USE OF *BACILLUS CIRCULANS* AS BIO-ACCELERATOR ENRICHING COMPOSTED AGRICULTURAL WASTES

II- Evaluation of produced compost under organic farming system

ABSTRACT

The quality of composted rice straw produced from the application of mixed culture of 4 effective *Bacillus circulans* strains as bioaccelerator plus animal manure as organic accelerator was evaluated. Assessment comprised a comparison of the effects of the composted rice straw with the recommended dose of chicken manure and the effects of applied rates (50, 100 and 200% of the recommended dose) of that compost on growth, yield and NPK contents of potato and broccoli grown under organic farming regulations. Data showed that application of the composted rice straw was always superior to that of chicken manure in enhancing total and specific microbial proliferation along with microbial activities in the rhizosphere of potato and broccoli, particularly after 60 days from cultivation. The superiority was also reflected on growth and yield parameters of both crops. The application of 200% of the recommended dose of the composted rice straw also gave more pronounced effects on all above-mentioned parameters compared with the recommended dose or 50% of that dose.

Key words: Organic farming, Composted rice straw, Chicken manure, Application rate, Potato, Broccoli.

INTRODUCTION

One of the major characteristics of Egyptian soils is the deficiency in organic matter content, being in the range of 0.2-1%. Organic matter amendment is also particularly important at the time being, due to the world movement toward natural resources.

At present, bio-organic agriculture is a concept that receiving greater consideration in various regions around the world. The importance of this technology is extended to be a potential solution to numerous problems facing present day agriculture. Accumulative research suggests that organic agriculture results in less erosion (**Reganold *et al.*, 1987**), less leaching of nutrients and higher carbon storage (**Drinkwater *et al.*, 1995**), promoting soil structure formation (**Pulleman *et al.*, 2003**), enhancing soil biodiversity (**Mäder *et al.*, 2002; Oehl *et al.*, 2004**), alleviating environmental stresses (**Horrigan *et al.*, 2002; Macilwain, 2004**).

Compost amendment is one of the major practices in bio-organic agriculture and is mainly responsible for most of the above-mentioned beneficial effects (**Giusquiani *et al.*, 1995; Pfotzer and Schu¨ ler, 1997; Bazzoffi *et al.*, 1998**). Since compost may be produced from different wastes it is very important to assess the quality of compost before its application in agriculture. Therefore, previous analyses of the material in relation to application of variable amendment demonstrate the quality of produced compost (**Masaguer *et al.*, 1999**).

This study, therefore, aimed to evaluate the effect of composted rice straw produced with the use of *B. circulans* plus animal manure as accelerating factors on growth, yield and NPK content of potato and broccoli grown under bio-organic farming system.

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MATERIAL AND METHODS

Bio-organically composted rice straw was selected **(Abd El-Fattah, 2006)** as an example for utilization of agricultural wastes in organic farming. Two field experiments were conducted at Bel-Hana (A-104 ECOA) organic farm situated at Dahshoer, Giza, during October 2004 and 2005 season to evaluate the effects of produced compost on growth, yield and chemical content of potato and broccoli. Soil analysis showed that it contained 0.099% total N, 0.070 % total K and 0.003% total P.

Plant materials

Tubers of potato (*Solanum tuberosum* L. cv. Diamont), along with the seed of broccoli (*Brassica oleracea* cv. hybrid) were used to evaluate the effect of bio-organically produced compost on their growth and yield.

Organic and raw fertilizers

Chicken Manure, was provided from the organic Farm under study, the chemical analysis of the used chicken manure showed that it contained 37.07% organic carbon, 4.56% total N, C/N ratio of 8.13:1, 0.10% total P and 1.95% Total K. Also Rock phosphate and feldspar were provided from Al-Ahram Company for Natural Products to be applied as phosphate and potassium fertilizers, respectively.

Experimental techniques

Evaluation of the effects of produced compost under organic farming system

A- Comparing the effects of produced compost with those of chicken manure.

This experiment comprised 3 treatments as fellows:

1- (Unamended control), i.e. cultivation without organic matter amendment but only the equivalent of recommended dose of rock phosphate (475Kg / fed) and feldspar (960Kg / fed).

2- Amendment with the recommended dose of bio-organically composted rice straw (2680Kg/ fed) plus the equivalent of recommended dose of rock phosphate and feldspar.

3- Amendment with the recommended dose of chicken manure (1589kg/ fed) plus the equivalent of recommended dose of rock phosphate and feldspar.

B- Evaluation of the effects of increased compost application rate

This experiment comprised 4 treatments as follows:

1- (Unamended control) i.e., cultivation without organic matter but only the equivalent of the recommended dose of rock phosphate and feldspar.

2- Amendment with the 50% of the recommended dose was applied (1340 kg/ fed) plus equivalent of the recommended dose of rock phosphate and feldspar.

3- Amendment with the recommended dose of bio-organically composted rice straw (2680kg/ fed) plus the equivalent to recommended dose of rock phosphate and feldspar.

4- Amendment with 200% of the recommended dose of compost (5360kg/ fed) plus the equivalent of the recommended dose of rock phosphate and feldspar.

Plant parameters

Shoot length (cm/ plant), stem diameter (mm/plant), number of leaves/plants, fresh and dry weight of shoot and root (g/plant) were recorded to determine dry weight, plants were dried by oven at 70°C until reaching a constant weight. The NPK content of potato tubers or fruit was recorded at harvested according to **Jackson (1973)**.

Densities of microbial populations in the Rhizosphere of tested plants

Total count of mesophilic bacteria, spore forming bacteria and K mobilizing bacteria were determined at 3 intervals (30, 60 and 90 days) from cultivation by plate count on soil extract agar medium **(Clark 1965)**, and modified Aleksandrov's medium **(Zahra, 1969)**, respectively with incubation at 30±2°C.

Rate of carbon dioxide evolution

The rate of CO_2 evolution was determined in the rhizosphere soil according to the methods described by **Promer and Schmid (1964)** and modified by **Shehata (1972).**

Statistical analysis:

The obtained data were statically analyzed according to **Snedecor and Cochran (1969)**. And the treatments were compared by using LSD at 0.05 levels probability.

RESULTS

Evaluation of the effects of produced compost under organic farming system

Comparative evaluation against chicken manure

Densities of microbial populations and activities in the rhizosphere

Total bacterial count and rates of CO_2 evolution:

Data plotted in Figure (1) obviously indicated the stimulatory response of total bacterial counts and rates of CO_2 evolution in the tested plants rhizosphere, due to compost or chicken manure compared with unamended control. Both parameters were considerably increased after 60 days of cultivation, and then sharply dropped. The maximum count were 23x 10^7, 26 x10^7 cfu/ g/ dry rhizosphere soil of potato and 29.8x10^7, 29x 10^7 cfu/ g/ dry rhizosphere, soil of broccoli amended with compost and chicken manure, respectively.

Densities of spore formers and K mobilizing bacteria:

Generally, spore forming bacteria and K mobilizing bacteria were remarkably stimulated in the presence of compost or chicken manure (Figure 2). Again, the maximum records of spore forming bacteria were obtained after 60 days from cultivation being 28.9 x 10^6, 24.3 x10^6 and 28.6 x10^6, 24.7 x 10^6 cfu/g dry rhizosphere soil of potato and broccoli plants, compared with control being 9.9x 106 cfu/g and 11x 10^6 cfu/g dry rhizosphere soil of potato and broccoli, respectively.

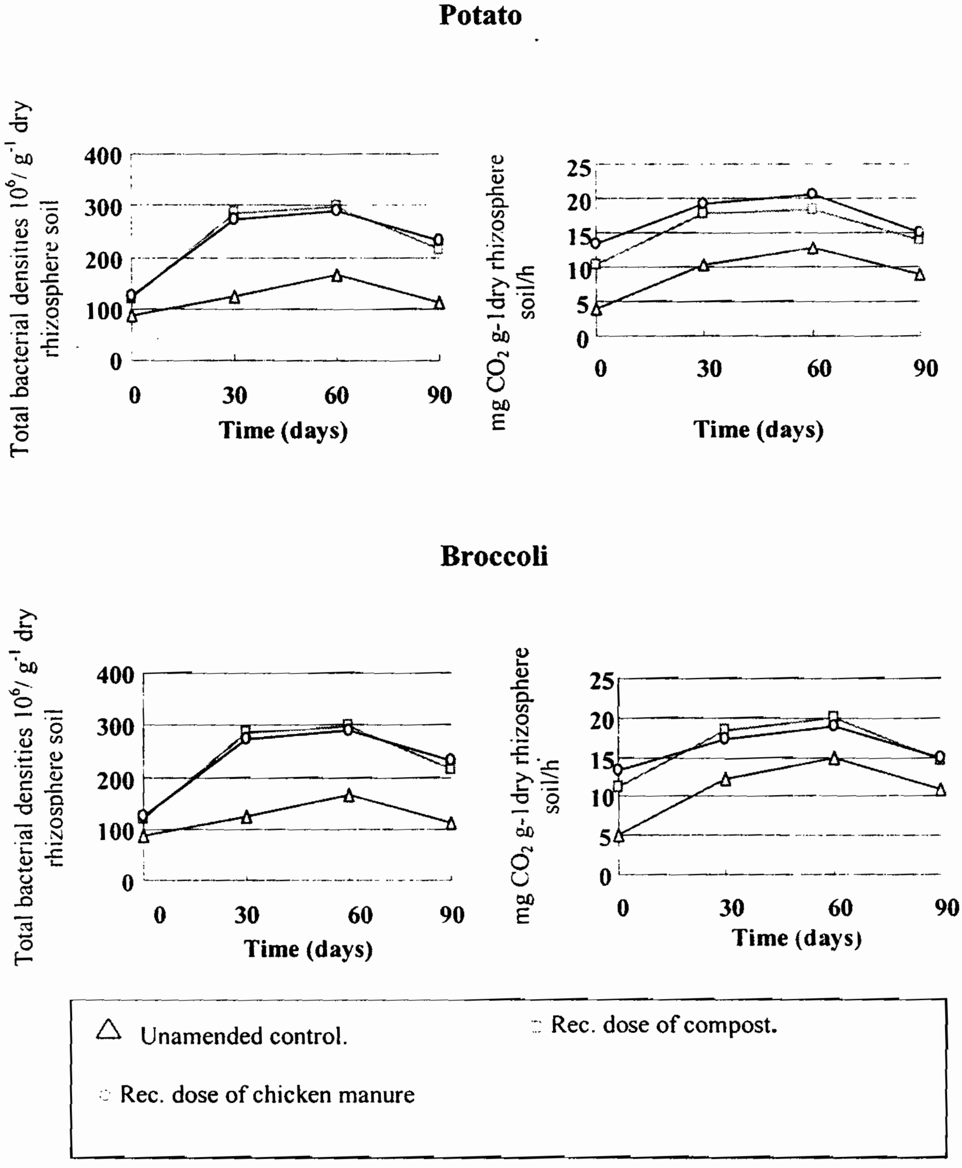

Figure (1): Total bacterial densities and rates of CO_2 evolution in the rhizosphere of potato and broccoli plants as influenced by composted rice straw or chicken manure.

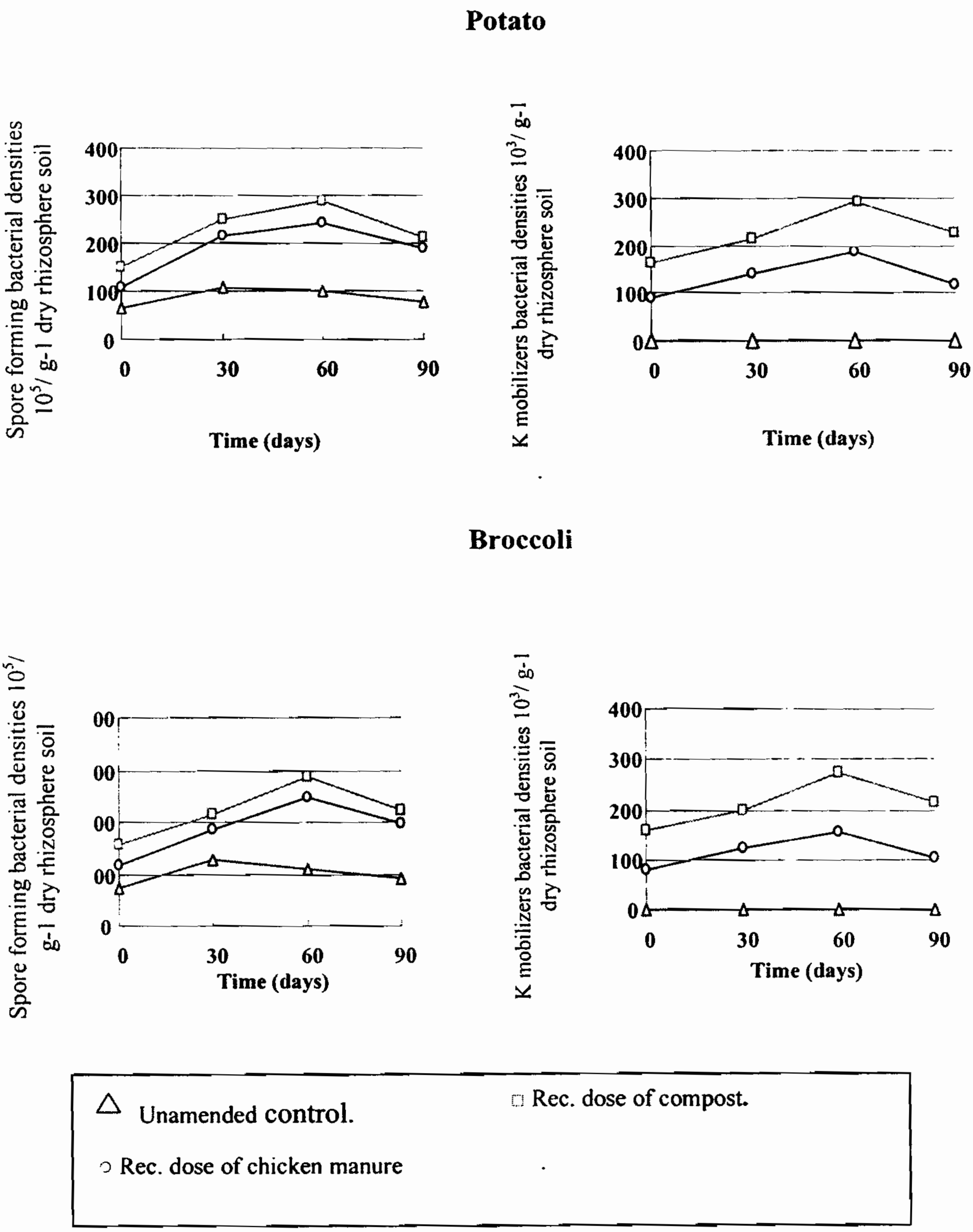

Figure (2): Densities of total spore forming bacteria and K mobilizing bacteria in the rhizosphere of potato and broccoli plants as influenced by composted rice straw or chicken manure

Table (1): Growth parameters of potato (*Solanum tuberosum* L. cv. Diamont) and broccoli (*Brassica oleracea* cy hybrid) plants amended with the recommended dose of composted rice straw and chicken manure after 90 days of cultivation

Parameters \ Treatments	Potato			Broccoli		
	Unamended control	Composted rice straw	Chicken manure	Unamended control	Composted rice straw	Chicken manure
Plant height (cm/ plant)	20.50	33.12	37.50	20.00	26.00	25.00
No. of branches (no./ plant)	9.00	13.00	15.00	13.00	17.00	19.00
Stem diameter (mm)	20.00	60.00	50.00	150.00	230.00	220.00
Shoot fresh weight (g/plant)	5.12	17.08	20.56	79.65	176.61	175.81
Shoot dry weight (g/plant)	2.46	4.19	5.82	33.13	43.98	28.25
Root fresh weight (g/plant)	4.30	7.01	8.38	40.22	62.46	53.14
Root dry weight (g/plant)	2.02	3.59	2.78	21.19	28.34	21.83
LSD	5% potato	5% broccoli		5% potato	5% broccoli	
Plant height	05.10	02.50	Shoot dry weight	02.38	05.880	
No. of branches	01.00	04.39	Root fresh weight	01.35	04.370	
Stem diameter	20.10	25.60	Root dry weight	0.34	04.190	
Shoot fresh weight	04.33	07.04				

* All parameters calculated per 3 plants from 3 replicates.

The recorded figures of K mobilizing bacteria are $29x10^4$, 18.6×10^4 and 27.4×10^4, 15.6×10^4 cfu/g dry rhizosphere soil in the same above-mentioned respective order. K mobilizing bacteria were not detected in the rhizosphere soil of unamended control plants during sampling intervals.

Growth responses

A highly significant differences were observed between the amended (compost or chicken manure) and unamended control plants except for shoot dry weight and No. of branches of potato and broccoli, respectively (Table 1). On the other hand, no significant differences were found between potato and broccoli treated with compost or chicken manure regarding growth parameter under investigation except for No. of branches of potato, shoot and root dry weight of broccoli.

Yield responses

A significant effect was reported with weight of tuber/plant and total weight of tuber / treatment. On the other hand there were no significant differences between the tested plants treated with composted rice straw or chicken manure with other yield parameters (Table 2).

As a general trend, application of compost or chicken manure on broccoli plants gave a highly significant effect on weight of fruit/plant and total weight of fruit/treatment, than those recorded in unamended control (Table 2). Maximum broccoli fruits/treatments were obtained with compost compared with chicken manure amendment being 75.12, 61.71 g/plant and 5760, 4659 g/ treatment for fruit weight and total fruit weight, respectively.

NPK content of potato tubers and broccoli fruits

Data in (Table 3) clearly show that NPK content of potato tubers and broccoli fruits were increased due to composted rice straw or chicken manure compared with unamended control. Application of compost gave NPK content of 1.50, 0.098 and 0.602 % against 15.430, 1.415 and 0.820% for potato and broccoli, respectively. Only higher N content of potato tuber was obtained from plants treated with chicken manure compared with composted rice straw (Table 3).

Table (2): Yield characteristics of potato (*Solanum tuberosum* L. cv. Diamont) and broccoli (*Brassica oleracea* cv. hybrid) amended with composted rice straw or chicken manure

Parameter / Treatments	Unamended control	Composted rice straw	Chicken manure
Potato			
No. of tuber/plant	7.5	6.0	5.5
Weight of tuber (g/plant)	300	600	495
Total no. of tuber/treatment	112.5	70.25	74.4
Total weight. of tuber (g/ treatment)	4500	7025	6696
Broccoli			
No. of fruit /plant	1	1	1
Weight of fruit (g/plant)	43.74	75.12	61.71
Total weight. of fruit (g /treatment)	3200	5760	4659

LSD potato	**5%**	**LSD broccoli**	**5%**	
No. of tuber/plant	0.5	Weight of fruit	2.19	
Weight of tuber	39.5	Total weight. of fruit	376.08	
Total no. of tuber	4.95			
Total weight. of tuber	1420.8			

*All parameters calculated per 3 plants from 3 replicates

Table (3): NPK content of potato tuber (*Solanum tuberosum* L cv. Diamont) and broccoli (*Brassica oleracea* cv. hybrid) fruit amended with composted rice straw or chicken manure

Parameters / Treatment		Unamended control	Composted rice straw	Chicken manure
Potato tuber				
Content%	N	0.94	1.50	1.60
	P	0.058	0.098	0.093
	K	0.236	0.602	0.518
Broccoli fruit				
Content%	N	1.410	5.400	7.727
	P	0.0492	1.145	0.974
	K	0.357	0.820	0.725

Table (4): Growth parameters of potato (*Solanum tuberosum* L.cv.Diamont) and broccoli (*Brassica oleracea* cv hybrid) plants amended with different rates of composted rice straw. after 90 days of cultivation.

Treatments / Parameters	Unamended control	Potato Compost application rate (%)			Unamended control	Broccoli Compost application rate (%)		
		50	100	200		50	100	200
Plant height (cm/ plant)	22.00	28.00	35.00	43.00	20.00	22.00	25.00	27.00
No. of branches (no./ plant)	10.00	12.00	15.00	20.00	19.00	21.00	24.00	29.00
Stem diameter (mm)	40.00	50.00	80.00	100.0	180.0	200.0	230.0	320.0
Shoot fresh weight (g/plant)	6.120	15.70	21.65	37.68	84.08	122.5	175.7	273.8
Shoot dry weight (g/plant)	3.750	7.250	7.66	10.23	23.86	22.86	35.07	76.86
Root fresh weight (g/plant)	4.910	7.810	8.81	8.93	38.89	50.21	84.76	92.74
Root dry weight (g/plant)	3.700	4.040	4.05	5.66	11.65	19.73	27.29	31.52

LSD	5% potato	5% broccoli	LSD	5% potato	5% broccoli
Plant height	7.59	4.60	Shoot dry weight	3.235	22.56
No. of branches	5.60	7.06	Root fresh weight	2.098	24.81
Stem diameter	34.2	64.39	Root dry weight	3.006	7.887
Shoot fresh weight	11.19	97.56			

* All parameters calculated per 3 plants from 3 replicates

Effects of increased rates of composted rice straw

Based on previous results, another trial was carried out to compare between the effects of different rates of composted rice straw on the same above mentioned parameters.

Densities of microbial populations and rates of CO_2 evolution in the rhizosphere of potato and broccoli

Data plotted in Figure (3) obviously indicated the stimulatory response of increased rates of composted rice straw on total bacterial counts and rates of CO_2 evolution in tested plant rhizosphere due to compost applied at three rates compared with unamended control. Both parameters were considerably increased after 60 days of cultivation, and then sharply decreased. The recorded figure at that interval reached $21.3x10^7$, $24x10^7$ and $29x10^7$, $21x10^7$, $24.2x10^7$ and $29x10^7$cfu/ g dry rhizosphere soil for potato and broccoli cultivated with 50, 100 or 200% of the recommended dose of compost, respectively.

Generally, densities of spore forming and K mobilizing bacteria in the rhizosphere of potato or broccoli were remarkably stimulated in the presence of either of the compost application rate (Figure 4). However, the maximum levels were obtained after 60 days from cultivation being 29.4 and 29.5 x 10^6 cfu of spore forming bacteria /g dry rhizosphere soil of potato and broccoli plants treated with 200% of compost, respectively. The corresponding figures for control plants were 9.9 x 10^6 and 11x 10^6 cfu/g dry rhizosphere soil of potato and broccoli plants, respectively

The recorded figures for K mobilizing bacteria were 22.1, 26.1 and 29x 10^4 and 19.3, 24.3 and 28.4 x 10^4 cfu/g dry rhizosphere soil of potato and broccoli obtained with 50, 100 or 200% of recommended dose of compost, respectively. K mobilizing bacteria were not detected in the rhizosphere of control plants at any of the examined intervals.

Growth responses

Significant differences were recorded between all growth parameters of potato plants due to application of different rates of composted rice straw compared with unamended control plants. The highest records of growth were obtained from potato plants treated with 200% of recommended dose (Table 4). Broccoli, on the other hand, gave significant responses with the application of 100 or 200% of compost recommended dose compared with the control. However, no significant differences were observed between the effects of applying 50 or 100% as well as 100 and 200% compost recommended dose for broccoli. (See Table 4).

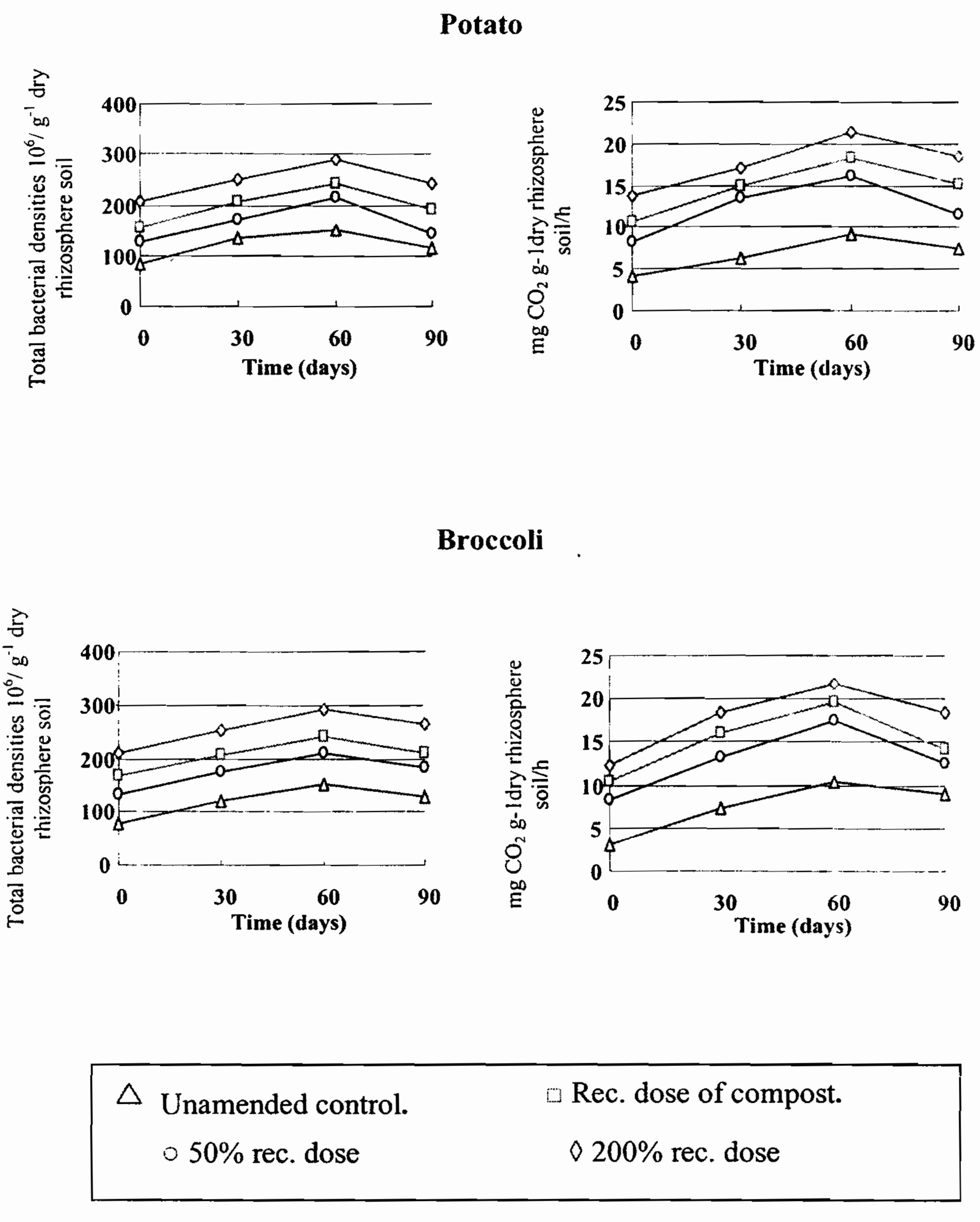

Figure (3): Total bacterial densities and rates of CO_2 evolution in the rhizosphere of potato and broccoli plants as influenced by different rates of composted rice straw.

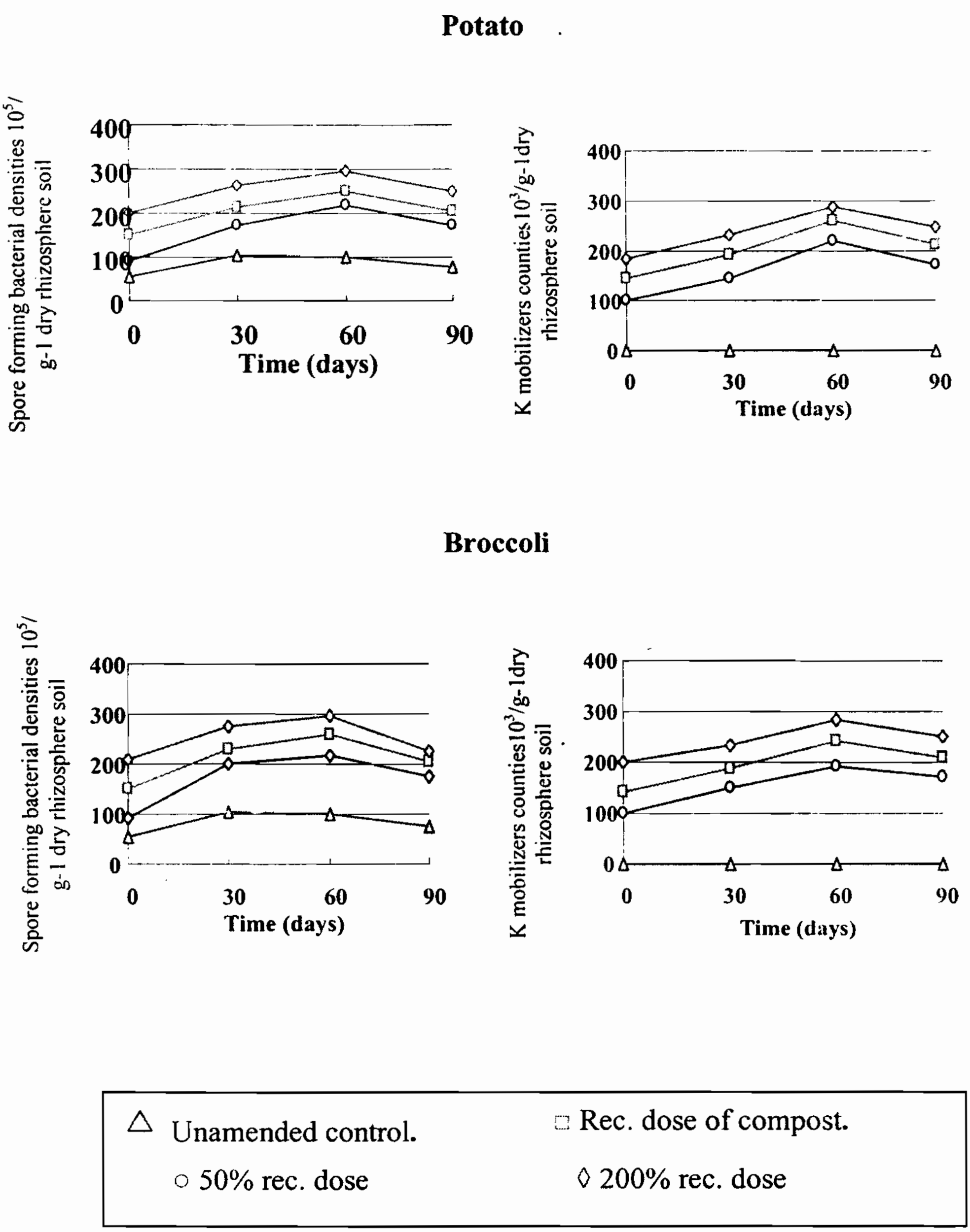

Figure (4): Densities of total spore forming bacteria and K mobilizing bacteria in the rhizosphere of potato and broccoli plants as influenced by different rates of composted rice straw.

Yield responses

Data in (Table 5) showed that no significant differences were found between the No of tubers / plant and No. of fruit/plant of amended or unamended potato and broccoli plants, respectively. Weight of tuber/plant, total weight of tubers/ plant and weight of Fruit /plant, total weight of fruits/treatment showed significant differences between 50, 100 or 200% of the recommended dose of compost.

NPK content of potato tubers and broccoli fruits

Data in (Table 6) clearly show that NPK contents of potato tuber and broccoli fruits were increased when treated with the three rates of composted rice straw compared with unamended control. However, application of the 200% rate of recommended dose of compost gave the highest content of NPK being 1.7, 0.12, 0.74 and 8.67, 2.31, 0.96% for potato tubers and broccoli fruits, respectively

DISCUSSION

Many studies revealed that *Bacillus circulans* harbor a wide array of traits that make it a good candidate for biological applications. The ability of the organism to produce extracellular enzymes such as amylase (**Kawn *et al.*, 1993**) cellulases **(Kim and Kim, 1993)**, chitinases **(Wiwat *et al.*, 1999)**, and protease which act mainly on plant material should enable *Bacillus circulans* to play an important role as a bio-accelerator to enhancing the maturity of composted material within a shorter time **Abd El-Fattah, (2006)**. This observation was practically reported by **Kubo *et al.* (1994)** who isolated and identified *Bacillus circulans* as one of microorganisms, which were able to degrade soybean lees efficiently.

The role of *Bacillus circulans* as a traditional bio-fertilizer used to mobilize soil potassium **(Mansour *et al.*, 1984; Groudeva and Groudev, 1987; Galgoczy, 1990)** and enhance K uptake **(Saber and El-Sherif, 1975; Balabel, 1997)** is well documented. However, **Berge *et al.* (1990)** reported that while *Azospirillum lipoferum* CRT1 showed a significant increase in the number of emerged maize plants, total dry matter and grain yield of field grown maize, the N_2 fixing *Bacillus circulans* RSA19 had a similar significant effect to that of *Azospirillum lipoferum* CRT1 in increasing nitrogen content of grain and total nitrogen exported with harvest. **Gaind and Gaur (1991)** also reported that seed inoculation of mung bean with *Bacillus circulans* (TT8) improved nodulation, the available P_2O_5 content of the alluvial soil, root and shoot biomass, straw as well as grain yield and phosphorus and nitrogen uptake of the crop.

Table (5): Yield characteristics of potato tuber (*Solanum tuberosum* L. cv. Diamont) and broccoli fruit (*Brassica oleracea* cv hybrid) amended with rates of composted rice straw

Treatments / Parameters	Unamended Control	Compost rate (%)		
		50	100	200
		Potato		
No. of tuber/plant	6.30	5.30	5.90	6.70
Weight of tuber (g/plant)	45.0	53.0	70.8	113.9
Total no. of tuber/treatment	100	100.6	61.11	67.7
Total weight. of tuber (g/treatment)	5000	5333	7334	11520
		Broccoli		
No. of fruit/plant	1	1	1	1
Weight of fruit (g/plant)	35.65	46.078	100.0	130.0
Total weight. of fruit (g/treatment)	2852	3686.24	8000	10400
LSD	5%	LSD		5%
No. of tuber	1.379	Total weight. of tuber		36.92
Weight of tuber	23.490	Weight of fruit		44.36
Total no. of tuber	324.13	Total weight. of fruit		345.7

*All parameters calculated per 3 plants from 3 replicates

Table (6): NPK content of potato tuber (*Solanum tuberosum* L .cv. Diamont) and broccoli fruit (*Brassica oleracea* cv. hybrid) amended by the rate of composted rice straw

Treatment / Parameters		Unamended control	Compost rate (%)		
			50	100	200
Potato plant					
Content %	N	1.04	1.39	1.67	1.70
	P	0.061	0.077	0.098	0.12
	K	0.401	0.563	0.653	0.74
Broccoli plant					
	N	1.423	4.250	6.128	8.673
Content %	P	0.057	0.092	1.252	2.314
	K	0.411	0.571	0.803	0.963

Due to its pronounced effect in composting of rice straw **(Abd El -Fattah, 2006),** a well known environmental pollutant at present, the material treated with *B. circulans* and animal manure was selected to show how it could be utilized in agriculture. The effects of rice straw compost application were strongly reflected on growth and yield parameter of the inoculated potato and broccoli. The dual manipulation of *B. circulans* for accelerating composting process and enriching the produced compost may also secure energy and carbon source for rhizosphere microorganisms of amended plants, thus overcoming the negative impact resulted from depletion of carbon in the absence of organic matter. This speculation is derived from the finding that in some cases of biofertilization practice, convincing data that pinpoint the importance of these bacteria are limited. This limitation was attributed to the inability of the bacteria to maintain their activity in the rhizosphere. Therefore, it is quite acceptable that organic matter should be applied in conjugation with the biofertilizers to secure their energy requirements and hence extend their effects on the host plant. Unfortunately, in many experiments, biofertilization and organic amendment are considered two separate treatments **(Dadarwal *et al.*, 1997).** Thus, the undetectable differences or negative effects of applied biofertilizers (**Boddy and Dobereiner, 1982**) could be replaced by an ensured positive effect on amended host due to the extending action of the biofertilizers. The later speculation was practically exercised in the two experiments planed to evaluate the effect of bio-organically composted rice straw on growth and yield of potato and broccoli under organic farming standards. In fact, the comparison between the effects of bio-organically composted rice straw and chicken manure as well as those of compost applied rates were always in favor of the compost amended plants. This observation was true for growth, yield parameters and NPK content of tubers and fruits.

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الملخص العربي

استخدام الباسلس سيركيولانس كمسرع حيوى يثرى المخلفات الزراعية المستخدمة

٢- تقيم الكومبوست المنتج تحت نظام الزراعة العضوية في انتاج الكومبوست

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تم تقييم الكومبوست الناتج من كمر قش الأرز بفعل إضافة خليط من أكفأ ٤ سلالات من ميكروب الـــ *Bacillus circulans* والذي تم استخدامه كمنشط حيوي مع روث الماشية كمنشط عضوي ولهذا الغرض تم عمل تجربتين حقليتين صممت الأولي لمقارنة استخدام المعدل الموصي بة من الكومبوست الناتج بالتسميد بزرق الدواجن , أما الثانية فقد صممت لتحديد أفضل معدل تسميدي من هذا السماد حيث استخدمت نصف الكمية والكمية الموصي بها وضعف هذه الكمية, خلال موسم النمو و تم تقييم تأثير الكومبوست علي النمو, الإنتاجية والمحتوي من النيتروجين والفسفور والبوتاسيوم علي نباتات البطاطس والبروكلي النامية تحت ظروف الزراعة العضوية.

أظهرت النتائج تفوق نباتات البطاطس والبروكلي المسمدة بالكومبوست عن النباتات المسمدة بزرق الدواجن, حيث ازدادت أعداد البكتريا الكلية والبكتريا المتجرثمة والميسرة للبوتاسيوم في ريزوسفير البطاطس والبروكلي وذلك بعد ٦٠ يوم من الزراعة وانعكس ذلك علي النمو الخضري والمحصول لكلا من المحصولين في حين حقق استخدام ضعف الكمية الموصي بها من الكومبوست الناتج من كمر قش الأرز أفضل تأثير علي جميع القياسات السابقة مقارنةً بالكمية أو نصف الكمية الموصي بها.