate [2]. The direct application of rock P and K minerals to soils may be more economically feasible than soluble chemical fertilizers [3].

The use of P and K solubilizing bacteria as biofertilizers has become a practical solution to supply the plants with both nutrients [4]. The co-inoculation of K and P solubilizing bacteria increased the soil phosphorus availability from 12 to 21% and the potassium availability from 13 to 15% as well as it improved their uptake by plants. They also showed that the harvested biomass and yield of the treated

plants were increased by 23 – 30% over the control [5].

Humic acid is the active constituent of organic humus. It can play a very greatest role in soil properties and plant growth [6]. Humic materials improve the soil structure, increase the soil water holding capacity, enhance the growth of beneficial microorganisms and serve as an a chelating agent to protect plant nutrients from being unavailable and loosing, [7]. They also enrich soil nutrients and display a higher cation exchange capacity (CEC) value [8,9]. They include humic acids, fulvic acids, and humins [10,11]. This study aims to evaluate the efficiency of rock phosphate and potassium feldspar applications combined with rock phosphate and potassium dissolving bacteria as well as humic substances on the availability of P and K, their uptake, growth and yield of canola plants.

2. MATERIALS AND METHODS

Two field experiments were performed during the winter seasons of 2014/2015 and 2015/2016 on the experimental farm of Faculty of Agriculture, South Valley University, Qena, Egypt which is located at the latitude of 26°11' 25 N", and the longitude of 32°44' 42" E.

The experiment layout was a randomized complete block design with three replications. The treatments included direct applications of rock phosphate and potassium feldspar with and without soil inoculation of both phosphate and potassium dissolving bacteria or their dual inoculation in presence or absence of humic substance applications. Some soil physical and chemical properties of the experimental farm are present in Table 1.

Table 1. Some soil physical and chemical properties of the experimental soil

Property	Value
Particle size distribution:	
Sand (%)	61.1
Silt (%)	27.2
Clay (%)	11.7
Texture class	Sandy loam
pH (1:1) in water	8.25
EC _e (dS/m)	3.06
Calcium carbonates (%)	8.21
Organic matter (%)	0.56
Total N (g/kg)	0.1
Total P (g/kg)	0.24
Total K (g/kg)	0.18
Available P (mg/kg)	7.11
Available K (mg/kg)	66.37

Each experimental plot had an area of 7.0 m² (2.0 m x 3.5 m). All plots received a composting filter mud cake, produced from the organic wastes of Quos sugarcane factory, at a level of 4 ton/fed. as general organic amendment (Table 2) and a commercial solid humic mixture material (Humi sunTM) was used as an organic amendment added at a level of 4 kg /fed. to the row before planting. It contained 60% humic acid, 15% (w/w) fulvic acid, 8% K₂O, 0.7% N, 0.06% P₂O₅, 3.98% Ca, 0.29% Mg, 1.89% Fe, 0.043% Mn, 0.013% Zn, 0.05% Cu and 0.048% B. Eighteen treatments were used in this study (Table 3).

Table 2. Some chemical characteristics of composting filter mud cake

Property	Value
pH (1:10)	6.7
EC (1:10) (dS/m)	5.6
Organic matter (%)	65.4
Organic carbon (%)	37.94
Total nitrogen (%)	2.31
C/N ratio	16.40
Total P (P ₂ O ₅ %)	2.51
Total K (K ₂ O %)	0.21

Rock phosphate (31% P₂O₅) and potassium feldspar (8% K₂O) were added at a level of 200 and 300 kg/fed., respectively, and mixed thoroughly with the soil in each plot. Canola (*Brassica napus* L.) CV. SERW 4 seeds were planted by using the row placement method along and inside the rows. The plantation was done in 40 cm row spacing with a 5 cm interplant distance in each row. Twenty mL of single or mixed liquid inoculants of PDB (4x10⁷ cells mL⁻¹)

and KDB (4x10⁷ cells mL⁻¹) were diluted in 1 liter of water and applied evenly into the open row after seed planting, and then, the row was covered with a thin soil layer and the experiment was directly irrigated.

Table 3. The experimental treatments

Treatment no.	Treatment
1	Control
2	Control (NPK full dose)
3	(RP+KF)
4	PDB*
5	KDB**
6	PDB + KDB
7	(RP+ KF)+ PDB***
8	(RP+ KF)+ KDB
9	(RP+ KF)+ PDB + KDB
10	1 + (Humi sun TM)
11	2 + (Humi sun TM)
12	3 + (Humi sun TM)
13	4 + (Humi sun TM)
14	5 + (Humi sun TM)
15	6 + (Humi sun TM)
16	7 + (Humi sun TM)
17	8 + (Humi sun TM)
18	9 + (Humi sun TM)

*PDB= Phosphate Dissolving Bacteria (*Paenibacillus polymyxa*; previously *Bacillus polymyxa*) were locally isolated in previous study by the author [12].

**KDB= Potassium Dissolving Bacteria (*Bacillus cereus*) were taken from Microbial Resource Centre, Faculty of Agriculture, Ain Shams University.

*** (RP+ KF) = Rock phosphate + potassium feldspar

Control treatments were done without any addition, and with supplying the full recommended dose of phosphorus (200 kg/ fed. of super phosphate, 15.5% P₂O₅), The full dose of potassium (50 kg/fed. of potassium sulphate, 48% K₂O) and nitrogen (150 kg/fed. of ammonium nitrate, 33% N) for all experimental plots.

2.1 Recorded Data

2.1.1 Growth measurements

At the age of 70 days of planting, plant samples were taken for the determination of vegetative growth characters. The roots were thoroughly washed with running water and separated from the shoots. Data of plant height, fresh and dry weights of shoots and roots as well as number of branches were recorded. The dried plant material samples were pulverized and analyzed for phosphorus and potassium contents.

Soil samples were collected from each plot at the same time of plant sampling, air-dried, passed through a 2 mm sieve and kept for analysis. The available P and K were estimated in the soil samples.

2.1.2 Yield measurements

At the harvest time, the plants of one row from each plot were harvested to estimate number of pods, seed yield, straw yield and 1000-seed weight.

2.1.3 Methods of soil analysis

- Particle size distribution was determined using the pipette method according to Jackson [13].
- The electrical conductivity (EC) was determined in a 1: 2.5 soil to water extract using conductivity meter according to Jackson [13].
- Organic carbon content was determined according to Walkely and Black's wet oxidation method according to Jackson [13].
- Total calcium carbonate was determined using Scheibler calcimeter according to Jackson [13].
- Available potassium was extracted using the ammonium acetate method and measured by flame photometer according to Jackson [13].
- Total P and K were determined according to the method described by Knudsen et al. [14].
- Available phosphorus was extracted using 0.5 M Na HCO₃ at pH 8.5. according to Olsen et al. [15], The extracted P was measured colorimetrically using the chlorostannus phosphomolybdic-sulfuric acid method as described by Jackson [13].
- Soil pH was measured in a 1: 2.5 soil to water suspension using Beckman pH meter as reported by McLean [16].

2.1.4 Methods of plant analysis

A half gram of ground canola plants was digested using 10 ml of H₂SO₄ and 2 ml of perchloric acid in a conical flask as described by Chapman and Pratt [17]. The digests were used to determine the following:

- The total phosphorus was spectrophotometrically determined using

the chlorostannus-phosphomolybdic - sulfuric acid method as described by Jackson [13].

- The total potassium was determined using the flame photometer method that was described by Knudsen et al. [14].

2.2 Statistical Analyses

All data obtained were analyzed using MSTAT-C (Russell, 1994) and one-way analysis of variance was applied. The differences between means of the different treatments were compared using the least significant difference (L.S.D.) at 5% and 1% probability [18].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Available phosphorus in soil

Table 4 showed that the soil sole inoculation with single PDB as well as the dual soil inoculation of PDB + KDB resulted in significant increases in the available soil P representing 20.6 and 19.13% over the control, respectively, while the soil sole inoculation with single KDB did not affect the availability of P in the soil. The results also showed that the combined addition of RP + KF with soil single or dual inoculation gave highly significant increases in the available soil P reaching 103.7, 97.07 and 108.1 % for PDB + (RP + KF), KDB + (RP + KF) and (PDB + KDB) + (RP + KF) treatments over the control, respectively. On the other hand, the results revealed that the application of humic substances plus all applied treatments caused significant increases in the P availability status of the soil. The highest increases in the available phosphorus of 114.0, 111.07 and 133.85% over the control, were recorded for the treatments of humic acid plus both of (RP + KF) + PDB, (RP + KF) + KDB, and (RP + KF) + PDB + KDB, respectively. Humic substances plus (RP + KF) + (PDB + KDB) treatment was significantly superior over all the other treatments. These results demonstrated that the synergistic effects of complete dual inoculation of P and K solubilizing bacteria integrated with the direct application of rock P and K feldspar plus humic substances. Data revealed an almost similar trend in the first season as that previously obtained in the second season.

Table 4. Effect of soil inoculation with both of phosphate and potassium solubilizing bacteria in combination with direct application of rock phosphate and potassium feldspar with addition of humic substances on available soil P and K after 70 days of canola planting in the first and second seasons

Treatment no.	Treatment	Available P (mg/kg)		Available K (mg/kg)	
		Season 1	Season 2	Season 1	Season 2
1	Control	29.01	30.19	134.83	138.62
2	Control (NPK full dose)	61.65	64.32	202.24	208.35
3	(RP+KF)	32.64	33.39	185.39	189.49
4	PDB*	34.99	33.07	143.25	146.41
5	KDB**	31.79	32.96	151.68	154.84
6	PDB + KDB	34.56	36.16	151.68	188.65
7	(RP+ KF)+ PDB***	59.09	59.41	185.99	189.60
8	(RP+ KF)+ KDB	57.17	59.31	185.39	191.71
9	(RP+ KF)+ PDB + KDB	60.37	61.44	193.81	194.87
10	1 + (Humi sun TM)	32.85	33.81	155.39	152.44
11	2 + (Humi sun TM)	73.39	74.56	219.09	221.09
12	3 + (Humi sun TM)	42.45	44.37	202.24	205.19
13	4 + (Humi sun TM)	44.80	46.40	168.53	171.59
14	5 + (Humi sun TM)	46.72	48.32	168.53	171.90
15	6 + (Humi sun TM)	51.84	52.37	176.96	178.86
16	7 + (Humi sun TM)	62.08	63.68	202.24	206.56
17	8 + (Humi sun TM)	61.23	62.61	202.00	206.24
18	9 + (Humi sun TM)	67.84	69.12	219.09	220.36
L.S.D 0.05		4.842	4.958	14.65	14.93
L.S.D .0.01		6.493	6.684	19.65	20.02

3.1.2 Available potassium in soil

With regard to the soil potassium availability, the results (Table 4) showed that, in both seasons, all treatments recorded highly significant increases in the available K over the control except the sole soil inoculation with PDB treatment. The highest increase in the availability of soil K occurred when RP + KF were mixed with the sole PDB, KDB and their dual inoculation, particularly with adding humic substances to the soil. These respective treatments significantly increase the availability of K by 37.94, 37.5 and 43.74 % over the control, respectively, without applying the humic substances. However, humic substance additions recorded increases of 50.00, 49.82 and 62.5% over the control for (RP + KF) + PDB, (RP + KF) + KDB, and (RP + KF) + PDB + KDB treatments, respectively. These respective treatments with humic substances exhibited relatively significant increases of 8.79, 8.96 and 13.04%, respectively, compared to these treatments without adding humic substances.

3.1.3 Canola growth parameters

Table 5 showed that highly significant increases were recorded in the plant height, number of

branches, plant fresh weight, plant dry weight, root fresh weight and root dry weight, as a result of applying the different biological treatments either with or without humic substance applications.

3.1.3.1 Plant height

In the first season, the single or dual soil inoculation of PDB and KDB did not display any significant increase in the plant height compared to the control treatment (Table 5). However, these inoculation treatments plus the application of rock phosphate (RP) and potassium feldspar (KF) showed highly significant increases of 18.43, 12.57 and 19.46% for the application of PDB plus (RP + KF), KDB plus (RP + KF) and a mixture of PDB and KDB plus (RP + KF) compared to the control, respectively. Greatest plant height values were recorded for the combined application of humic substances with all investigated treatments than those without humic substance applications. The increases reached 14.56, 14.40, 19.06, 20.54, 27.59, 28.52 and 31.43% for (RP + KF), PDB, KDB, (PDB+KDB), (RP + KF) +PDB, (RP + KF) +KDB, and (RP + KF) +PDB+KDB treatments compared to the control, respectively. However, the relative increases associated with applying

humic substances were 10.96, 11.17, 15.18, 17.36, 7.74, 14.16 and 10.02% compared to using the previous treatments without humic material applications. The results of the second season showed the same trend as that obtained in the first one (Table 6).

3.1.3.2 Number of branches

Table 5 revealed that, in the first season, the sole application of RP+KF did not record any significant increase in the number of branches/plant. However, significant increases in number of branches/plant were recorded with the soil inoculation using PDB or KDB and their dual inoculation in a combination with RP+KF compared to the untreated control treatment. These increases were 35.97, 38.42 and 40.1% for the previous respective treatments. However, the application of humic substances to all treatments exhibited significantly highest values of number of branches except the (RP + KF) treatment. The highest number of branches/plant was found in the plots treated with humic substances plus PDB, KDB, (PDB + KDB), (RP + KF) + PDB, (RP + KF) +KDB, and (RP + KF) +PDB+KDB treatments which showed 36.2, 40.33, 42.51, 48.23, 49.6 and 54.5% compared to the untreated control, respectively. These results revealed no significant differences among these treatments. Also, similar results were obtained in the second season (Table 6).

3.1.3.3 Plant fresh weight

The shoot fresh weight of canola plants had highly significant increases with the application of RP + KF combined with the single and dual soil inoculation with PDB and KDB and recorded increases of 86.38, 101.1 and 122.2% for (RP + KF) + PDB, (RP + KF) + KDB and (RP + KF) + PDB + KDB treatments, respectively (Table 5). However, the sole addition of RP+KF and the sole soil inoculation with PDB and KDB or their dual inoculation recorded slightly non-significant increases. Also, the combination of the previously respective treatments with humic substances showed highly significant increases in the shoot fresh weight over the control of 101.64, 91.36, 125.0, 120.16, 155.56, 171.20 and 198.36% for the application of humic substances plus (RP + KF), PDB, KDB, (PDB + KDB), (RP + KF) + PDB, (RP + KF) + KDB, and (RP + KF) + PDB + KDB treatment, respectively. On the other hands, these respective treatments

displayed highly significant increases over those without the humic substance addition of 73.76, 62.38, 90.49, 83.46, 37.12, 34.85 and 34.26%, respectively.

3.1.3.4 Plant dry weight

Tables 5 and 6 indicated that the dry weight of canola plants was affected by the addition of the different treatments in presence or absence of humic substances. Almost, a similar trend to that previously obtained in the plant fresh weigh occurred for all treatments which received humic substances. The application of humic substances had a higher marked effect on the canola dry weight than without adding them. The highest increase in the plants dry weight was found when the humic substances were added plus (RP + KF) + PDB, (RP + KF) + KDB, and (RP + KF) +PDB + KDB, which recorded increases in the first season of 105.99, 96.24 and 107.51% respectively, compared to the untreated control treatment and 107.46, 97.1 and 109.81%, respectively, in the second season.

3.1.3.5 Fresh and dry weight of roots

Table 5 showed that the root fresh and dry weights in the first season had the highest significant increases using the treatments that received humic substances plus the single and dual inoculation with PDB or KDB compared to the control. In this respect, the highest increases in root fresh weight were 95.6, 82.4, and 153.4% over the control treatment when humic substances were added with (RP + KF) + PDB, (RP + KF) + KDB, and (RP + KF) + PDB + KDB treatments, respectively. On the other hand, the increases in the root dry weight were 72.5, 88.62 and 110.77% for the previously respective treatments without adding humic substances, respectively. The root fresh and dry weights in the second season revealed almost a similar trend to those previously obtained in the first season.

3.1.4 Yield and yield component of canola

The results of yield and yield components of canola plants influenced by the direct application of rock P and K feldspar with the single or dual inoculation of PDB and KDB in presence or absences of humic substances are present in Tables 5 and 6 for the first and second seasons, respectively.

Table 5. Effect of soil inoculation with both of phosphate and potassium solubilizing bacteria in combination with direct application of rock phosphate and potassium feldspar with addition of humic substances on growth parameters and yield of canola (first season)

Treatment no.	Treatment	Plant height (cm)	No. branches /plant	Plant fresh weight (g/plant)	Plant dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Pod no./plant	Weight of 1000 Seeds (g)	Seed yield (kg/fed)
1	Control	72.45	3.67	45.00	9.85	5.00	1.67	121.58	2.56	838.32
2	Control (NPK full dose)	77.20	4.22	69.82	13.80	7.64	2.65	203.86	3.05	1411.76
3	(RP+KF)	74.80	3.67	52.22	11.11	5.94	1.95	134.88	2.62	974.40
4	PDB*	74.56	4.50	53.03	13.33	6.67	1.99	151.93	2.68	1089.76
5	KDB**	74.89	4.70	53.15	13.55	5.99	1.89	148.29	2.78	1071.28
6	PDB + KDB	74.41	4.56	54.00	13.33	6.56	2.00	158.56	2.90	1154.72
7	(RP+ KF)+ PDB***	85.80	4.99	83.87	16.11	7.90	2.03	202.60	3.12	1485.68
8	(RP+ KF)+ KDB	81.56	5.08	90.50	14.54	7.74	2.04	201.93	3.10	1481.76
9	(RP+ KF)+ PDB + KDB	86.55	5.14	100.00	15.46	8.27	2.04	247.27	3.10	1827.84
10	1 + (Humi sun TM)	80.22	4.82	108.89	14.63	8.14	2.11	148.60	3.01	1094.24
11	2 + (Humi sun TM)	86.63	5.22	134.69	17.96	8.89	3.15	275.63	3.26	1958.32
12	3 + (Humi sun TM)	83.00	4.67	90.74	15.04	8.52	2.61	194.86	3.12	1398.32
13	4 + (Humi sun TM)	82.89	5.00	86.11	15.56	7.65	2.35	194.85	3.07	1462.72
14	5 + (Humi sun TM)	86.26	5.15	101.25	18.33	8.33	2.32	207.06	3.10	1563.52
15	6 + (Humi sun TM)	87.33	5.23	99.07	17.78	8.96	2.38	232.72	3.20	1655.92
16	7 + (Humi sun TM)	92.44	5.44	115.00	20.29	9.78	2.88	306.38	3.54	2335.76
17	8 + (Humi sun TM)	93.11	5.49	122.04	19.33	9.12	3.15	327.83	3.33	2444.40
18	9 + (Humi sun TM)	95.22	5.67	134.26	20.44	12.67	3.52	401.69	3.42	3102.96
	L.S.D 0.05	3.839	1.299	19.71	4.217	3.110	0.4156	49.77	0.4222	369.4
	L.S.D .0.01	5.147	1.648	26.43	5.654	4.171	0.5573	66.74	0.5661	495.4

Table 6. Effect of soil inoculation with both of phosphate and potassium solubilizing bacteria in combination with direct application of rock phosphate and potassium feldspar with addition of humic substances on growth parameters and yield of canola (second season)

Treatment no.	Treatment	Plant height (cm)	No. branches /plant	Plant fresh weight (g/plant)	Plant dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Pod no./plant	Weight of 1000 Seeds (g)	Seed yield (kg/fed)
1	Control	75.02	3.81	46.83	10.19	5.18	1.72	99.13	2.25	614.76
2	Control (NPK full dose)	82.00	4.42	72.71	14.39	7.98	2.77	180.93	2.91	1221.95
3	(RP+KF)	77.52	3.83	54.26	11.51	6.15	2.02	121.43	2.47	754.92
4	PDB*	78.64	4.74	55.77	14.04	7.03	2.10	126.57	2.45	893.77
5	KDB**	77.64	4.90	55.24	14.03	6.20	2.08	119.03	2.43	865.14
6	PDB + KDB	78.00	4.75	56.32	13.94	6.81	2.08	128.48	2.43	949.74
7	(RP+ KF)+ PDB***	89.40	5.05	87.74	16.79	8.34	2.13	180.02	2.87	1296.05
8	(RP+ KF)+ KDB	84.87	5.27	94.42	15.22	8.66	2.12	176.89	2.97	1295.51
9	(RP+ KF)+ PDB + KDB	89.87	5.36	139.19	16.17	8.62	2.13	228.14	2.94	1674.36
10	1 + (Humi sun TM)	82.93	4.99	113.04	15.15	8.80	2.19	123.17	2.47	882.12
11	2 + (Humi sun TM)	90.34	5.46	139.26	18.74	9.25	3.28	242.25	2.98	1789.28
12	3 + (Humi sun TM)	85.89	4.83	93.56	15.58	8.88	2.70	166.36	2.92	1203.52
13	4 + (Humi sun TM)	86.89	5.24	90.09	16.42	8.09	2.47	171.06	2.71	1286.32
14	5 + (Humi sun TM)	89.36	5.36	104.66	19.11	8.68	2.18	187.38	2.99	1374.99
15	6 + (Humi sun TM)	90.74	5.45	107.68	18.55	9.35	2.48	201.71	3.04	1467.35
16	7 + (Humi sun TM)	96.38	5.66	120.82	21.14	10.22	3.01	325.42	3.21	2531.73
17	8 + (Humi sun TM)	97.05	5.73	126.61	20.08	9.50	3.27	373.22	3.23	2925.48
18	9 + (Humi sun TM)	99.57	5.90	140.01	21.38	13.21	3.68	420.70	3.34	3297.92
	L.S.D 0.05	5.874	1.129	20.71	4.373	3.226	0.4505	53.37	0.2342	383.0
	L.S.D .0.01	7.876	1.514	27.77	5.863	3.796	0.6040	71.57	0.314	513.6

3.1.4.1 Number of pods per plant

The number of pods / plant in both seasons showed non-significant increases as the result of the sole treatments of RP + KF, PDB, KDB and PDB + KDB, while the rest of treatments recorded highly significant increases in the number of pods/plant compared to the control (Tables 5 and 6). The results also revealed that the addition of humic substances to all treatments caused highly significant increases with most treatments over the same treatments without humic substance additions.

In the first season, the highest number of pods per plant was recorded for adding humic substances with (RP + KF) + PDB + KDB treatment followed by (RP + KF) + KDB and (RP + KF) + PDB treatment which had increases of 230.39, 169.64 and 152.0% over the control, respectively, and relative increases of 62.45, 62.35 and 51.22% compared to the same treatments without humic substance addition. A similar trend was observed in the second season, where the maximum increases in the number of pods/plant of 324.39, 276.5 and 228.28% were obtained for the application of humic substances with (RP + KF) +PDB+KDB, (RP + KF) + KDB and (RP + KF) +PDB treatments, respectively compared to the control.

3.1.4.2 Weight of 1000 seeds

Tables 5 and 6 showed that the 1000- seed weight was significantly affected by all treatments except of the sole treatments of RP+KF, PDB, KDB and PDB+KDB which had slightly non-significant increases in this parameter. The results also showed that the combination of rock P and K feldspar with single or dual dissolving microorganisms in presence of humic substances recorded the greatest values of 1000-seeds weight and relative increases of 38.28, 30.08 and 33.59% over the control treatment for the application of humic substances to RP + KF + PDB, RP + KF + KDB and RP + KF + PDB + KDB treatments, respectively, in the first season. A similar trend was recorded in the second season as that obtained in the first one where the increases of 42.67, 43.56 and 48.44 % over the control, respectively, were found for previously respective treatments.

3.1.4.3 Seed yield

The application of (RP + KF) alone and with single or dual inoculation of PDB and KDB did

not show significant increases in canola seed yield in both seasons compared to the untreated control (Tables 5 and 6). On the other hand, the application of (RP + KF) plus single and dual soil inoculation recorded highly significant increases in seed yield of 77.22, 76.75 and 118.04% for (RP + KF) + PDB, (RP + KF) + KDB and (RP + KF) + PDB + KDB treatments, respectively, compared to the control. Also, the seed yield of canola plants recorded high values when humic substances were added in combination with the previously respective treatments where the increases reached to 178.62, 191.58 and 270.14% compared to the control in the first season. Also, in the second season, the application of humic substance treatments in a combination with P and K materials and P or K dissolving bacteria exhibited highest increases in the seed yield of 311.82, 375.87 and 436.46% for adding humic substances with (RP + KF) + PDB, (RP + KF) + KDB and (RP + KF) + PDB + KDB, respectively, compared to the control.

3.1.5 Phosphorus uptake by canola plants

In the first season, the single or dual soil inoculation of PDB, KDB plus the application of rock phosphate (RP) and potassium feldspar (KF) showed highly significant increases in the P uptake by canola plants of 30.77, 20.51 and 33.33 % for PDB plus (RP + KF), KDB plus (RP + KF) and both of of PDB and KDB plus (RP + KF) respectively, compared to the control, while applying these bacteria solely recorded the lowest values (Table 7). Also, the application of humic substances plus previously treatments displayed higher P uptake by canola plants than those without humic substance applications which recorded increases of 48.72, 38.46 and 61.54 % for the previously respective treatments compared to the control. In the second season, increases of 47.62, 38.1 and 54.76 % were obtained for the previously respective treatments.

The P uptake by canola plants had the highest significant increases using the complete combination of humic substances and (RP + KF) with single or dual soil inoculation with PDB and KDB. Increases occurred in the P-uptake of 209.32, 173.68 and 237.85% over the control for humic substance additions to PDB + (RP + KF), KDB + (RP + KF) and (PDB + KDB) plus (RP + KF) treatments respectively, in the first season. However, in the second season they were 208.34, 172.22 and 227.70% over the control for the previously respective treatments (Table 8).

Table 7. Effect of soil inoculation with both of phosphate and potassium solubilizing bacteria in combination with direct application of rock phosphate and potassium feldspar with addition of humic substances on P and K content and uptake by canola plant after 70 days of planting (first season)

Treatment no.	Treatment	P% In plant	P uptake (mg/plant)	K% In plant	K uptake (mg/plant)
1	Control	0.39	37.99	1.79	176.18
2	Control (NPK full dose)	0.58	79.37	2.42	333.79
3	(RP+KF)	0.43	47.77	2.05	227.74
4	PDB	0.48	63.91	2.11	281.56
5	KDB	0.43	57.97	2.11	283.67
6	PDB + KDB	0.46	61.67	2.16	288.75
7	(RP+ KF)+ PDB	0.51	81.21	2.11	338.44
8	(RP+ KF)+ KDB	0.47	68.23	2.21	321.65
9	(RP+ KF)+ PDB + KDB	0.52	80.64	2.21	341.46
10	1 + (Humi sun TM)	0.43	62.88	2.00	292.14
11	2 + (Humi sun TM)	0.69	123.19	3.00	537.77
12	3 + (Humi sun TM)	0.49	74.04	2.26	340.38
13	4 + (Humi sun TM)	0.53	81.79	2.26	351.67
14	5 + (Humi sun TM)	0.49	90.55	2.26	415.90
15	6 + (Humi sun TM)	0.50	87.09	2.32	407.24
16	7 + (Humi sun TM)	0.58	117.51	2.42	490.62
17	8 + (Humi sun TM)	0.54	103.97	2.42	467.00
18	9 + (Humi sun TM)	0.63	128.35	2.63	537.31
L.S.D 0.05		0.052	19.08	0.11	107.07
L.S.D .0.01		0.070	25.58	0.16	144.50

Table 8. Effect of soil inoculation with both of phosphate and potassium solubilizing bacteria in combination with direct application of rock phosphate and potassium feldspar with addition of humic substances on P and K content and uptake by canola plant after 70 days of planting (second season)

Treatment no.	Treatment	P% In plant	P uptake (mg/plant)	K% In plant	K uptake (mg/plant)
1	Control	0.42	42.34	1.89	191.91
2	Control (NPK full dose)	0.62	88.86	2.52	361.58
3	(RP+KF)	0.46	52.64	2.11	241.86
4	PDB*	0.51	72.12	2.22	312.84
5	KDB**	0.46	63.88	2.34	325.95
6	PDB + KDB	0.49	68.64	2.28	318.05
7	(RP+ KF)+ PDB***	0.54	91.08	2.22	372.19
8	(RP+ KF)+ KDB	0.50	76.29	2.33	354.33
9	(RP+ KF)+ PDB + KDB	0.55	89.53	2.30	370.83
10	1 + (Humi sun TM)	0.46	69.28	2.11	318.33
11	2 + (Humi sun TM)	0.74	137.58	3.16	590.95
12	3 + (Humi sun TM)	0.52	81.36	2.35	365.78
13	4 + (Humi sun TM)	0.57	92.63	2.33	381.58
14	5 + (Humi sun TM)	0.52	100.07	2.36	451.52
15	6 + (Humi sun TM)	0.53	97.50	2.44	448.09
16	7 + (Humi sun TM)	0.62	130.55	2.53	533.45
17	8 + (Humi sun TM)	0.58	115.26	2.51	502.01
18	9 + (Humi sun TM)	0.65	138.75	2.69	573.05
L.S.D 0.05		0.05237	21.02	0.1888	105.7
L.S.D .0.01		0.07022	28.19	0.2523	141.7

3.1.6 Potassium uptake by canola plants

Tables 7 and 8 revealed that all treatments under investigation displayed highly significant increases in K content and uptake by canola plants. Also, the results showed that all treatments that received humic substances recorded higher K content and uptake values than those without humic substances. The dual inoculation of PDB and KDB with investigated treatments almost showed higher K content and uptake values than those for single soil inoculation.

In the first season, the maximum K content and uptake were found for the dual inoculation with PDB and KDB plus the humic substance application and (RP + KF) materials, followed by the same treatments with the single inoculation of both PDB and KDB which recorded maximum increases of 35.2, 35.2 and 46.93% in the K content and 178.48, 165.07 and 204.98% in the K uptake using the previously respective treatments compared to the control. In the second season a similar trend was obtained as in the first season, where the increases in K plant content with the previously respective treatments were 33.86, 32.8 and 42.33% respectively, while the increases in plant K uptake were 177.97, 161.59 and 198.60%, respectively compared to the control treatment.

3.2 Discussion

3.2.1 Soil phosphorus and potassium availability

Regarding to the direct addition effect of rock phosphate and potassium feldspar as well as the different biological or organic treatments on the soil availability of phosphorus and potassium, the results demonstrated the effects of the dual inoculation of P and K solubilizing bacteria combined with applying rock P and K feldspar directly with and without humic substance application on P and K availability. In this respect, the bacterial inoculation, could enhance the P and K availability in soils through producing organic acids that dissolve P and K bearing materials which stimulate growth and nutrient uptake by plants [12,19,20]. Also, the integration of P and K mineral materials with inoculation of P- and K-solubilizing bacteria increased the available P from 12 to 21% and the available K from 13 to 15% in soils cultivated with hot pepper (*Capsicum annuum* L. as compared to the control [5].

The application of rock P and K minerals material with the co-inoculation of phosphate dissolving bacteria might provide a readily continuous supply of phosphorus and potassium for the optimal plant growth. [21,22]. Also, The dual inoculation of PDB and KDB in a combination with applying rock phosphate and potassium mineral materials to the soil increased the phosphorus and potassium availability, shoot and root growth as well as their uptakes by maize plants [23]. The biologically increase of phosphorus and potassium availability in soils occurred with the inoculation of plant growth promotion rhizobacteria combined with rock material applications may lead to increases in the P uptake and plant growth [20,24,25,26].

Humic substances play important roles in the nutrient availability in different soils [11]. Also, other results indicated that the humic substances may improve soil structure, increase the soil available water, activate the growth of beneficial soil microorganisms and increase the absorbed nutrients [7].

3.2.2 Growth and yield parameters

From the above results of growth and yield parameters, the positive effects of the humic substances combined with PDB, KDB or their dual inoculation may be due to increasing the soil fertility and in turn, number of such microorganisms that accelerate the microbial activity in the rhizosphere of the inoculated soil. It may be also explained on the basis of the beneficial effects of these applied bacteria on the nutrient availability which, in turn, they stimulate the plant growth. The present results of plant growth are comparable with those reported by co-inoculation study of PSB and KSB which resulted in high amounts of NPK uptake leading to high plant growth [2]. Similar results were also obtained by other investigator [4]. In addition, the maize yield significantly increased and also the soil properties such as the organic content improved due to the co-inoculation with KSB and PSB [27]. The present results also agree with those of Blasubramaniam and Subramanian [28] who indicated that the increase in rice grain yield due to the plant growth promotion using rhizobacteria (silicate solubilizing bacteria) was 18.1% compared to the control. Also, the seed inoculation with the phosphate dissolving bacteria (PDB) was reported to cause remarkable increases in most parameters of mung bean plants as compared to adding the natural rock phosphate fertilizer [29,30].

Humic acid is the active component of organic humus, which can play a very vital role in plant growth and soil conditioning [6]. Combined application of both plant growth promotion rhizobacteria and humic acid improved the growth and yield of canola more than the single application of each one alone [31]. Moreover, applied humic acid addition to the tested soil significantly increased the yield of oil per hectare of *Ocimum basilicum* while the combination of plant growth promotion rhizobacteria with the humic acid increased the oil yield more than adding the humic acid alone [32]. Nitrogen, phosphorus and potassium uptakes by the hot pepper (*Capsicum annuum* L.) plants improved as a result of the combined application of rock phosphate and potassium feldspar with the inoculation of phosphate and potassium solubilizing bacteria which increased the plant photosynthesis by 16% and the leaf area by 35% compared to control plants [5]. Also, the biomass harvest and fruit yield of the treated plants were 23% and 30%, respectively, higher compared to the control. In this respect, additions of organic materials promote the metabolic activity inside plants and enhance the movement of the metabolites through the roots and stems toward the leaves. So the nutrient content of the plants may increase [33]. The application of yeast bio-fertilizers with chicken manure to the soil gave the most effective increases in the plant height and number of branches/plant of thyme plants [34].

Also, broad bean plants amended with a mixture of rock phosphate, sulphur, and organic manure exhibited the highest N, P and K contents than those obtained from adding each amendment separately [35].

Soil amendments of organic materials enhanced the NPK uptake by wheat plants. Also, the application of K and P solubilizing bacteria inoculants with humic substances maximizes the favorable effect on the growth and yield of canola plants than applying each inoculant separately [33].

In this regard, the combined soil inoculation with cultures of potassium solubilizing bacteria (KSB) and phosphate solubilizing bacteria (PSB) as well as adding humic acid showed greater growth and yield, and higher available phosphorus and potassium level in the soil compared to those of the control and sole inoculation treatment with KSB or PSB [36].

In general, the application of the complete treatment of humic substances plus PDB + KDB and (RP + KF) in this study recorded the highest average increases in the content and uptake of P and K by canola plants over the control treatment but these increases did not significantly differ from the NPK mineral fertilizer treatment. It means that the application of complete biological and organic treatment shows the same effect as that of NPK mineral fertilizers treatment. This finding coincides with those obtained in other study [5].

4. CONCLUSION

Potassium and phosphorus which are major essential macronutrients for plant growth and development are commonly added as fertilizers to optimize yield.

Phosphate dissolving bacteria (PSB) have a phosphate dissolution ability to convert the insoluble rock P material into a soluble available forms for plant uptake. Also, potassium solubilizing bacteria (KSB) are able to solubilize K minerals through the production and excretion of organic acids. Humic acid also plays an important role in improving soil properties and enhancing the microbial activity.

These results demonstrated the synergistic effects of complete dual-inoculation of P and K solubilizing bacteria integrated with the direct application of P and K minerals as well as humic substances which they did not significantly differ from the application of P and K chemical fertilizers.

In conclusion, the direct additions of phosphorus and potassium mineral materials in a combination with phosphate and potassium dissolving bacteria as well as humic substances as an organic form to the sandy loam soil improve the estimated parameters including phosphorus and potassium availability in the soil and different growth parameters of canola plants and give a significantly positive effect than the control.

The combination of these amendments gave highly significant increases in yield and nutrient uptake of canola compared to their individual applications. The most effective treatment was (RP + KF) + (PDB + KDB) + humic substances which achieved the highest growth and yield parameters as well as nutrient uptakes by canola plants compared to the control.

Therefore, the rock phosphate and potassium feldspar combined with solubilizing bacteria are considered as promising alternative sustainable fertilizers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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