J. of Plant Production, Mansoura Univ.,Vol 12 (5):541 - 551, 2021

Journal of Plant Production

Journal homepage: www.jpp.mans.edu.eg
Available online at: www.jpp.journals.ekb.eg

The Role of New Transformants Phosphate Bio-Stimulates (PBS) Bacteria Inoculates on Growth, Yield and Quality of Kohlrabi Plants

Soltan, H. A. H. [1]*; S. A. M. Osman[2] and I. A. A. Tantawy[3]

[1]Central Laboratory of Organic Agriculture, Agriculture Research Center (ARC), Giza 12619, Egypt.
[2]Department of Genetics, Faculty of Agriculture, Minia University, El Minia, Eg-61519, Egypt
[3]Department of Horticulture, Faculty of Agriculture, Minia University, El Minia, Eg-61517, Egypt.

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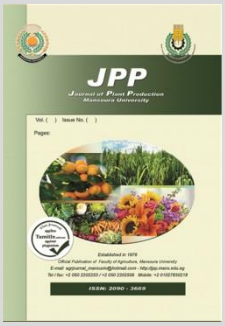

ABSTRACT

Producing new transformants phosphate bio-stimulate (PBS) bacterial inoculates in addition to study their potential effect on growth, yield and quality of kohlrabi plants at two successive seasons 2017/2018 and 2018/2019 were studied in this present work. The obtained results revealed that using the three tested phosphate bio-stimulate inoculates (PBS1, PBS2 and PBS3) alone or combined with different doses of phosphate mineral fertilizer PMF (calcium superphosphate) increase considerably growth, yield and quality of kohlrabi plants as compared to control. Among all tested PBS inoculates treatments, plants treated with PBS2 was superior and gave the best results in all studied traits when used alone or in combination with PMF doses at both seasons. Applying PBS (1,2 and 3) inoculates alone or combined with PMF doses improved the quality of kohlrabi knobs by increasing the values of total soluble solid TSS%, carbohydrates% and L-ascorbic acid% content in the same time, it decreased significantly the fiber% and nitrate content in knob as compared to the control in both seasons. So it could be recommended that to reduce the actual hazard of mineral fertilizer on soil and human health and to produce safe and healthy vegetable for local market and exportation it should be used PBS inoculates alone or combined with low PMF doses.

Keywords: Kohlrabi, Transformants phosphate, Mineral fertilizer hazards

INTRODUCTION

Kohlrabi (*Brassica oleracea* var. *gongylodes* L.) is a vegetable crop belongs to the family Brassicaceae. It is closely related to cabbage, broccoli, cauliflower and Brussels sprouts (Combs and Ernst, 2014). It has a short growing season and it can be grown for the early, summer and autumn market periods (Arin *et al.*, 2003). It has enlarged stem (knob) which can be eaten raw, cooked or preserved while, its young leaves can be used for salads or cooked like spinach (Liebster, 1991). According to Nagar, 2016 one hundred gram of their knob contains 92.7 g moisture, 1.1 g protein, 0.2g fat, 0.7g minerals, 1.5g fiber, 3.8g carbohydrates, 25 cal. Energy, 20mg calcium, 18mg magnesium, 10mg oxalic acid, 35mg phosphorus, 0.4mg iron, 0.12mg sodium,37mg potassium, 0.09mg copper, 143mg sulphur, 36 I.U. vitamin A, 0.12mg riboflavin, 0.5mg nicotinic acid, 0.05mg Thiamin, and 85mg Vitamin C as well as, it has various medicinal properties due to its rich in glucosinolates which known to have anti carcinogenic activity (Tjeertes , 2004 and Chauhan *et al.*, 2016).

The system of sustainable agriculture contains three major nutrients sources (chemical, organic and bio-fertilizers) which have various advantages such as, enhancing rapidly the availability of essential nutrients, improving the physical, chemical, and biological properties of the soil, fixing the atmospheric nitrogen and transforming the nutrients from unavailable to available forms (Singh *et al.*, 2019). Recently, there is great attention to use bio-fertilizer as a promising and alternative component in agriculture integrating nutrient supply system. Both bio and organic fertilizers may be present as good solution for decreasing pollution and high cost of mineral fertilizers (Salem *et al.*, 2010). Bio-fertilizers are those substances that contain living microorganisms and they colonize the rhizosphere of the plant and increase the supply or availability of primary nutrient and/or growth stimulus to the target crop (Ahmed, 2017). Phosphorus is one of the plant macronutrient which constitutes about 0.2 % of a plant's dry weight. It is play important role in synthesis of nucleic acids, phospholipids and ATPs as well as, involved in several plant functions such as: controlling enzyme reactions, regulation of metabolic pathways, energy transfer, photosynthesis, transformation of carbohydrates and oils formation (Marschner, 1995).

Phosphate Bio-Stimulates bacteria (PBS) are important microbes which release phosphorus from unavailable form to available form by colonizing the root surface of growing plant root. It is also promoting plant growth due to increasing nutrient uptake particularly phosphorus, zinc and other micro-nutrients, production of growth promoting substances and resistance to plant pathogen (Choudhary *et al.*, 2017).

Nowadays, there are great efforts paid for cultivation and production untraditional vegetables such as: kohlrabi, brussels sprouts, sweet fennel, broccoli and chinese cabbage to the Egyptian agriculture for the local consumption and export purposes. In this direction many researches were carried out on kohlrabi (Saber, 2011, Shams, 2012, Saleh *et al.*, 2013, El-Bassiony *et al.*, 2014, Osman, and salim, 2016, Abou El Magd, 2019 and Morsy,

*** Corresponding author.**
E-mail address: hassan_sultan@cloa.arc.gov.eg
DOI: 10.21608/jpp.2021.178930

2019). So, the aim of this investigation is to evaluate the promoting effect of three transformants of phosphate bio-stimulate bacterial inoculates on yield and quality of Kohlrabi plant.

MATERIALS AND METHODS

Laboratory experiments

Laboratory experiments were carried out at Microbial Genetics Laboratory, Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Egypt.

Stains

Two different bacterial strains *Bacillus megatherium var. phosphaticum* as a donor and *Azotobacter vinelandii* as a recipient were prepared in Microbial Genetics Lab., Dept., of Genetics, Faculty of Agriculture, Minia University.

Media

Complete medium were used for growing *Bacillus megatherium var. phosphaticum* (Xiufang *et al*., 2006) and Burk's medium were prepared according to (Dos Santos, 2011) for *Azotobacter vinelandii*.

DNA isolation

Total genomic DNA was extracted from donor isolate (*Bacillus megatherium var. phosphaticum*) according to protocol of GeneJET genomic DNA purification Kit [Mini] obtained from Thermo scientific. The additional requirements [96%~100% Ethanol, Sterile DNase -free pipette tips and micro-centrifuge tubes Epindorph, RNase (50mg/ml), freshly prepared Lysozyme buffer (20mg/ml lysozyme; 20 mM Tris-HCl, 2 mM EDTA pH 8.0 and 0.1% triton X-100] were used.

Antibiotic resistance

Antibiotic resistance properties is used to select the transformants *Azotobacter vinelandii* due to ampicillin resistance of Azotobacter and sensitive of Bacillus isolates in the same concentration of antibiotic.

Transformation procedure

According to Rafaat *et al*., (1974) DNA of donor strain was added to the recipient cultures with a final concentration 30mg/ml. Lithium acetate was also added (1ml 0.3 w/v) for culture (Ali and Hafez,1991). The mixture was then incubated at 30ºC for 4 hours. Transformants were examined by plating on CM of *Bacillus megatherium* supplemented with 50mg/ml Ampicillin and incubated at 30 ºC for 48 hours. The transformed Azotobacter strains were obtained using Page and Von Tigerstrom (1979) procedure.

Field experiments

Field experiments were carried out at private Farm, Talla village, Minia governorate, Egypt during the two successive seasons of 2017/2018 and 2018/2019.

Plant material

Purple kohlrabi (*Brassica oleracea* var. *gongylodes* L.) seeds cv. "AZUR STAR" were introduced from Tamar Organics, Carthamartha Farm, Rezare, Launceston, Cornwall, UK.

Inoculant strains

Three phosphate bio-stimulates (PBS) bacteria namely (PBS1, PBS2 and PBS3) were kindly provided by Genetics Department (Microbial Genetics Lab.), Fac., Agric., Minia Univ., Egypt.

Field experiments

Seeds of purple kohlrabi were sown in the 10th and 15nd of November in winter seasons of 2017/2018 and, 2018/2019 respectively. The soil texture is clay and the physical and chemical properties of the used soil are listed in Table (1).

Table 1. Physical and chemical properties of the used soil at 0-30 cm depth used during the two seasons of 2018/2019 and 2019/2020.

Constituents	Value	
	1st season	2nd season
Sand (%)	27.19	27.16
Silt (%)	24.56	25.02
Clay (%)	48.25	47.82
Soil type	Clay	Clay
Organic matter (%)	1.78	1.47
$CaCo_3$ (%)	7.71	4.57
pH(1:2.5)	7.54	7.56
E.C.(m mhos/cm)	3.08	2.90
Total N (mg/kg)	286.33	297.73
Total P (mg/kg)	51.66	48.13
Total K^+ (mg/g)	876.33	862.15
Available Ca^{++} (mg/100g)	32.71	31.80
Available Na^+ (mg/100g)	2.42	2.30
Available micronutrients (EDTA, ppm):		
Fe	7.52	7.22
Cu	2.15	2.02
Zn	1.99	1.87
Mn	8.44	8.39

The experiments were arranged in a Randomized Complete Block Design (split plot) with three replicates. Four levels (0, 25, 50, 75 and 100 kg/fed) of phosphate mineral fertilizer (PMF), and three treatments of transformants phosphate bio-stimulates (PBS) bacterial inoculants (PBS1, PBS2 and PBS3) and control (non-treated treatment) were tested. Therefore, the total of the tested treatments were 20 treatments. The plot area was 10.5 m^2 (3m in width x 3.5 m in length) included 4 ridges, 60 cm apart. Each treatment was separated by one guard row. Potassium treatments were in the form of potassium sulfate (48% K_2O), ammonium sulfate (20.6 % N) at the rate of 60 kg N/feddan and calcium superphosphate (16% P_2O_5) at the rate of 100 kg PMF/fed. The first dose of calcium superphosphate was applied during soil preparation while, the second dose was added with the first dose of N and K fertilizers after one month from planting, and the second dose of N and K fertilizers was added after two months from planting. Other agricultural practices were conducted according to the recommendations of the Egyptian Ministry of Agriculture, Agriculture Research Center (ARC). Regarding, PBS inoculates treatments; they have been applied as follow:

1. Control (without PMF and PBS).
2. PBS1 inoculant at 5 ml /plant.
3. PBS2 inoculant at 5 ml /plant.
4. PBS3 inoculant at 5 ml /plant.
5. 25 Kg/fed PMF.
6. 25 Kg/fed PMF + PBS1 inoculant at 5 ml /plant.
7. 25 Kg/fed PMF + PBS2 inoculant at 5 ml /plant.
8. 25 Kg/fed PMF + PBS3 inoculant at 5 ml /plant.
9. 50 Kg/fed PMF
10. 50 Kg/fed PMF + PBS1 inoculant at 5 ml /plant.

11. 50 Kg/fed PMF + PBS2 inoculant at 5 ml /plant.
12. 50 Kg/fed PMF + PBS3 inoculant at 5 ml /plant.
13. 75 Kg/fed PMF
14. 75 Kg/fed PMF + PBS1 inoculant at 5 ml /plant.
15. 75Kg/fed PMF + PBS2 inoculant at 5 ml /plant.
16. 75Kg/fed PMF + PBS3 inoculant at 5 ml /plant.
17. 100 Kg/fed PMF
18. 100 Kg/fed PMF + PBS1 inoculant at 5 ml /plant.
19. 100 Kg/fed PMF + PBS2 inoculant at 5 ml /plant.
20. 100 Kg/fed PMF + PBS3 inoculant at 5 ml /plant..

After three months from transplanting, samples of 10 plants from each plot were taken to determine N, P and K concentrations in knobs. Knob samples were separated, and oven dried at 70 ºC for 72 hours till constant weight, then fine grounded and wet digested.

N, P and K were determined in the plant digests according to AOAC (2000) as follows: N by micro-Keldahl, P by spectrophotometer (Jenway 6705 UV/Vis) using ammonium molybdate and ascorbic acid reagents and K by flame photometer (Jenway pfp7). Chlorophyll a, b and carotenoids in leaves were estimated according to methods of Metzner *et al.* (1965). At the harvesting time (120 days from transplanting), the total yield as ton/fed. was calculated. In the same time, samples of 10 plants from each experimental plot were taken to record vegetative growth parameters (plant height, number of leaves per plant and shoot fresh weight), yield components (root length, root diameter and root weight), Total Soluble Solids TSS (measured by hand Refractometer) content in root were determined according to methods mentioned by Umiel and Gabelmoii (1971). Nitrate content in knobs and fiber were determined according to the method described by Cataldo *et al.* (1975) and AOAC (2000), respectively. TSS and L-ascorbic acid were determined according to AOAC (2000). Carbohydrates content was determined according to Merrill and Watt (1973).

Statistical analysis

The statistical analysis of variance of the obtained data was performed according to (Gomez and Gomez, 1984) due to the MSTAT-C microcomputer statistical program.

RESULTS AND DISCUSSION

1- Producing transformants PBS bacterial inoculates

Transformation procedures were conducted directly by inoculating recipient (*Azotobacter vinelandii)* by DNA of the donor bacteria (*Bacillus megatherium).* Numbers and percentages of transformed clones which exhibited appropriate antibiotic resistance after 5 days in incubation at 30ºC were presented in Table 2. Data showed that from 10^5 only 15 colony showed antibiotic resistance and introduced as transformant colonies with 0.015%. Moreover, the extracted DNA from donor cells (*Bacillus megatherium*) gave the ampicillin resistance ability to the recipient (*Azotobacter vinelandii*) in addition to, phosphate solubilizing character on the tested plates.

These results indicated that numbers of transformed *Azotobacter vinelandii* were obtained by mixing DNA of *Bacillus megatherium* at 30ºC for 4 hours incubation. This may be attributed to the competence of the recipient cells (Ali, *et al*., 1980, Nassif, 1992 and Dakhly *et al*., 1997).

Table 2. Number and percentage of *Azotobacter vinelandii* and *Bacillus megatherium var. phosphaticum* transformants.

donor	recipient	Count	transformants no.	transformants %
Bacillus megatherium	*Azotobacter vinelandii*	10^5	15	0. 015

1-Vegetative parameters

The potential effect of three new phosphate bio-stimulate PBS bacterial inoculates, phosphate mineral fertilizer PMF and their combinations on growth and yield parameters of kohlrabi plants were shown in Table 3 and 4.

Data showed that treatment with 100kg/fed PMF gave the highest value of plant height (PH) at both seasons (59.5 and 60.3cm, respectively) as compared with all other PMF doses and all PBS inoculants alone. Plants treated with PBS2 was superior than those treated with the other PBS inoculates(PBS1 and PBS3) in PH trait at both seasons (54.3 and 55.2cm ,respectively) and also exhibited insignificant decrease with 100kg/fed PMF treatment. Almost all PBS, PMF combinations gave considerable increase in PH trait as compared with using PMF or PBS alone one the same side, mixing the three PBS inoculates with the high dose of PMF (100kg/fed) gave the highest values of PH as compared with all other treatment at both seasons.

The obtained results showed that there was significant difference between all PMF treatments and control (zero PMF and PBS) in leaves numbers /plant (L.N.) at the two seasons. As shown in Table 3. Plant treated with the two inoculates PBS2 and PBS1 showed significant increase in L.N. trait in the both seasons (13.1, 12.8 and 13.3, 12.9, respectively) as compared with all PMF and control treatments. The best values of L.N. trait were obtained after treated plants with PBS2+PMF combinations at the two seasons as compared with all other tested treatments.

A knob diameter (K. Di.) was varied with treatments and growing seasons (Table 3). All treatments were significantly increased K.Di except the two treatments (25 Kg/fed PMF and PBS1) as compared with control during all of seasons. Meanwhile, the highest K.Di values were given by PBS2+ 100 Kg/fed PMF treatment in 1st and 2nd growing seasons (10,71 and 10.11, respectively) followed by PBS3+100 Kg/fed PMF that recorded (10,12 and 10.02) in 1st and 2nd growing seasons, respectively.

As shown in Table 3 plants treated with 75Kg/fed PMF and 100kg/fed PMF gave a high values in leaf area (L.A) as compared with all other PMF doses and all PBS treatments at the two seasons. The largest value of leaf area was obtained by PBS3+ 100 Kg/fed PMF (47.33 and 44.00 cm^2) in the 1st and 2nd seasons, respectively followed by PBS1+75 Kg/fed PMF treatments at both seasons (42.50

and 41.80, respectively) while, the narrowest leaf area was obtained by PBS3+25 Kg/fed PMF treatments (23.57 and 24.00cm^2) in the 1st and 2nd seasons, respectively. The obtained results in Table 4 indicated that plant treated with 100kg/fed PMF gave the best values of leaves fresh weight LFW, leaves dry weight LDW, knob fresh weight KFW and knob dry weight KDW at the 1st season (210.58, 54.13, 402.82 and 39.57g, respectively) and at 2nd season (233.28, 59.20, 417.12 and 39.18g, respectively) as compared with all PMF, PBS and control treatments.

Among all tested PBS inoculates treatments the PBS2 was superior to the others in all previous traits at both seasons. As general, using PBS+PMF combination treatments gave a considerable increase in all tested traits and were more efficient than using PBS or PMF alone. Regarding total knob yield (TKY) trait, it increased gradually by increasing PMF dose at both seasons. As well as, PBS2 treatment gave high values of TKY in both seasons (20.72 and 20.85 t.ha^{-1}) as compared with the other PBS treatments. All PBS + PMF combination treatments increase TKY at the both seasons and were superior to using PBS or PMF alone. PBS2+all PMF dose combinations treatments gave the best results of TKY at both seasons compared with all PBS+PMF combination treatments. The highest values of TKY were obtained after treatment with PBS2+100kg/fed PMF at both seasons (24.18 and 24.90 t.ha^{-1}, respectively) followed by PBS2+75 Kg/fed PMF which gave (23.20 and 23.67 t.ha^{-1}, respectively).

Our results revealed that using phosphate bio-stimulate bacterial inoculates PBS alone or combined with phosphate mineral fertilizer PMF increased vegetative growth characters and total yield of kohlrabi. These improvements might be due to increase the activity and number of useful soil microbial flora population by adding bio-fertilization (Zaki *et al*., 2019).

Table 3. Effect of three transformants phosphate bio-stimulate (PBS) inoculants, phosphate mineral fertilizer (PMF) treatments and their combinations on plant height (PH), leaves number (L.N.), knob diameter (K.Di.), and leaves area (L.A.) traits of kohlrabi at two successive seasons.

Treatment		P.H. (cm)	L. N.	K. Di. (cm)	L.A.(cm^2)
Zero PMF and PBS	1st season (2018/2019)	36.3	12.0	7.11	28.46
25 Kg/fed PMF		38.2	12.0	7.55	28.65
50 Kg/fed PMF		43.4	12.0	8.16	32.05
75 Kg/fed PMF		52.8	12.0	8.42	40.55
100 Kg/fed PMF		59.7	12.0	9.10	40.86
PBS1		49.6	12.8	7.35	35.17
PBS2		54.3	13.1	8.89	34.02
PBS3		47.2	11.5	8.71	34.15
PBS1+25 Kg/fed PMF		38.3	13.0	8.05	29.53
PBS1+50 Kg/fed PMF		42.7	12.9	8.79	37.67
PBS1+75 Kg/fed PMF		55.0	12.3	9.13	42.50
PBS1+100 Kg/fed PMF		63.7	13.0	9.33	35.10
PBS2+25 Kg/fed PMF		47.7	13.1	9.11	34.50
PBS2+50 Kg/fed PMF		57.0	12.8	9.38	30.00
PBS2+75 Kg/fed PMF		60.7	13.2	9.96	41.47
PBS2+100 Kg/fed PMF		67.0	13.5	10.71	38.17
PBS3+25 Kg/fed PMF		45.0	11.3	8.97	23.57
PBS3+50 Kg/fed PMF		52.3	12.0	9.48	29.20
PBS3+75 Kg/fed PMF		58.7	11.3	9.67	37.17
PBS3+100 Kg/fed PMF		61.0	11.7	10.12	47.33
Mean		51.53	12.13	8.90	35.01
Zero PMF and PBS	2nd season (2019/2020	37.5	12.0	7.19	29.50
25 Kg/fed PMF		41.2	12.0	7.53	28.60
50 Kg/fed PMF		47.5	12.0	8.36	33.40
75 Kg/fed PMF		54.5	12.0	8.51	39.40
100 Kg/fed PMF		60.3	12.0	9.19	40.80
PBS1		51.8	12.9	7.85	35.50
PBS2		55.2	13.3	8.91	40.20
PBS3		50.2	11.5	8.69	37.90
PBS1+25 Kg/fed PMF		42.3	12.7	8.45	30.30
PBS1+50 Kg/fed PMF		48.8	13.0	8.69	36.80
PBS1+75 Kg/fed PMF		55.0	12.3	9.16	41.80
PBS1+100 Kg/fed PMF		61.0	12.3	9.43	37.70
PBS2+25 Kg/fed PMF		49.2	13.3	9.22	34.20
PBS2+50 Kg/fed PMF		59.0	13.0	9.48	36.80
PBS2+75 Kg/fed PMF		64.7	13.3	9.86	41.40
PBS2+100 Kg/fed PMF		69.0	13.7	10.11	36.70
PBS3+25 Kg/fed PMF		47.0	12.0	8.95	24.00
PBS3+50 Kg/fed PMF		51.8	10.7	9.58	29.30
PBS3+75 Kg/fed PMF		56.2	12.3	9.77	41.10
PBS3+100 Kg/fed PMF		62.1	11.3	10.02	44.00
Mean		53.07	12.36	8.95	36.47
$LSD_{0.5}$		7.33	0.76	1.23	7.13

Table 4. Effect of three transformants phosphate bio-stimulate (PBS) inoculants, Phosphate Mineral Fertilizer (PMF) treatments and their combinations on leaves fresh weight (L.F.W), leaves dry weight (L.D.W.), Knob fresh weight (K.F.W), Knob dry weight (K.D.W.) and total knob yield (T.K.Y.) traits of kohlrabi at two successive seasons.

Treatment	Season	L.F.W. (g)	L.D.W. (g)	K.F.W. (g)	K.D.W. (g)	T.K.Y. (t ha^{-1})
Zero PMF and PBS	1st season (2018/2019)	142.24	36.56	225.30	22.33	11.80
25 Kg/fed PMF		155.33	39.52	287.50	28.24	15.05
50 Kg/fed PMF		173.25	44.53	305.95	30.05	16.02
75 Kg/fed PMF		186.75	48.10	375.66	36.91	19.67
100 Kg/fed PMF		210.58	54.13	402.82	39.57	21.09
PBS1		162.88	41.87	325.54	31.98	17.05
PBS2		191.85	49.21	395.63	38.86	20.72
PBS3		181.40	46.63	357.03	35.07	18.69
PBS1+25 Kg/fed PMF		168.00	43.18	340.99	33.49	17.85
PBS1+50 Kg/fed PMF		185.67	47.72	375.77	36.91	19.68
PBS1+75 Kg/fed PMF		193.00	49.61	405.47	39.83	21.23
PBS1+100 Kg/fed PMF		213.33	54.83	425.13	41.46	22.26
PBS2+25 Kg/fed PMF		178.67	45.92	394.43	38.74	20.65
PBS2+50 Kg/fed PMF		182.67	46.95	405.24	39.75	21.22
PBS2+75 Kg/fed PMF		203.67	52.35	443.18	43.53	23.20
PBS2+100 Kg/fed PMF		220.33	56.63	461.84	45.36	24.18
PBS3+25 Kg/fed PMF		162.33	42.72	378.45	37.17	19.82
PBS3+50 Kg/fed PMF		178.67	45.92	394.73	38.77	20.67
PBS3+75 Kg/fed PMF		193.33	49.69	400.22	39.31	20.96
PBS3+100 Kg/fed PMF		200.67	51.18	423.30	41.58	22.16
Mean		184.23	47.36	376.21	36.95	19.70
Zero PMF and PBS	2nd season (2019/2020)	144.52	37.96	235.23	22.40	12.32
25 Kg/fed PMF		165.13	43.17	276.57	25.98	14.48
50 Kg/fed PMF		179.21	48.07	315.25	29.73	16.51
75 Kg/fed PMF		193.14	50.73	385.33	36.28	20.18
100 Kg/fed PMF		233.28	59.20	417.12	39.18	21.84
PBS1		193.22	50.75	344.21	32.37	18.02
PBS2		201.15	52.83	398.13	37.32	20.85
PBS3		187.30	49.20	363.33	34.14	19.02
PBS1+25 Kg/fed PMF		178.01	46.76	351.19	32.99	18.39
PBS1+50 Kg/fed PMF		198.59	52.16	385.66	36.23	20.19
PBS1+75 Kg/fed PMF		200.00	52.53	425.54	39.98	22.28
PBS1+100 Kg/fed PMF		206.60	53.88	444.11	41.72	23.25
PBS2+25 Kg/fed PMF		189.62	49.81	403.48	37.91	21.13
PBS2+50 Kg/fed PMF		192.61	50.59	425.14	39.94	22.26
PBS2+75 Kg/fed PMF		213.69	56.13	452.1	42.48	23.67
PBS2+100 Kg/fed PMF		225.39	62.27	475.63	44.69	24.90
PBS3+25 Kg/fed PMF		189.33	49.73	381.15	35.81	19.96
PBS3+50 Kg/fed PMF		172.51	45.31	400.33	37.61	20.96
PBS3+75 Kg/fed PMF		199.21	52.33	414.92	38.98	21.73
PBS3+100 Kg/fed PMF		205.13	54.27	419.37	39.40	21.96
Mean		193.38	50.88	385.69	36.26	20.19
$LSD_{0.5}$		29.87	11.76	87.99	11.09	4.53

Inoculation the soil with phosphate dissolving bacteria (PDB) decreases the rhizosphere pH through production of acids in soil and consequently favoring the solubility of calcium phosphates and micronutrients (Follett *et al*., 1981) which effect on the proliferation and elongation of meristematic cells, improving nutrient absorption (Joo *et al*., 2005) as reflected in knob diameter. It can also cause a positive effect on growth and yield characters due to increase the nutrients uptake particularly phosphorus, zinc and other micro-nutrients, production of growth promoting substances (enzymes and phytohormones) and resistance to plant pathogen (Choudhary *et al.,* 2017). In addition, these bio-fertilizers are organic in origin and thus, are absolutely safe. Our results are in accordance with the findings of Zaki *et al*., 2019 on sweet fennel, Uddin *et al*., 2009, El-Bassiony *et al*., 2014, Osman and Salim, 2016, Choudhary *et al*., 2017 and Morsy, 2019 on Kohlrabi, Nassal, *et al*. (2018) on tomato , Shams El-Deen, *et al*., (2020) on wheat and Ahmed, (2017) on Celery and Dill.

2. Photosynthesis pigments on leaves of kohlrabi

The effect of different PBS bacterial inoculants and PMF doses and their combinations on photosynthesis pigments (total chlorophyll a, b, and carotenoids) contents in leaves of kohlrabi were shown in Table (5).

Data showed that among all PMF treatments, only 100kg/fed PMF and 75kg/fed PMF gave high values of total chl a, b and carotenoids content in leaves at 1st and 2nd seasons as compared to the other PMF, PBS and control treatments.

Generally, using PBS, PMF combination treatments were more efficient than those of PBS or PMF alone due to the results of total chl a, b and carotenoids content at the both tested seasons. The best results of total chl a, b and carotenoids have been obtained from plants treated with PBS2+ PMF combinations at both seasons followed by those treated with PBS1+ PMF combinations.

Table 5. Effect of three transformants phosphate bio-stimulate (PBS) inoculants, phosphate mineral fertilizer (PMF) and their combinations treatments on total chlorophylls a, b and carotenoids in leaves of kohlrabi at two successive seasons.

Treatment	Season	T. Chl. A in leaves mg/g of FW	T. Chl. b in leaves mg/g of FW	T. Carotenoids in leaves mg/g of FW
Zero PMF and PBS	1st season (2018/2019)	0.331	0.102	0.128
25 Kg/fed PMF		0.445	0.116	0.131
50 Kg/fed PMF		0.405	0.222	0.140
75 Kg/fed PMF		0.603	0.549	0.149
100 Kg/fed PMF		0.658	0.695	0.151
PBS1		0.552	0.407	0.162
PBS2		0.592	0.480	0.172
PBS3		0.454	0.331	0.146
PBS1+25 Kg/fed PMF		0.429	0.189	0.150
PBS1+50 Kg/fed PMF		0.563	0.283	0.153
PBS1+75 Kg/fed PMF		0.611	0.620	0.170
PBS1+100 Kg/fed PMF		0.675	0.755	0.181
PBS2+25 Kg/fed PMF		0.571	0.128	0.159
PBS2+50 Kg/fed PMF		0.575	0.580	0.165
PBS2+75 Kg/fed PMF		0.653	0.772	0.175
PBS2+100 Kg/fed PMF		0.690	0.789	0.184
PBS3+25 Kg/fed PMF		0.333	0.089	0.135
PBS3+50 Kg/fed PMF		0.392	0.141	0.138
PBS3+75 Kg/fed PMF		0.570	0.641	0.153
PBS3+100 Kg/fed PMF		0.658	0.710	0.165
Mean		0.538	0.430	0.155
Zero PMF and PBS	2nd season (2019/2020)	0.366	0.110	0.138
25 Kg/fed PMF		0.460	0.180	0.145
50 Kg/fed PMF		0.408	0.230	0.146
75 Kg/fed PMF		0.600	0.553	0.156
100 Kg/fed PMF		0.660	0.700	0.157
PBS1		0.554	0.404	0.164
PBS2		0.594	0.485	0.174
PBS3		0.459	0.337	0.149
PBS1+25 Kg/fed PMF		0.431	0.130	0.152
PBS1+50 Kg/fed PMF		0.568	0.190	0.156
PBS1+75 Kg/fed PMF		0.618	0.627	0.172
PBS1+100 Kg/fed PMF		0.678	0.772	0.183
PBS2+25 Kg/fed PMF		0.577	0.287	0.163
PBS2+50 Kg/fed PMF		0.588	0.588	0.170
PBS2+75 Kg/fed PMF		0.661	0.759	0.181
PBS2+100 Kg/fed PMF		0.694	0.792	0.186
PBS3+25 Kg/fed PMF		0.335	0.090	0.140
PBS3+50 Kg/fed PMF		0.397	0.145	0.141
PBS3+75 Kg/fed PMF		0.574	0.646	0.155
PBS3+100 Kg/fed PMF		0.662	0.719	0.167
Mean		0.544	0.437	0.160
$LSD_{0.5}$		0.215	0.133	0.004

The highest values of total chl a, b and carotenoids were obtained by PBS2+100Kg/fed PMF (0.690, 0.789 and 0.184 mg/g of FW, respectively in 1st season and 0.694, 0792 and 0.186 mg/g of FW, respectively in 2nd season), while the lowest values were obtained from Zero PMF and PBS treatment (0.311, 0.102 and 0.128 mg/g of FW, respectively in 1st season and 0.366, 0.110 and 0.138 mg/g of FW, respectively in 2nd season, Table 5).

Increasing of photosynthetic pigments content in leaves may be due to increase maentration in plant tissues (Opera and Asigebu 1996) and induction of chlorophyll related enzymes and this leads to increase in the photosynthesis and improved growth (Kang *et al.* 2014). The previous results in agreement, more or less, with these of Rashed (2002) on parsley, Marulanda-Aguirre *et al.* (2008) on lettuce and Mohammad *et al*. (2012) on *Pimpinella anisum*.

3- N, P and K contents in kohlrabi knob.

The effect of different PBS bacterial inoculants and KMF treatments and their combinations on N, P and K contents in leaves of kohlrabi were shown in Table (6).

Among all PMF treatments, the 100 Kg/fed PMF dose gave the highest values of N, P and K (2.44, 0.35 and 1.94%, respectively) at 1st season and (2.46, 0.36 and 1.99%, respectively) at 2nd season as compared with all other PMF doses. On the other side, the PBS2 inoculant was superior to all other PBS inoculants for increasing the percentage of N, B and K contents in leaves at both seasons. All PBS+ low PMF doses treatments (25 and 50 Kg/fed) exhibited considerable increase of N, P and K contents as compared to PMF dose at both seasons.

Plants treated with PSB2+100 Kg/fed PMF gave the highest values of N, P and K (2.62, 0.35 and 2.15%, respectively) at 1st season and (2.62, 0.36 and 2.17%, respectively) at 2nd season as compared with all other treatments.

Using PBS inoculates alone or combined with low doses of PMF (25 and 50kg/fed) increase the N, P and K content due to the quick and readily availability of major nutrients to plants at earlier stages of plant growth (Angadi *et al*., 2017). These finding are in harmony with Manjarrez *et al*., 2000 and Srivastava *et al*., 2002 whose reported that application of biofertilizer caused significant increases in the population of beneficial microorganism such as mycorrhizal fungi and phosphate dissolving bacteria and fungi in the soil which increased the availability of nutrients such as; N, P, K, Mg required for the plants.

Table 6. Effect of three transformants phosphate bio-stimulate (PBS) inoculants, phosphate mineral fertilizer (PMF) and their combinations treatments on N, P and K contents in knob of kohlrabi at two successive seasons.

Treatment		N%	P%	K %
Zero PMF and PBS	1st season (2018/2019)	2.01	0.25	1.93
25 Kg/fed PMF		2.18	0.27	1.94
50 Kg/fed PMF		2.21	0.28	1.93
75 Kg/fed PMF		2.31	0.33	1.94
100 Kg/fed PMF		2.44	0.35	1.95
PBS1		2.24	0.26	1.93
PBS2		2.55	0.29	1.98
PBS3		2.21	0.26	1.97
PBS1+25 Kg/fed PMF		2.33	0.26	1.96
PBS1+50 Kg/fed PMF		2.32	0.27	1.95
PBS1+75 Kg/fed PMF		2.47	0.29	1.99
PBS1+100 Kg/fed PMF		2.52	0.31	1.97
PBS2+25 Kg/fed PMF		2.50	0.28	2.02
PBS2+50 Kg/fed PMF		2.57	0.28	2.05
PBS2+75 Kg/fed PMF		2.60	0.33	2.12
PBS2+100 Kg/fed PMF		2.62	0.35	2.15
PBS3+25 Kg/fed PMF		2.28	0.28	1.98
PBS3+50 Kg/fed PMF		2.30	0.27	1.97
PBS3+75 Kg/fed PMF		2.39	0.29	1.98
PBS3+100 Kg/fed PMF		2.47	0.31	1.97
Mean		2.38	0.29	1.98
Zero PMF and PBS	2nd season (2019/2020)	2.03	0.27	1.95
25 Kg/fed PMF		2.19	0.28	1.97
50 Kg/fed PMF		2.23	0.29	1.95
75 Kg/fed PMF		2.32	0.35	1.97
100 Kg/fed PMF		2.43	0.36	1.99
PBS1		2.27	0.27	1.95
PBS2		2.57	0.31	1.98
PBS3		2.23	0.28	1.96
PBS1+25 Kg/fed PMF		2.34	0.25	1.95
PBS1+50 Kg/fed PMF		2.35	0.27	1.96
PBS1+75 Kg/fed PMF		2.46	0.27	1.98
PBS1+100 Kg/fed PMF		2.53	0.32	1.98
PBS2+25 Kg/fed PMF		2.52	0.27	2.03
PBS2+50 Kg/fed PMF		2.56	0.29	2.05
PBS2+75 Kg/fed PMF		2.61	0.34	2.13
PBS2+100 Kg/fed PMF		2.62	0.36	2.17
PBS3+25 Kg/fed PMF		2.27	0.28	1.97
PBS3+50 Kg/fed PMF		2.32	0.28	1.98
PBS3+75 Kg/fed PMF		2.38	0.31	1.98
PBS3+100 Kg/fed PMF		2.45	0.32	1.96
Mean		2.38	0.30	1.99
$LSD_{0.5}$		0.19	0.05	0.05

4- Quality and nitrate accumulation of kohlrabi knobs

The effect of different transformants phosphate bio-stimulate (PBS) inoculants, phosphate mineral fertilizer (PMF) and their combinations treatments on knob chemical characteristics, data in Table (7) revealed that plants treated with PBS inoculants gave high values of TSS% as compared to all PMF treatments at two seasons. The PBS2 treatment gave the highest value of TSS% as compared with other PBS inoculants (PSB1 and PBS3) at two seasons (8.10 and 7.95, respectively). Plants treated with PBS+PMF combinations showed a clear increase in TSS% as compared with the 100 Kg/fed PMF at two seasons. The highest values of TSS% were found in plant treated with PBS2+100 Kg/fed PMF in the 1st and 2nd seasons (8.59 and 8.47, respectively).

Concerning, carbohydrates contents in knob, considerable increase were found in the plants treated with different doses of PMF particularly 100 and 75 Kg/fed (7.79 and 6.55%, respectively) in 1st season and (7.15 and 6.81%, respectively) in the 2nd season. There were non-significant difference between PBS2 and high dose of PMF treatments at the both seasons (6.21 and 6.64 %, respectively). Almost all of PBS+PMF combination treatments were better than those of PBS or PMF alone treatments in the percentages of carbohydrate content at both seasons.

Data showed that plant treated with PBS inoculates reduce significantly nitrate content and fibers in kohlrabi knob in both seasons as compared to all PMF and control treatments on contrast, there were significant increase in ascorbic acid content in knob treated with PBS inoculates at both tested seasons. All PBS+PMF combination treatments gave considerable increase in ascorbic acid content at the both seasons as compared to PMF doses treatments. The highest values of nitrate content were found in knob treated with 100 Kg/fed PMF (49.13 and 49.73 mg 100 g^{-1} DW) at the both seasons, respectively while, the lowest values were obtained by PBS3 treatment at the two seasons (42.31 and 42.63 mg 100 g^{-1} DW, respectively). As mentioned in Table 7, using PBS+PMF combinations reduced the nitrate content in knobs at both seasons as compared to apply high doses of PMF.

Table 7. Effect of three transformants phosphate bio-stimulate (PBS) inoculants, phosphate mineral fertilizer (PMF) and their combinations treatments on knob quality of kohlrabi at two successive seasons.

Treatment	Season	TSS %	Carbohydrates (%)	Fiber (%)	L-ascorbic acid (mg 100 g^{-1} F.W)	Nitrate content (mg 100 g^{-1} DW)
Zero PMF and PBS	1st season (2018/2019)	6.12	5.98	1.61	45.13	45.55
25 Kg/fed PMF		6.04	5.55	1.69	44.58	47.29
50 Kg/fed PMF		5.64	6.13	1.75	46.13	48.86
75 Kg/fed PMF		6.73	6.55	1.85	52.01	48.55
100 Kg/fed PMF		6.34	6.79	1.91	54.11	49.13
PBS1		7.20	5.66	1.53	56.82	44.11
PBS2		8.10	6.21	1.59	64.22	43.58
PBS3		7.52	5.48	1.41	59.13	42.31
PBS1+25 Kg/fed PMF		6.92	6.27	1.45	59.33	44.35
PBS1+50 Kg/fed PMF		7.31	6.66	1.63	61.21	45.22
PBS1+75 Kg/fed PMF		7.64	6.45	1.69	57.12	45.75
PBS1+100 Kg/fed PMF		7.40	6.92	1.75	55.33	46.12
PBS2+25 Kg/fed PMF		7.52	6.76	1.68	62.10	44.20
PBS2+50 Kg/fed PMF		7.58	6.81	1.75	65.12	44.55
PBS2+75 Kg/fed PMF		8.27	7.01	1.85	61.78	45.89
PBS2+100 Kg/fed PMF		8.59	7.25	1.87	60.18	46.01
PBS3+25 Kg/fed PMF		6.92	6.32	1.71	60.71	44.55
PBS3+50 Kg/fed PMF		7.36	6.49	1.68	62.58	44.71
PBS3+75 Kg/fed PMF		7.65	6.85	1.79	61.88	45.30
PBS3+100 Kg/fed PMF		7.59	6.69	1.83	62.24	45.89
Mean		7.19	6.43	1.63	57.54	45.60
Zero PMF and PBS	2nd season (2019/2020)	6.36	6.02	1.59	46.73	46.15
25 Kg/fed PMF		6.23	5.75	1.71	44.18	47.55
50 Kg/fed PMF		6.68	6.55	1.77	45.44	48.75
75 Kg/fed PMF		6.75	6.81	1.87	51.11	48.95
100 Kg/fed PMF		6.84	7.15	1.95	55.43	49.73
PBS1		7.19	5.78	1.51	58.95	44.32
PBS2		7.95	6.64	1.53	62.11	43.88
PBS3		7.66	5.88	1.47	60.12	42.63
PBS1+25 Kg/fed PMF		7.30	6.53	1.56	58.51	44.75
PBS1+50 Kg/fed PMF		7.32	6.68	1.67	62.33	45.33
PBS1+75 Kg/fed PMF		7.86	6.79	1.73	59.13	45.19
PBS1+100 Kg/fed PMF		7.78	7.10	1.72	57.08	46.53
PBS2+25 Kg/fed PMF		7.86	6.87	1.83	61.23	45.36
PBS2+50 Kg/fed PMF		7.66	7.14	1.78	67.18	45.77
PBS2+75 Kg/fed PMF		8.18	7.55	1.87	65.88	45.95
PBS2+100 Kg/fed PMF		8.47	7.40	1.92	61.29	46.75
PBS3+25 Kg/fed PMF		6.92	6.48	1.76	61.17	44.15
PBS3+50 Kg/fed PMF		7.25	6.64	1.72	61.58	44.66
PBS3+75 Kg/fed PMF		7.71	6.76	1.86	62.81	45.66
PBS3+100 Kg/fed PMF		7.69	6.98	1.89	63.42	46.09
Mean		7.31	6.66	1.70	58.28	45.91
$LSD_{0.5}$		0.93	0.63	0.18	5.69	1.89

Applying PBS (1,2 and 3) inoculates alone or combined with PMF doses improved the quality of kohlrabi knobs by increasing the values of total soluble solid TSS%, carbohydrates% and L-ascorbic acid% content in the same time, it decreased significantly the fiber% and nitrate content in knob as compared to the control in both seasons.. The increment of carbohydrates% might be related to increase the root surface area per unit of soil, water-use efficiency, and photosynthetic activity, which directly affects the physiological processes and utilization of carbohydrates (Elsayed *et al*., 2020). These results are in harmony with those reported by of El-Shafie *et al*. (2010), Hellal *et al*. (2011) El-Gohary *et al*., (2013) and Aly *et al*.(2015) indicated that applying bio-fertilizers treatments alone or combined with chemical fertilizer increased significantly carbohydrates content. The reduction of nitrate contents in plants treated bio-fertilizer were due to the slow release of nutrients (nitrogen form) in soil organic matter which are slower than that in mineral fertilizers which were adequate to meet the requirements of plants (Elsayed *et al*., 2020). Our finding matched well with those of Shaimaa (2010) on celery plants, Ghazal and Shahhat (2012) on fennel, Zeinab *et al*. (2015) on sweet fennel, and Massoud *et al*. (2019) on parsley.

CONCLUSION

Generally, the obtained data revealed that treatment of purple kohlrabi (*Brassica oleracea* var. *gongylodes* L.) cv. "AZUR STAR" plants by phosphate bio-stimulate bacterial inoculates (PBS1, PBS2 and PBS3) alone or combined with different doses (25, 50, 75 and 100kg/feddan) of phosphate mineral fertilizer PMF (calcium superphosphate) increased significantly vegetative growth traits and total yield as well as, improving knob quality of kohlrabi at both tested seasons. The best results were found in plants treated with PBS2 inoculate alone or combined PMF. Using PBS inoculates alone or combined with low PMF doses (25 and 50 kg/feddan) decrease nitrate accumulation in knobs. So it could be recommended that to reduce the actual hazard of mineral fertilizer on soil and human health and to produce safe and healthy vegetable for local market and exportation it should be used PBS inoculates alone or combined with low PMF doses.

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دور اللقاحات البكتيرية الميسرة للفسفور والمحولة وراثيا على نمو وإنتاجية وجودة نباتات الكرنب أبو ركبه.

حسن أحمد حسن سلطان[1]، سيد عبد المقصود عثمان[2] و أسماعيل أبو بكر طنطاوى[3]

[1]المعمل المركزي للزراعة العضوية - مركز البحوث الزراعية - الجيزة - مصر - رمز بريدي 12619

[2]قسم الوراثة - كلية الزراعة - جامعة المنيا - المنيا - مصر - رمز بريدي 61519

[3]قسم البساتين - كلية الزراعة - جامعة المنيا - المنيا - مصر - رمز بريدي 61519

أجريت هذه الدراسة لإنتاج لقاحات جديدة من البكتريا الميسرة للفوسفور(PBS) والمحولة وراثيا بالإضافة إلى دراسة تأثيرها المحتمل على نمو وإنتاج وجودة نباتات الكرنب أبو ركبة خلال موسمين متتاليين 2017/2018 و 2018/2019. وقد أظهرت النتائج ان استخدام الثلاثة لقاحات من البكتيرية الميسرة للفسفور (PBS1 و PBS2 و PBS3) وحدها أو مخلوطة مع الجرعات المختلفة من السماد المعدني الفسفاتي PMF (سوبر فوسفات الكالسيوم) يزيد بشكل كبير من نمو وإنتاجية وجودة نباتات الكرنب أبو ركبه . من بين جميع الملقحات المختبرة ، كانت النباتات المعاملة بـ PBS2 متفوقة وأعطت أفضل النتائج في جميع الصفات المدروسة سواء استخدمت بمفردها أو بعد خلطها مع جرعات التسميد المعدني PMF في كلا الموسمين. معاملة النباتات باللقاحات البكتيرية بصورة مفردة او بعد خلطها بجرعات من السماد الفسفوري المعدني PMF عملت على تحسين جودة الساق المتضخمة knobs لنباتات الكرنب ابو ركبة عن طريق زيادة قيم إجمالي نسبة المواد الصلبة الذائبة TSS٪ والكربوهيدرات٪ ومحتوى حامض الاسكربيك L-ascorbic acid٪ و في نفس الوقت ، انخفض بشكل ملحوظ نسبة الألياف ومحتوى النترات في الساق المتضخمة knobs مقارنة بالكنترول في كلا الموسمين. لذلك يمكن التوصية بأنه لتقليل المخاطر الفعلية للأسمدة المعدنية على التربة وصحة الإنسان ولإنتاج خضروات آمنة وصحية للسوق المحلي والتصدير ، يجب استخدام PBS لتلقيحها بمفردها أو مع جرعات منخفضة من PMF.