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COMBINED EFFECT OF ORGANIC MANURING AND BIOFERTILIZATION ON CHEMICAL AND BIOLOGICAL ACTIVITIES IN THE RHIZOSPHERE OF FABA BEAN

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ABSTRACT

A pot experiment was conducted in the green house of National Research Center to study the effect of organic manuring (composted town refuse) and organic manure associated with dual biofertilizers on rhizosphere of faba bean plants, as well as the enzymatic activities of soil as indicators to the biological activity.

An empirical formula was suggested to evaluate the effects of different treatments applied under the rhizosphere conditions. These effects could be indexed by determining the Efficiency of Rhizosphere Treatment (ERT) i.e., the net effect of rhizosphere treatment divided by the net effect of the same treatment in root – free soil. Organic amendment (2% of composted town refuse) alone or associated with dual biofertilizers (*Rhizobium leguminosarum*+ Phosphate dissolving bacteria) (PDB) wrer used. The rhizosphere effect and the ERT were periodically determined (2, 4, 6, 8 weeks) for the unamended and amended soil respectively. The changes in soil organic carbon, total nitrogen, rate of CO_2 evolution, populations of total microbial flora, *Rhizobium leguminosarum* And PDB, as well as soil enzymatic activities of invertase, phosphatase, and dehydrogenase were used as chemical, microbiological and biological parameters under these conditions.

The results revealed that the biofertilizers treatment recorded the higher figures of ERT for all parameters studied as compared to the organic amendment (CTR) alone or the unamended soil under the rhizosphere of faba been *(Vicia faba)*.

INTRODUCTION

The rhizosphere is currently a subject of intensive investigations, which widely varied in their objectives. Many comprehensive reviews had shown that the rhizosphere of legumes is always rich with organic materials through the root exudates and debris of root tissues (Rovira and Davey, 1974 and Hale *et al.*, 1978)

The use of organic manures in agriculture performs a crucial role in clean farming system. They are precious renewable resources, which complete the nutrient elements cycle, improving the biological and chemical properties of soil as a source of energy for soil ecosystem. Various organic amendments containing mixed cultures of indigenous microorganisms are usually applied recently in clean farming which termed as biofertilizers. Some species of these microorganisms tend to survive longer than others as they tolerate the environments. However most of the amended organisms following the peak of growth and greater activity resulting from utilization and depletion of available substrates in the soil by these microflora. These inoculated microorganisms are overwhelmed by the native soil of microflora through competion and antagonism (Higa and Windana, 1991 and Higa,

1994). The repeated applications of organic amendment, or mixed culture inoculants, while the level of microbial growth and their activities still high above the baseline, they can maintain a high inoculum's density of the inoculated microorganisms for longer extended period.

This approach can help to ensure that the numbers of beneficial microbial cultures that promote plant growth and protection will remain high during the first 3-4 weeks after planting a crop. This is the period in which the young seedlings and plants are so vulnerable environmental stress (Saber, 2001).

The aim of this work is to evaluate the combined effect of organic manuring (composted town refuse) associated with dual biofertilization (phosphate dissolving bacteria, *Rhizobium leguminosarum*) on some chemical and biological properties of rhizosphere of faba bean *(Vicia faba,* L) rhizosphere.

These parameters included organic carbon, total – N, total microbial counts, counts of PDB, *Rhizobium leguminosarum* as well as, CO_2 evolution and enzymatic activities of invertase, phosphatase and dehydrogenase. The growth and uptake of N and P in plants were also determined as affected by the treatments.

MATERIALS AND METHOD

A pot experiment was carried out in the green house of National Research Center (NRC) to study the effect of organic manuring (composted town refuse, CTR) and organic manuring, (CTR) + dual biofertilizers (DBF) (*Rhizobium leguminosarum* + Phosphate dissolving bacteria, PDB) on biological activities of rhizosphere of faba bean, in relation to its growth.

The experiment was done in plastic pots (25 cm in diameter) filled with 7 kg of a clay loam soil collected from Giza Governorate (organic carbon 0,580%, total-N 0,072%, total-P 122 ppm, E.C. 0.55 dsm^{-1} and C/N ratio 8.05)

The pots were divided into two groups A and B. The group A was kept unplanted, whereas the group B was sown with *vicia faba* seeds (var. Giza 402) by the rate of 4 seeds per pot. Each group divided to 3 sub treatments:

1- Unamended soil (control).
2- Amended soil with 2% composted town refuse, CTR (0.C. 29,8%, T.N. 2,16 %, C/N = 13,8).
3- Amended soil with 2% composted town refuse (CTR) + dual biofertilizers (DBF) containing a efficient strains of rhizobia *(Rhizobium leguminosarum)* and phosphate dissolving bacteria (*Bacillus megaterium* var. *phosphaticum*, independently grown in nutrient broth for 48 hours at 30°C in a rotary shaking incubator. Liquid broth cultures containing 8 $x10^7$ and $7x10^8$ viable cells/ml, respectively. In biophertilization treatments, 30 ml of either tested microorganisms added to the soil in each biophertilized pot. These strains were obtained from our department of Soil Microbiology Type Culture Collection. Each treatment

was replicated 4 times and soil moisture was raised up to 60% of its water holding capacity and kept constant through daily compensation of evaporating losses by re-weighed periodically to restore any loss in soil humidity. All pots were kept for 8 weeks; representative soil samples were taken from each treatment, initially and after 2,4,6 and 8 weeks of manuring and sowing for different analyses. Each sample was well mixed and analyzed chemically for total organic carbon, total nitrogen (Black et *al.*, 1965) and Co_2 evolution (Monib et *al.*, 1981).

Total bacteria, rhizobia and phosphate dissolving bacteria (PDB were enumerated under different treatments by the serial dilution method (Allen, 1953). Enzymatic activities of invertase (Balasubramanyan et *al.*, 1970), phosphatese (Khaziev, 1968) and dehydrogenase (Skujins, 1973) were also assayed. Plant analysis was carried out in samples collected after 8 weeks of planting for fresh and dry weight of plants (g/pot), N and P content (%) and their uptake.

The actual effects of different treatments applied under the rhizosphere conditions were determined by the Efficiency of Rhizosphere Treatment (ERT) i.e. the net effect of rhizosphere treatment divided by the net effect of the same treatment in root free soil (Ishac *et al.*, 1986). This could be estimated according to the following equation:

ERT = Rt-Rc /St-Sc

Whereas: ERT =efficiency of rhizosphere treatment

Rt = treated rhizosphere

Rc = control rhizosphere

St = treated soil

Sc = control soil

Plant analysis were exposed to the proper statistical analysis and least significance difference (L.S.D at 5%) (Snedcor and Cochran, 1990).

RESULTS AND DISCUSSION

Effect of biofertilization on chemical properties of soil :

The effects of organic amendment (CTR) and of biofertilization (CTR +D BF) on soil organic carbon, total nitrogen and Co_2 evolved are presented in Table (1). The data showed that the organic manure (CTR), and organic manure (CTR) + dual biofertilizers increased the organic carbon content of soil than in unamended soil which reflected on the higher efficiency of rhizosphere treatments (ERT recorded an average 1.06 for the organic manure treatment (CTR) and 1.07 for the dual biofertilizers treatment (CTR +DBF). This increase may be due to the nursing effect of added organic matter, which enriched the microflora in rhizosphere of faba bean rather than in soil apart from root plants (control). This beneficial effect of organic manure and biofertilizers may be attributed to its favorable effect on microbial populations and nutrient availability (Khalifa *et al.*, 1997 and Moharram, 1999).

Table (1): Effects of organic amendment (CTR) and organic amendment with dual biofertlizers (CTR + DBF) on chemical properties of rhizosphere of faba bean

Treatment / Time (Weeks)	Unamend-ed Soil		Rc/Sc*	Amended with CTR		ERT1*	Amended with CTR + DBF		ERT2*
	Rc	Sc		Rt_1	St_1		Rt_2	St_2	
(1) Organic carbon (%)									
0	-----	0.58	-----	-----	1.18	------	-----	1.18	-----
2	0.52	0.55	0.94	1.03	1.10	0.93	1.10	1.13	1.00
4	0.50	0.54	0.92	0.95	1.02	0.94	1.02	1.05	1.02
6	0.48	0.53	0.90	0.92	0.93	1.11	0.97	0.93	1.22
8	0.47	0.52	0.89	0.90	0.88	1.25	0.95	0.90	1.10
Average			0.92			1.06			1.07
(2) Total nitrogen (%)									
0	-----	0.07	-----	-----	0.12	-----	-----	0.12	-----
2	0.12	0.08	1.43	0.26	0.14	2.23	0.29	0.13	3.15
4	0.09	0.08	1.19	0.22	0.15	1.76	0.24	0.14	2.37
6	0.08	0.07	1.09	0.16	0.14	1.07	0.26	0.14	1.76
8	0.07	0.03	1.01	0.13	0.13	0.85	0.17	0.13	1.61
Average			1.18			1.47			2.22
(3) CO_2 evolution (mg/100 g soil /24hour)									
0	-----	-----	-----	-----	-----	-----	-----	-----	-----
2	72.7	26.4	2.75	145.0	36.9	6.88	138.0	34.8	7.77
4	54.7	36.2	1.45	116.8	60.2	2.59	109.2	54.1	3.04
6	41.8	30.5	1.37	100.3	55.1	2.38	91.9	49.1	2.70
8	36.0	28.5	1.26	87.1	50.3	2.34	79.8	45.0	2.65
Average			1.71			3.55			4.04

* ERT = efficiency of rhizosphere treatment = treated rhizosphere – control rhisosphere / treated soil – control soil ERT = Rt – Rc / St - Sc
ERT1 = Rt1- Rc1 / St1-Sc1 ERT2 = Rt2-Rc2 / St2 –Sc2

A gain in total-N was recorded in rhizosphere of faba bean after amended the soil with composted organic manure alone (ERT_1 = 1,47) or with dual biofertilizers (ERT_2 = 2,22) comparing to the unamended soil. These results indicate that there is a greater response of rhizosphere of faba bean to biofertilizers amendment more than to the organic manuring. These results are in agreement with the findings of Saber (2001) who explained these positive effect to the role of biofertilizers in relation to plant growth i.e. symbiotic and a asymbiotic N-fixation mobilizing plant nutrient elements and / or secreting plant growth promoting principles and biocontrolling soil born diseases.

Addition of organic manure (CTR) alone, or associated with biofertilizers resulted in a pronounced increase in Co_2 evoluted the rhizophere (Table 1) The recorded figures of ERT for the rhizosphere treatments were 3,55 for manuring soil and 4,04 for biofertilized one. This increasing reflected the high microbial activities in the rhizosphere of

amended soils rather than in the unamended ones (Table 1). Lou and Sun, (1994) reported that this increase might be due to available carbon sources on which organisms live besides conserving soil moisture and maintaining favorable soil temperature.

Behaviors of tested microorganisms in rhizosphere of faba bean plants:

Data in Table (2) showed that the counts of total bacteria in rhizosphere of amended soils recorded high densities more than in unamended one. (ERT for organic manure was 2.20 whereas ERT for biofertilizers treatment was 2.76. This means that the density of total microbial flora increased 2.20 and 2.76 times in the rhizosphere of faba bean of amended soils as compared to the unamended planted soil.

Rhizobia and phosphate dissolving bacteria (PDB) occurred in higher densities in biofertilized rhizosphere of faba bean plants during the grown in soil amended with composted organic manure (CTR) as compared to unamended soil.

Table (2): Effect of organic amendment (CTR) and organic amendment with dual biofertilizers (CTR +DBF) on microbial populations of rhizosphere of faba bean

Treatment Time (Weeks)	Unamended Soil		Rc/Sc*	Amended with CTR		ERT1*	Amended with CTR +DBF		ERT2*
	Rc	Sc		Rt_1	St_1		Rt_2	St_2	
(1) Total count (counts $x10^{5}$)									
0	-----	38.5	-----	-----	38.5	-----	-----	38.5	-----
2	96.83	43.19	2.24	210.9	81.1	3.01	197	73.1	3.38
4	72.92	52.27	1.40	199.7	89.0	3.44	188	78.8	3.37
6	51.53	44.29	1.16	113.5	81.3	0.81	102	70.4	1.94
8	42.46	33.13	1.28	93.1	66.2	1.52	77	55.4	2.35
Average			1.52			2.20			2.76
(2) Rhizobia (counts x 10^{5})									
0	-----	0.80	-----	-----	0.80	-----	-----	0.80	-----
2	8.25	2.84	2.90	25.52	8.14	3.24	37.0	12.7	2.90
4	6.16	2.92	2.11	21.69	9.02	2.54	33.5	14.3	2.42
6	7.48	2.19	3.56	15.84	7.50	1.57	28.8	11.7	1.52
8	5.22	1.92	2.72	13.80	5.46	2.77	19.5	8.05	2.31
Average			2.07			2.51			2.28
(3) PDB (counts x 10^{5})									
0	-----	0.50	-----	-----	0.50	-----	-----	0.50	-----
2	6.38	1.96	3.48	27.09	8.17	3.27	20.4	4.91	4..62
4	4.58	2.14	2.15	25.34	7.85	5.41	19.5	6.13	3.73
6	3.51	1.26	2.79	17.92	4.15	4.32	13.6	3.62	4.32
8	2.53	0.98	2.58	7.04	2.35	3.00	10.6	3.23	3.92
Average			2.75			3.80			4.15

* ERT = efficiency of rhizosphere treatment = treated rhizosphere – control rhisosphere / treated soil – control soil ERT = Rt – Rc / St - Sc

ERT1 = Rt1- Rc1 / St1-Sc1 ERT2 = Rt2-Rc2 / St2 –Sc2

Enzymatic activities under faba bean plants

It is worthy to state that, the composted town refuse (CTR) showed distinguishable different responses on the counts of tested microorganisms in rhizosphere of faba bean Table 2). Generally, the amendment of soil with composted town refuses, in the presence of biofertilizers, recorded higher counts of tested microorganisms in the rhizosphere as compared to unamended soil.

The obtained results of enzyme activities of invertase, phosphatase and dehydrogenase are shown in Table (3). Addition of composted organic manure (CTR) alone or associated with DBF, highly increased the invertase activity in the rhizosphere of faba bean over the control (ERT_1 3.98, ERT2 4.0 respectively. The same trend was observed in phosphatase activity (ERT_1 3.31, ERT_2 3.91) and dehydrogenase activity (ERT_1 3.02, ERT2 3.20) respectively. In all enzymes studied, the higher activities were recorded during the fourth week of experimental period. These results are in agreement with the findings of Tarafdar and Claassen (1988), Mamada and Okino, 1989 and Fox and Comerford (1992). They studied the transformation of phosphatases produced by plant roots and microorganisms in the rhizosphere of some strategic plants (clover, barely and wheat). They reported that there was an increase in phosphatases in the rhizosphere of these plants.

Table (3): Effect of organic amendment (CTR) and organic amendment with dual biofertilizers (CTR+DBF) on enzyme activities of rhizosphere of faba bean

Treatment Time (Weeks)	Unamended Soil		Rc/Sc*	Amended with CTR		ERT1*	Amended with CTR + DBR		ERT2*
	Rc	Sc		Rt_1	St_1		Rt_2	St_2	
(1) Invertase activity (mg glucose/100g soil / day)									
0	-----	5.60	------	-----	5.60	-----	-----	5.60	-----
2	9.16	7.40	1.24	20.37	9.44	7.44	16.76	8.04	7.44
4	11.99	9.17	1.31	22.01	11.35	4.07	18.66	10.8	4.07
6	8.67	7.63	1.14	18.54	10.45	3.47	16.02	9.42	3.47
8	6.40	4.90	1.08	16.38	9.19	1.40	11.43	6.34	1.40
Average			1.19			4.00			3.98
(2) Phosphatase activity (mg P2O5 /100g soil /day)									
0	-----	43.9	-----	-----	43.9	-----	-----	43.9	-----
2	125.1	116.8	1.07	162.2	132.2	2.41	144.2	123.9	2.78
4	154.7	137.8	1.12	198.5	143.5	7.61	180.1	141.3	7.22
6	58.0	58.8	1.13	77.3	71.7	1.5	78.1	64.9	3.31
8	53.7	30.21	1.24	82.2	46.9	1.70	76.9	40.3	2.31
Average	97.9	85.7	1.14	130.0	98.5	3.31	119.9	92.6	3.91
(3) Dehydrogenase activity (ul H2 /g soil /day)									
0	-----	5.10	------	-----	5.10	-----	-----	5.10	-----
2	10.04	763	1.32	18.37	9.56	4.31	25.38	10.61	5.15
4	12.36	8.42	1.47	20.51	10.11	4.82	27.09	11.84	4.30
6	11.35	6.26	1.81	15.35	9.72	1.36	21.26	10.26	2.44
8	10.56	5.13	2.06	12.15	6.12	1.60	14.02	8.94	0.91
Average	11.28	6.86	1.67	16.60	8.88	3.02	15.67	10.42	3.20

* ERT = efficiency of rhizosphere treatment = treated rhizosphere – control rhisosphere / treated soil – control soil ERT = Rt – Rc / St - Sc

ERT1 = Rt1- Rc1 / St1-Sc1 ERT2 = Rt2-Rc2 / St2 –Sc2

Mu JinMig (1997) and Shen Hong et *al.*, (1999) reported that the invertase activity in the soil amended with maize stubble was higher than that in soil amended with Soya bean stubble. This increase was reached the maximum value during 30-60 days after seeding.

Dehydrogenase activity was studied by Rao *et al.*, (1990) in arid land crops. They studied the distribution of dehydrogenase activity in the rhizosphere of four varieties of cluster bean, mung bean, mouth bean and pearl millet grown in pots and reported that rhizosphere soils showed higher activity than other soils, and the activity was significantly higher in the rhizospheres of legumes than in those of other plants.

Plant growth as affected by different treatments:

Data presented in Table (4) showed that the organic manure CTR) treatment alone or in presence of biofertilizers (DBF) has surpassed that of unamended soil in increasing the dry matter accumulation in faba bean plants. These also were correlated with the increase in N and P% as well as their uptake by the plants as affected by the treatments under study. These findings indicated the vital role of biofertilizers which release more available nutrient elements to be adsorbed by plant roots and this in turn ,increase the dry matter content in different organs of different plants (Saber and Kabesh ,1990 on lentil plants, Ahiabor and Hirata, 1994 on peanut. Also, the ability of organic compost to produce such positive effects of plant characteristics varied greatly with the type of organic wastes used. Abd El-Moez, (1996) stated that the application of composted rice straw associated with biofertilizers exhibited higher values of dry weight of different peanut organs as compared to the other organic wastes.

Table 4:Effect of organic amendment (CTR) and organic amendment with biofertilizers on growth of faba bean plants and their N and P contents

Treatment	Dry weight g/pot	Nitrogen		Phosphorus	
		%	Up take mg/plant	%	Up take mg/plant
Unamended soil	3.84	2.491c	18.56 b	0.177	0.106 c
Soil + 2% TR	4.44	2.849 b	18.83 b	0.185	0.156 b
Soil + 2%TR + BF	5.64	3.169 a	29.16 a	0.191	0.174 a
LSD at 5%		0.099	0.789	-	0.009

It could be concluded that the beneficial effects of organic manuring associated with the biofertilizers on the characters of the rhizospheres, as well as, their microbial activities were reflected on the vegetative characters of faba bean plants as well as their content of N and P (%) and their uptake.

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التأثير المشترك للتسميد العضوى والحيوى علىالنشاط البيولـوجى لريزوسـفير الفول البلدى وعلى نمو النباتات

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أجريت تجربة أصص بالصوبة فى المركز القومى للبحوث لدراسة التـأثير المشـترك للتسميد العضوى (٢% سماد قمامة المدن) والتسميد الحيوى (مخصب حيـوى مـن ميكروبـات الريزوبيوم + الميكروبات المذيبة للفوسفات) على النشاط الحيوى لريزوسفير نباتات الفول البلـدى وكذلك على الوزن الجاف ومحتواها من النيتروجين والفوسفور.

اقترحت معادلة لتقييم التأثيرات الناجمة عن المعاملات المختلفة التى تم اجراؤها تحـت ظروف الريزوسفير وذلك من خلال ما أطلق عليه "كفاءة معاملة الريزوسفير" وهى تعنى التـأثير النهائى الناتج من نفس المعامل تحت ظروف التربة الخالية من الجذور النباتية.

لهذا الغرض أجيت تجربة اصص لنبات الفول البلدى مع قياس تأثير المعـاملات المختلفـة علـى الريزوسفير للنباتات النامية فى التربة غير العامة والمعاملة بالسماد العضوى والسماد الحيوى.

واستخدمت لذلك قياس التغيرات الحادثة فى الكربون العضوى والنيتروجين الكلى فـى التربة، كذلك قياس كمية ثانى أكسيد الكربون المنطلقة من التربة – هذا بجانب التغيرات فى الاعداد الكلية للميكروبات واعداد الريزوبياء والميكروبات المذيبة للفوسفات كمؤشـرات ميكروبيولوجيـة وكيميائة تحت هذه الظروف. كذلك استخدم قياسات النشاط الانزيمى لكل من الانفرتيز والفوسفاتيز والديهيدروجنيز كمؤشرات للنشاط الحيوى للريزوسفير.

كذلك تم تقدير محتوى المادة الجافة فى نباتات الفول البلدى ومحتواها مـن النيتـروجين والفوسفات وامتصاصها فى كلتا النوعين من التسميد.

أظهرت نتائج هذه الدراسة أن تأثير التسـميد العضـوى + التسـميد الحيـوى علـى الريزوسفير يفوق بصفة عامة التسميد العضوى بمفرده حيث كانت أرقام جميع القياسات "لكفـاءة معاملة الريزوسفير" بالنسبة لكل المؤشرات المختبرة عالية.