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RESPONSE OF SWEET PEPPER GROWN ON RICE STRAW SUBSTRATE TO APPLICATION OF ROCK PHOSPHATE AND PHOSPHATE SOLUBILIZING BACTERIA

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ABSTRACT: *Plastic house experiment was conducted during 2013/2014 and 2014/2015 seasons, at the experimental site of Central Laboratory for Agricultural Climate (CLAC), Agriculture Research Center, Giza, Egypt. This work aims to investigate the effect of application of rock phosphate and phosphate solubilizing bacteria on growth and yield of sweet pepper (Slevit F_1 Hybrid) grown on rice straw substrate. Phosphate solubilizing bacteria were applied as mixture of Bacillus polymyxa and Bacillus megaterium (1:1) for once, twice or thrice times (after 1; 1 and 3 or 1, 3 and 5 weeks from transplanting respectively). The control treatment received mineral phosphate fertilizer (calcium super phosphate) without phosphate solubilizing bacteria. The results clearly showed that the highest values of all vegetative characteristics, mineral percent (NPK) and yield of pepper were recorded with rock phosphate + inoculation of phosphate solubilizing bacteria for twice or thrice times treatments. The lowest values of all parameter were resulted by using rock phosphate without phosphate solubilizing bacteria. There were not significant differences between rock phosphate with inoculation of phosphate solubilizing bacteria for once and control treatment. This work showed that applying rock phosphate with phosphate solubilizing bacteria produced sweet pepper plants superior in growth and yield comparing with those fertilized by calcium super phosphate fertilizer, as well as provides an environmentally friendly method to manage rice straw as agriculture substrate instead of burning.*

Key words: *Rice straw, Rock phosphate, Phosphate solubilizing bacteria, Pepper*

INTRODUCTION

Sweet pepper (*Capsicum annuum L*) is a member of the solanaceous vegetables group. It is one of the most important, popular and favorite vegetable crops cultivated in Egypt for local consumption and exportation. The cultivated area from sweet and hot pepper for all seasons was about 95 thousand feddans, produced about 651 thousand tons on annual basis with an average of 6.8 tons/feddan (Ministry of Agriculture and Land Reclamation, 2013).

The problem of agricultural wastes in Egypt became very obvious especially after the harvest of summer crops. Egyptian farmers get rid of these wastes by burning. Burning not only is considered an economic loss but also has harmful effects on the environment, i.e. emission of poison gases to the air and reducing the microbial activities in the soil. Therefore, utilization of agriculture wastes in any other environmentally friendly way is very important (Abou Hussein and Sawan, 2010). one of these methods are growing vegetables on rice straw as substrate.

Rice straw could be used as a growing media for cultivation of vegetable crops instead of soil. Also, pepper grown on rice straw bales showed better growth and increased fruit number and weight compared with those grown in natural soil. Besides, the pH values around the roots in straw bales ranged from 5.5 to 6.5. So, sowing on rice straw can solve the conditions of alkalinity and salinity in the rhizosphere (El-Marzoky and Abdel-Sattar, 2008).

Phosphorus (P) is one of the major plant growth-limiting nutrients, Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch and transporting of the genetic traits (El-Gizawy and Mehasen, 2009). It is usually supplied to the plant in many different forms some of which are manufactured, i.e., phosphoric acid and calcium super phosphate, while some others are common in nature form such as rock phosphate. The appropriate utilization of rock phosphate as P source can contribute to sustainable agricultural intensification, particularly in developing countries endowed with rock phosphate resources, in addition, minimizing environmental pollution in countries where rock phosphate are processed industrially. The rock phosphate products are an economically sound alternative P input to manufactured superphosphates (Zapata and Roy, 2004; Schneider *et al.,* 2010).

The phosphate solubilizing bacteria increased P availability and other nutrients in rhizosphere zone, thus inoculated plants were able to absorb nutrients from solution at faster rates than uninoculated plants resulting in accumulation of more N, P and K in the leaves (El-Tantawy and Mohamed, 2009; Premsekhar and Rajashree 2009).

Inoculation with phosphate solubilizing microorganisms along with rock phosphate can substitute the chemical fertilizer in alkaline soil and help in improving the crop production (Khan *et al.,* 2009; Singh and Reddy, 2011). Some bacteria such as *Bacillus polymyxa* and *Bacillus megaterium* provide plants with growth promoting substances and play major role in phosphate solubilizing (Abou-Aly *et al.,* 2006; Rai, 2006; Saharan and Nehra, 2011). Application of rock phosphate with phosphate solubilizing bacteria had beneficial effects on dehydrogenase, phosphatase activities and vegetative characters, as well as improved the content of photosynthetic pigments, nutrients and carbohydrates which eventually must have been reflected on the yield (Abou El-Yazeid and Abou-Aly, 2011).

The present work aimed to overcome problems of rice straw residual and provide a friendly environment alternative for synthetic phosphorus fertilizers, by studying the response of sweet pepper plants grown on rice straw substrate to inoculations of phosphate solubilizing bacteria in presence of rock phosphate.

MATERIALS AND METHODS

Experiment location

The experiment was carried out into plastic house during the two growing seasons of 2013/2014 and 2014/2015 at Central Laboratory for Agricultural Climate (CLAC), Agriculture Research Center, Egypt, to investigate the effect of phosphate solubilizing bacteria in presence of rock phosphate on growth and yield of sweet pepper grown in rice straw substrate.

Plant material

Sweet pepper (*Capsicum annuum L.*) plants (Slevit F_1 Hybrid) were transplanted in the horizontal polyethylene bags (25 cm wide x 60 cm length x 20 cm height) on 11 and 3 of September in the first and second seasons, respectively.

Methods

The trial was conducted in rice straw as substrate into bags, each bag included 25 liter (1500 g) of rice straw in combination with 200 g compost. Two plants were planted in each bag, the space between plants was 30 cm, which irrigated by drip irrigation system, emitter discharge rate was 4 l/hr. The main chemical analyses of rice straw and compost are shown in Table (1) and (2) respectively.

Table 1: Chemical composition of rice straw

pH 1:5	C/N ratio	DM	Ash	O.C	N	P	K
		%					
6.46	64.73	91.42	17.56	31.16	0.46	0.058	1.35

Table 2: Chemical analyses of compost

pH 1:5	EC 1:10 dS/m	O.M (%)	Macro elements (%)					Micro elements (ppm)			
			N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
7.81	4.62	30.58	1.12	0.82	1.05	0.76	0.33	2465	78	128	96

Preparation of treatments

Bacillus polymyxa and *Bacillus megaterium* as phosphate solubilizing bacteria (PSB) were kindly provided by the Microbiology Dept. Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The mixture of PSB (1:1) as liquid culture (1 ml contains 10^8 cell), were diluted by water without Chlorine at 1 : 20 and added to rice straw substrate in bags at a rate of 20 ml/plant according to Mashhoor *et al.* (2002). The inoculation by mixed PSB was applied for once, twice or thrice times (at 1; 1 and 3 or 1, 3 and 5 weeks after transplanting respectively).

Recommended dose of NPK as mineral fertilizers were applied according to Ministry of Agriculture and Land Reclamation (2009) as follow: 150 kg N/fed as 730 kg ammonium sulphate (20.5% N), 48 kg K_2O/fed as 100 kg potassium sulphate (48% K_2O) and 60 kg P_2O_5/fed as 267 kg rock phosphate (22.5% P_2O_5) or 387 kg calcium superphosphate (15.5 % P_2O_5) as control treatment. All quantities of Calcium superphosphate and rock phosphate were added to rice straw in bags once before transplanting. While, ammonium sulphate and potassium sulphate were added at four equal portions, before transplanting, then after 2, 4 and 6 weeks from transplanting.

Treatments

The Experimental Treatments were as follow:

1. Recommended dose of P_2O_5 as calcium super phosphate (Ca S) as a control.
2. Recommended dose of P_2O_5 as rock phosphate (RP).
3. Rock phosphate + inoculation of phosphate solubilizing bacteria for once after 1 week of transplanting (RP + PSB_1).
4. Rock phosphate + inoculation of phosphate solubilizing bacteria for twice after 1 and 3 weeks of transplanting (RP + PSB_2).
5. Rock phosphate + inoculation of phosphate solubilizing bacteria for thrice times after 1, 3 and 5 weeks of transplanting (RP + PSB_3).

Experiments design

The experiment was laid out in a completely randomized block design with three replicates for each treatment. Each replicate included 5 bags.

Measurements

After 60 days from transplanting, three plants per replicate were randomly chosen to measure plant height, number and area of leaves/plant. Chlorophyll reading in the fourth upper leaf was measured by using Minolta Chlorophyll Meter Spad 501. Total nitrogen, phosphorous and potassium percent were determined in the dry matter of fourth upper leaf according to Cottenie *et al.* (1982).

Total nitrogen was determined by Kjeldahl method according to the procedure described by FAO (1980). Phosphorus percent was determined using spectrophotometer according to Watanabe and Olsen (1965). Potassium percentage was determined spectrometrically using Phillips Unicum Atomic Absorption Spectrometer as described by Chapman and Pratt (1961). Fresh and dry shoot weight was measured at harvesting. Total yield, number of fruits per plant were recorded after each harvesting accumulatively, average of mature fruit weight was measured, as well.

Data of the two seasons were arranged and statistically analyzed by the analysis of variance using one way ANOVA according to Snedecor and Cochran (1980) with SAS package. Comparison of treatment means was done using Tukey test at significance level 0.05.

RESULTS AND DISCUSSION

Vegetative Characters

Vegetative characteristics of sweet pepper plants as affected by the different treatments are presented in Tables (3 and 4). Data showed that the highest values of all growth characteristics (fresh and dry shoot weight, plant height, number and area of leaves/plant) were obtained with rock phosphate + inoculation of phosphate solubilizing bacteria for twice or thrice times treatments compared to calcium super phosphate treatment only (control). On the contrary, the lowest values were resulted when rock phosphate was applied without phosphate solubilizing bacteria. While there were no significant differences between application of rock phosphate + phosphate solubilizing bacteria for once treatment and control treatment. These results were true in two seasons. The enhancing effect of phosphate solubilizing bacteria on growth may be due to the activity of P solubilization caused by the used strain and increased further mineral availability uptake as was described by Abou El-Yazeid and Abou-Aly (2011). These are in agreement with those obtained by Abou-Aly *et al.* (2006), Rai (2006) and Saharan & Nehra (2011) they reported that adding *B. polymyxa* and *B. megaterium* could solubilize phosphate and enhanced the plant growth promotion. Also, the increase in growth characters might be due to the fact that inoculated plants with phosphate solubilizing bacteria were able to absorb nutrients from soil solution at faster rates than uninoculated plants (El-Tantawy and Mohamed 2009; Premsekhar and Rajashree 2009).

The maximum reading of chlorophyll in pepper leaves was observed in plants treated with rock phosphate and inoculated by phosphate solubilizing bacteria for twice or thrice times in both seasons. This positive effect on chlorophyll may be due to that application of rock phosphate with phosphate solubilizing bacteria improved the content of photosynthetic pigments in the plant leaves as was suggested by Abou El-Yazeid and Abou-Aly (2011). Similar results were reported by Abou-Aly and Gomaa (2002) they stated that biofertilizers increased both nutrient content and leaf chlorophyll concentration than control.

Table 3: Effect of different treatments on fresh, dry weight and plant height of sweet pepper plants during 2013/2014 and 2014/2015 seasons.

Treatments	Fresh weight g/plant		Dry weight g/plant		Plant height cm	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Ca S (control)	467.47 b	492.23 b	101.63 b	105.50 b	109.50 bc	114.32 b
RP	444.80 c	465.07 c	96.37 c	98.67 c	105.00 c	107.95 c
RP + PSB_1	470.90 b	495.87 b	102.37 b	105.93 b	111.56 b	114.80 b
RP + PSB_2	518.30 a	549.10 a	113.03 a	117.30 a	118.67 a	123.89 a
RP + PSB_3	525.57 a	550.77 a	114.23 a	118.57 a	120.67 a	125.98 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

Ca S = Calcium superphosphate
RP = rock phosphate
PSB_1 = phosphate solubilizing bacteria once time
PSB_2 = phosphate solubilizing bacteria twice times

PSB_3= phosphate solubilizing bacteria thrice times

Table 4: Effect of different treatments on leaf No, leaf area and chlorophyll reading of sweet pepper plants during 2013/2014 and 2014/2015 seasons.

Treatments	Leaf No. / plant		Leaf area cm^2 / plant		Chlorophyll reading Spad	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Ca S (control)	193.33 b	198.75 b	10452 b	10931 b	52.00 b	54.96 b
RP	182.33 c	188.14 c	9441 c	9883 c	49.00 b	50.81 b
RP + PSB_1	195.00 b	199.79 b	10458 b	10938 b	52.33 b	55.32 b
RP + PSB_2	212.00 a	218.55 a	12474 a	12887 a	60.00 a	62.22 a
RP + PSB_3	214.67 a	220.62 a	12605 a	13021 a	60.67 a	62.91 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

Ca S = Calcium superphosphate
RP = rock phosphate
PSB_1 = phosphate solubilizing bacteria once time
PSB_2 = phosphate solubilizing bacteria twice times
PSB_3 = phosphate solubilizing bacteria thrice times

Micronutrients content

The nutritional status in pepper plants is shown in Table (5). The obtained results in both seasons showed that the highest concentrations of N, P and K were preceded by rock phosphate + inoculation with phosphate solubilizing bacteria for twice or thrice times treatments. The treatments of rock phosphate + inoculation of phosphate solubilizing bacteria for once and calcium super phosphate only came in the second order, whereas the application of rock phosphate without phosphate solubilizing bacteria gave the lowest concentrations.

These results are in harmony with those obtained by El-Tantawy and Mohamed (2009) and Singh and Reddy (2011). They reported that the phosphate solubilizing bacteria increased P availability and other nutrients, thus inoculated plants were able to absorb nutrients from solution at faster rates than uninoculated plants resulting in accumulation of more N, P and K in the leaves. These findings may be due to use rice straw as substrate, where the decomposition of this organic material improves the nutrient cycling and availability to the plants (Abdulla, 2007; Dai *et al.,* 2010).

Yield components

Data illustrated in Table (6) show that total yield, number of fruits per plant and fruit weight were significantly increased in response to use rock phosphate plus inoculation of phosphate solubilizing bacteria for twice or thrice times. These treatments gave the highest values of these parameters in the both seasons. On the other hand, application of rock phosphate without phosphate solubilizing bacteria produced the lowest values. Whereas, yield component values upon application of rock phosphate plus inoculation of phosphate solubilizing bacteria at once and calcium super phosphate without phosphate solubilizing bacteria treatments were moderate. In this respect, similar results were reported by El-Tantawy and Mohamed (2009), Premsekhar and Rajashree (2009) and Mohamed and Ibrahim (2011) on tomato. These increases may be attributed to beneficial effects of rock phosphate inoculated with phosphate solubilizing bacteria on dehydrogenase, phosphatase activities and vegetative characters, as well as improved the content of photosynthetic pigments, nutrients and carbohydrates as described by Abou El-Yazeid and Abou-Aly (2011). These positive effects were clearly reflected on the yield and yield components.

Table 5: Effect of different treatments on NPK percent of sweet pepper leaves during 2013/2014 and 2014/2015 seasons.

Treatments	N		P		K	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Ca S (control)	3.827 b	4.050 b	0.297 b	0.318 b	4.150 b	4.375 b
RP	3.383 c	3.580 c	0.183 c	0.196 c	3.617 c	3.830 c
RP + PSB_1	3.830 b	4.053 b	0.310 b	0.332 b	4.167 b	4.397 b
RP + PSB_2	4.050 a	4.193 ab	0.407 a	0.436 a	4.433 ab	4.590 ab
RP + PSB_3	4.123 a	4.227 a	0.420 a	0.450 a	4.483 a	4.653 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

Ca S = Calcium superphosphate
RP = rock phosphate
PSB_1 = phosphate solubilizing bacteria once time
PSB_2 = phosphate solubilizing bacteria twice times
PSB_3 = phosphate solubilizing bacteria thrice times

Table 6: Effect of different treatments on yield, fruit No. and fruit weight of sweet pepper plants during 2013/2014 and 2014/2015 seasons.

Treatments	Yield Kg/plant		Fruit No. /plant		Fruit weight g	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Ca S (control)	2.37 b	2.49 b	42.33 bc	44.23 bc	54.33 b	57.37 b
RP	1.88 c	2.09 c	37.67 c	38.96 c	50.33 c	52.25 c
RP + PSB_1	2.38 b	2.51 b	43.00 b	45.28 b	55.00 b	57.19 b
RP + PSB_2	2.76 a	2.88 a	48.00 a	50.65 a	60.63 a	62.15 a
RP + PSB_3	2.79 a	2.90 a	51.33 a	52.05 a	61.33 a	62.62 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

Ca S = Calcium superphosphate
RP = rock phosphate
PSB_1 = phosphate solubilizing bacteria once time
PSB_2 = phosphate solubilizing bacteria twice times
PSB_3 = phosphate solubilizing bacteria thrice times

CONCLUSIONS

It could be concluded that inoculating phosphate solubilizing bacteria for twice or thrice with application of rock phosphate as phosphate fertilizer under rice straw substrate culture, produce good growth and yield of sweet pepper plants. Generally phosphate solubilizing bacteria play an important role in plant nutrition and allow the use of phosphorus source is nature and cheaper such as rock phosphate instead of super phosphate. As well as, this work provides an environmentally friendly method to manage rice straw instead of burning.

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استجابة الفلفل الحلو النامى فى بيئة قش الارز لاضافة صخر الفوسفات والبكتريا الميسرة للفوسفات

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الملخص العربى

أجريت تجربة صوبة بلاستيكية خلال موسمى 2014/2013 و 2015/2014 بالموقع البحثى للمعمل المركزى للمناخ الزراعى – مركز البحوث الزراعة – الجيزة – جمهورية مصر العربية. هذا العمل يهدف لدراسة تأثير استخدام صخر الفوسفات والبكتريا الميسرة للفوسفات على نمو ومحصول الفلفل الحلو (هجين سلفيت) النامى فى بيئة قش الارز. استخدمت البكتريا الميسرة للفوسفات كمخلوط من الباسلس بوليمكسا والباسلس ميجاتيريم (1:1) لمرة واحدة ومرتين وثلاث مرات (بعد 1 و3 و5 اسابيع من الشتل). معاملة المقارنة فى هذه الدراسة عبارة عن التسميد بالفوسفات المعدنى (سوبر فوسفات الكالسيوم) مع عدم اضافة البكتريا الميسرة للفوسفات.

النتائج اظهرت بوضوح ان اعلى القيم فى كل الصفات الخضرية ونسبة العناصر المغذية ومحصول الفلفل سجلت مع معاملة صخر الفوسفات والتلقيح بالبكتريا الميسرة للفوسفات مرتين او ثلاث مرات. اقل القيم لكل الصفات المختبرة نتجت باستخدام صخر الفوسفات مع عدم التلقيح بالبكتريا الميسرة للفوسفات. لم يوجد اختلافات معنوية بين معاملة صخر الفوسفات مع التلقيح بالبكتريا الميسرة للفوسفات لمرة واحدة وبين معاملة المقارنة. اظهر

هذا العمل ان استخدام صخر الفوسفات مع التلقيح بالبكتريا الميسرة للفوسفات انتج نباتات فلفل حلو تفوقت فى النمو والمحصول على تلك المسمدة بالسوبر فوسفات الكالسيوم، كما تقدم طريقة صديقة للبيئة لادارة قش الارز كبيئة للزراعة بدلاً من الحرق.