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EFFECT OF RHIZOBACTERIA AND MINERAL FERTILIZATION ON YIELD AND ITS COMPONENTS OF *MELILOTUS ELEGANS* PLANTS UNDER CONDITIONS OF EL-HAMAM AREA – EGYPT

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ABSTRACT

The aim of this study was approach a maximum yield of *Melilotus* plants by integration between bio-fertilizers and mineral fertilizers especially P fertilizers.

A field experiment was conducted for two successive years (2018 and 2019) using completely randomized field experiments with three replications for each treatment in El-Hamam area, Marsa Matrouh Governorate, Egypt (between the intersection of the longitude 30º 34' 51" N and the altitude 30º 15' 40" E). *Melilotus elegans* was the investigated crop, sown in the in plots (3×4m) in rows. Biofertilization treatments were Rhizobia, phosphate dissolving bacteria (*Bacillus megatherium* and *Pseudomonas putida*). The mineral fertilization was applied as a general treatment using three rates of 15 , 30 and 45 kgP_2O_5/fed. as calcium super phosphate(15.5%P_2O_5) mixed with the soil during soil preparation . N and K fertilizers were added at one rate of 80 kg N/fed. as NH_4NO_3 and 40 kg K_2O as potassium sulphate divided into two equal doses applied at seedling and after cut one stages. The dose of 10m^3 organic manure was added.

Obtained results clearly showed that, mixed biofertilization treatment recorded highest values for yield and its components as well as total N,P and oil % in content of shoot and leaves of *Melilotus* plant for two cuts and during two growing seasons followed by *P.putida*, and then PDB while mineral P increase yield parameters with increase rates of P up to P3.

From the obtained results we can cocluded that, mixed biofertilization treatment combined with P fertilization was superior treatment for *Melilotus* plants under El-Hamam soil conditions .

Key Words: Rhizobacteria, Mineral fertilization, *Melilotus elegans* , phosphate dissolving, nitrogen fixers

INTRODUCTION

Marasco *et al.*, (2012) , reported that plant growth promoting rhizobacteria (PGPR) naturally associated with plants, have been shown

to be essential partners for improving plant tolerance to stressful conditions. **Cherif *et al*., (2015)** and **Yaish (2016)** found that endophytic bacteria species cultured from date palm roots had positive effects on plants growing under saline and /or drought conditions. These organisms may facilitate plant growth in a variety of ways including improving the availability of some nutrients such as nitrogen, phosphorus, potassium, iron and calcium or modulating plant hormone levels, providing plants with phytohormones such as auxins, cytokinins or gibberellins or by lowering plant ethylene levels (**Ryan *et al*., 2008** and **Glick, 2012**). Root-associated bacterial (rhizosphere) communities in date palm have previously been studied under saline conditions (**Ferjani *et al*., 2015**).

The effect of integration mineral fertilizers with bio fertilizers on yield components of crops were determined by **Fawy *et al*.**, (**2015**) who reported that, the integration treatment (Bio and mineral fertilizers) for yield, nutrients and biochemical components contents of wheat was (P4+Zn1 plus Mycorrhizae + Azotobacter) which achieved 5.45 and 2.21ton/fed for straw and grains respectively in sandy soil, while being 9.5 and 4.16 ton/fed in clay soil of New Valley, Egypt. **Attia *et al*.,(2015)** reported that the integration between bio and mineral fertilizers was P_2+ (AZ)+ (SD)+(PDB)+ Zn_1 under conditions of the irrigation of every 10 days which gave 2.34, 11.1, 0.99 and 1.82 for weight straw, seeds, oil and fiber (Mg/ha^{-1}) of flax plant respectively in the first season. While in the second season it achieved 2.48, 11.4, 1.09 and 1.89 (Mg/ha^{-1}). **EL-Sharabasy *et al*., (2018)** reported that, the application of bio fertilizer at rate 1:1/4:1/4 (v/v) induced significant increases in the leaf nutrient elements content (N, P, K, Fe, Mn, Zn, and Cu over control treatment. So, it can be recommended to use plant growth promoting rhizobacteria (PGPR) as a source of nitrogen (*Azotobacter chroococcum and Azospirillum brasilense*), phosphorus (*Bacillus megatherium*) and potassium (*Bacillus circulans*) at rate 1:1/4:1/4 (v/v) to improve the vegetative growth, increase chemical compositions in leaves and improved nutrients uptake of date palm plants grown under saline stress conditions.

Species belonging to the genus *Melilotus* have recently received renewed attention for use in Australian farming systems due to the need for a broader range of leguminous species suitable for saline soils (**Nichols *et al*., 2007**; **Dear and Ewing 2008**). Melilotus albus Medik. has shown considerable potential (**Evans and Kearney 2003**) and recently the cultivar Jota was released (**Evans** and **Thompson 2006**). The

potential of M. siculus (Turra) Vitman ex B. D. Jacks. (Syn M. messanensis) as a pasture species was also outlined by **Nichols *et al.* (2008)** and **Rogers *et al.*** (**2008**). When developing new pasture species it is important to know of the presence of secondary plant compounds, especially if there are animal health concerns associated with them (**Revell** and **Revell 2007**), if they affect feed intake or if they may cause tainting of food products. High concentrations of a secondary plant compound, coumarin, are a major limiting factor in the use of Melilotus species in Australia (**Evans and Kearney 2003**). Coumarin has been associated with di coumarol production upon spoilage by fungi in M. albus (**Poulton *et al.*, 1980**).

The purpose of this research was to study the effect of bio-fertilizers, and mineral fertilizers application especially P on *Melilotus elegans* plants yield under El Hamam soil conditions.

MATERIALS AND METHODS

A field experiment was conducted at two successive years (2018 and 2019) completely randomized field experiments with three replications for each treatment in El-Hamam area, Marsa Matrouh Governorate, Egypt. (between the intersection of the longitude 30º 34' 51" N and the altitude 30º 15' 40" E). Field experiment was irrigated by Nile water from Nasr canal (410 ppm). Some physico-chemical properties and available nutrients of the studied soils are reported in Table (1) according to **Page *et al,* (1982) .**

Table (1): Physico- chemical properties and available nutrients of the experimental soil*.

Depth cm	pH	E.C dS/m	OM	$CaCO_3$	Sand	Silt	Clay	CEC Cmol/kg	Texture
			%						
0-30	8.36	1.51	2.97	27.4	68.09	16.02	15.89	12.13	Sandy loam
30-60	8.44	1.65	2.15	30.6	60.48	21.16	18.36	15.10	

Soluble cations and anions in soil (me/L)							
Depth	Na	K	Ca	Mg	HCO_3-1	Cl^{-1}	$SO4^{-2}$
0-30	3.87	0.58	4.90	5.75	0.80	9.67	4.63
30-60	4.56	0.60	5.39	5.95	0.85	10.44	5.21

Available nutrients in soil (µg/g)							
Depth	N	P	K	Fe	Mn	Zn	Cu
0-30	43.4	10.4	81	4.47	3.03	0.89	0.37
30-60	41.1	8.81	87.5	5.54	3.47	1.05	0.41

Effect of rhizobacteria and mineral fertilization on yield and its components of *Melilotus elegans* plants under conditions of El-Hamam area Egypt was studies. *Melilotus elegans* was the investigated crop. Plants were sown in the plots (3×4m) in rows. The mineral fertilization was applied as a general treatment using three rates of 15, 30 and 45 kgP_2O_5/fed. as calcium super phosphate(15.5%P_2O_5) mixed with the soil during soil preparation . N and K fertilizers were added at one rate of 80 kgN/fed. as NH_4NO_3 and 40 kg K_2O as potassium sulphate divided into two equal doses applied at seedling and after cut one stages. The dose of $10m^3$ organic manure was added by mixing with 0-20 surface layer before sowing. Physical and chemical analysis of the soil are presented in Table 1.

Bio-fertilizers treatments: four different bio-fertilizers treatments (control, *P. putida & Bacillus megatherium* (PDB) and mixed bio-fertilizers treatments (*P. putida* +PDB) were performed.

Isolation of *P.putida* and Phosphate dissolving bacteria:

For isolation of *P. putida* and PDB, different soil samples were collected from soil at different sites of South Sinai and El-Hamam area.

The highest for phosphate solubilization were selected for further study according to **De Freitas *et al.*, (1997)**. The highest rhizobial isolate for nitrogen fixation according to **Page *et al.*, (1982)** and nitrogenase activity was determined according to **(Haahtela *et al.*, 1981)** for examining most active rhizobial isolate. Each isolate were grown on its specific medium containing different sodium chloride concentrations (2,4,6,8,10%), also, at different incubation temperature (25,30,40,45,50°C) and different pH (5-9) .The growth was measured at 600nm. Selected *P.putida* and PDB isolates were purified and identified according to **Bergey's Manual of Determinative Bacteriology (1994)**. The selected isolates (*P.putida* and *Bacillus megatherium*) were subjected to different biochemical tests for screening their hormonal (**Rizzolo *et al.*, 1993**) and enzymatic activity (**Barrow** and **Veltham, 1993**).

Molecular identification of bacterial isolates

Bacterial isolates were cultured in sterile test tubes containing 10 ml of nutrient broth media **(Zimbro *et al.*, 2009).** Cultures were incubated at 28^oC for 48 hours prior sending to the molecular Biology Research Unit, Assiut University for DNA extraction. Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea was used. The extracted DNA samples were sent to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing. PCR was performed using two universal primers

namely 27F (5'- AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'- GGTTACCTTGTTA CGACTT-3'). The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. Purified amplicons were sequenced in the sense and antisense directions using 27F and 1492R primers with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Sequences were further analyzed using Basic

Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done using MegAlign (DNA Star) software version 5.05.

Fresh liquid culture of *P.putida* and *Bacillus megatherium* were used for soil and foliar applications single or in combination at the rate of 10^8colony forming unit(cfu/ml).

Rhizosphere soil samples were collected at heading and harvesting stages. The samples were analysed for total counts of microorganisms according to **Nautiyal (1999)**. Counting and growing phosphate dissolving bacteria were carried out using Pikovskaya's agar medium (PVK) **Goenadi, (2000)**. Estimates of number of Pseudomonas by MPN technique were calculated using Cochran's Table. .

Soil samples were analyzed and Nitrogenase activity was measured using a standard acetylene reduction assay as described by **Haahtela *et al.*, (1981)**. For determination of phosphatase activity disodium phenylphosphate served as enzyme substrate (**Őhlinger, 1996**). Plant samples were taken at harvesting from each treatment, dried at 70°, and ground using stainless steel equipment for the determination of N,P, K, Mg, Ca and Na. Plant nutrients were determined as follows: Total nitrogen using the micro kjeldahl method **(AOAC ,1985)**. Phosphorus, potassium, calcium, magnesium and sodium using dry ashing technique according to **Cottenie *et al.*, (1982)**.

Growth parameters: at first and second cuts plants were taken from each plot for estimating plant height, fresh and dry weight.

Statistical analysis: all the obtained data from each season were exposed to the proper statistical analysis of variance according to **Gomez and Gomez (1984**). LSD at 0.05 level of significance was used for the comparison between means.

RESULTS AND DISCUSSION

Biochemical activities of Rhizobial isolates: Microbes under study known to produce a number of secondary metabolites (Table 2) which may affect growth, health of plants, and the relationships between rhizosphere soil microorganisms. Nitrogen fixation and Nitrogenase enzyme, as shown in Table 2, the microorganisms exhibited variable results in beneficial action in field **(El-Saidy and Abd El-Hai, 2011).**

Table (2): Nitrogen fixing ability for Rhizobial isolate total nitrogen and nitrogenase enzyme in nodule .

Sample No.	Total Nitrogen %	Nodule/hr MLC_2H_2/g^{-1}dry
M 1	1.69	1768
M 2	1.98	1837
M 3	1.41	1510
M 4	1.85	1804
M 5	1.87	1807
M 6	1.91	1828
M 7	1.99	1836
M 8	1.29	1436
M 9	1.37	1458
M 10	2.45	2391
M 11	2.15	2360
M 12	1.98	1837
M 13	1.60	1680
M 14	1.70	1810
M 15	2.03	2210

Phosphate solubilizing activities for bacillus and pseudomonas isolates were measured by means of inhibition zone diameter as shown in Tables 3 and 4 . Obtained results in Tables 3 and 4 proved that, the most active isolates in the phosphate solubilization was P7 and Ps4. These isolates can be selected as potentially efficient biofertilizer. Obtained results are in agreement with those obtained by **Abd El-Gawad (2014) and El-Shazly, (2020).**

Table (3): Phosphate dissolving activity for *B. megatherium* and *P.putida* isolates qualitatively (inhibition zone diameter cm) .

B. megatherium	P- dissolving activity			*P.putida*	P- dissolving activity		
	Z (cm)	C (cm)	C/Z (cm)		Z (cm)	C (cm)	C/Z (cm)
P 1	1.76	1.33	1.55	Ps 1	2.13	1.76	1.21
P 2	1.28	0.50	3.64	Ps 2	1.43	0.50	2.86
P 3	0.94	0.38	2.47	Ps 3	2.26	0.95	2.37
P 4	1.30	0.38	3.42	Ps 4	1.33	0.79	1.68
P 5	1.43	0.44	3.25	Ps 5	2.44	0.78	3.07
P 6	1.13	0.50	2.26	Ps 6	1.53	0.79	1.93
P 7	4.52	3.14	1.43	Ps 7	1.76	0.95	1.85
P 8	0.50	0.13	3.84	Ps 8	0.79	0.28	2.68
P 9	2.83	2.27	1.24	Ps 9	1.54	1.13	1.36
P 10	2.00	1.53	1.31	Ps 10	0.95	0.50	1.90

Z = Diameter of clear zone (cm)

C = Diameter of the developed colony (cm)

Spectrophotometer O. D. = 600 nm.

Table (4): Soluble phosphate activity of the tested strains *Bacillus megatherium* and *Pseudomonas putida* (quantitatively).

B. megatherium							*P.putida*						
	5	6	7	8	9	10		5	6	7	8	9	10
P 1	630	660	680	680	680	680	Ps 1	620	640	660	660	660	660
P 2	690	770	770	770	770	770	Ps 2	660	710	730	730	730	730
P 3	660	690	720	720	720	720	Ps 3	660	690	720	720	720	720
P 4	680	770	780	780	780	780	Ps 4	650	680	690	690	690	690
P 5	660	730	740	740	740	740	Ps 5	680	770	680	680	680	680
P 6	650	690	720	720	720	720	Ps 6	650	690	720	720	720	720
P 7	630	665	670	670	670	670	Ps 7	650	680	640	690	690	690
P 8	730	780	820	820	820	820	Ps 8	660	700	720	720	720	720
P 9	620	640	660	660	660	660	Ps 9	620	640	670	670	670	670
P 10	620	630	660	660	660	660	Ps 10	650	690	720	720	720	720

Field experiment

After the application of different fertilizers of bio and mineral treatments, the following exhibit will deal with the response of *Melilotus* yield. So, the effect of enhanced fertility status of soil nutrients will be examined to furnish the fertilizer treatment design on the basis of sufficient level of each nutrient under conditions of integration bio and mineral system during two successive seasons.

Effect of biofertilization treatments and phosphate levels on microbial determinations in rhizosphere of *Melilotus elegans* :

Data shown in Table 5. presented the effect of bio and mineral fertilizer treatments under study on total microbial counts. All fertilizer treatments proved to be significantly higher during two seasons. The mixed bio fertilizer treatment gave the highest effect on total microbial counts in soil than other sources (PDB , *P.putida*) followed by *P.putida* and then PDB which was the last effect on microbial activity. The total microbial count was increased with increases mineral P fertilizers rate in the two seasons. The second season had higher effect on total count microbial activity than first season. The superior fertilizer treatment was (Bio mixed+ P3) which achieved the highest count and microbial activity during the two seasons.

Table (5): Effect of biofertilization treatments and phosphate levels on microbial determinations in rhizosphere of *Melilotus elegans* :

Treatment		Total count 10^6 CFU / g dry soil .			
		C 1 S 1	C 1 S 2	C 2 S 1	C 2 S 2
PDB	Control	18	23	25	26
	P 1	50	64	67	69
	P 2	75	80	128	94
	P3	105	105	135	122
P.putida	Control	28	31	36	35
	P 1	80	86	98	91
	P 2	86	92	147	112
	P3	113	110	153	141
Mixed	Control	35	37	44	46
	P 1	99	104	119	120
	P 2	112	119	198	163
	P3	128	130	205	182
LSD 0.05 Bio-fertilizer		1.27	1.23	2.07	1.71
LSD 0.05 P fertilizer		1.55	1.50	2.54	2.10
LSD0.05 2 factors		2.20	2.13	3.59	2.96

C : Cut (C 1 : First cut – C 2 : Second cut), S : Season (S 1 : First season – S 2 : Second season), **PDB** : P-dissolvers, ***P.putida***, **Mix** : PDB + *P.putida*

The results obtained at Table 6 indicated that the *P.putida* had higher activity than PDB to dissolves and densities of *P.putida*. The fertilizers treatments studied take the same trend in total microbial activity. The present results agree with that obtained by **Revillas *et al.*, (2005)** and **Yousefi *et al.*, (2011).**

Table (6): Effect of biofertilization treatments and phosphate levels on densities of Pseudomonas and P-dissolvers. in rhizosphere of *Melilotus elegans.*

Treatment		*P.putida*×10^2 CFU / g dry soil .				P-dissolvers×10^2 CFU / g dry soil .			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	17	19	23	26	14	17	21	23
	P 1	48	52	62	68	40	46	58	61
	P 2	60	65	100	110	56	57	92	103
	P3	84	90	130	164	78	79	118	148
P.putida	Control	26	27	37	42	22	22	34	38
	P 1	75	75	100	110	62	62	93	100
	P 2	122	128	192	218	110	113	179	199
	P3	165	167	215	250	151	159	202	241
Mix	Control	42	44	64	70	37	42	58	65
	P 1	120	122	173	184	105	116	158	171
	P 2	185	187	235	258	168	169	214	236
	P3	193	198	270	290	172	181	255	266
LSD 0.05 Bio-fertilizer		2.12	2.13	2.83	3.13	1.93	1.99	2.64	2.91
LSD 0.05 P fertilizer		2.59	2.61	3.46	3.83	2.37	2.43	3.23	3.56
LSD0.05 2 factors		3.66	3.69	4.90	5.41	3.35	3.44	4.57	5.04

Effect of biofertilization treatments and phosphate levels on organic carbon content %

The results in Table 7 reported that the organic carbon % increase with the increasing of P fertilizer rates and bio-fertilizers but the most effective treatment was (bio Mixed + P3) which gave the highest values than others treatments. The organic carbon % Tooke the same trend of previous studied parameters.

Table (7): Effect of biofertilization treatments and phosphate levels on organic carbon %

Treatment		Time			
		C 1 S 1	C 1 S 2	C 2 S 1	C 2 S 2
PDB	Control	0.046	0.068	0.052	0.068
	P 1	0.130	0.190	0.140	0.180
	P 2	0.230	0.310	0.250	0.280
	P3	0.290	0.380	0.320	0.320
P.putida	Control	0.063	0.086	0.067	0.080
	P 1	0.180	0.240	0.180	0.210
	P 2	0.330	0.480	0.340	0.460
	P3	0.380	0.510	0.400	0.480
Mix	Control	0.077	0.104	0.089	0.095
	P 1	0.220	0.290	0.240	0.250
	P 2	0.460	0.610	0.520	0.560
	P3	0.540	0.680	0.560	0.650
LSD 0.05 Bio-fertilizer		0.005	0.007	0.006	0.007
LSD 0.05 P fertilizer		0.007	0.009	0.007	0.008
LSD0.05 2 factors		0.010	0.012	0.010	0.012

Effect of bio and mineral P levels on yield components of *Melilotus elegans* plants

Results in Tables (8, 9 ,10,11,12 and 13) for yield parameters of Melilotus plants as number of nodules & dry weight of nodules, nodules nitrogen % and nodules, plant height (cm), shoot fresh & dry weight (kg/4plants), leaves fresh & dry weight (kg / 4 plants) and yield fresh & dry weight (kg / 4 plants).

Table (8): Effect of bio and mineral P levels on yield fresh and dry weight (kg / 4 plants) of *Melilotus elegans* plants

Treatment		Fresh weight				Dry weight			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.95	1.37	2.08	2.56	0.12	0.30	0.53	0.67
	P 1	2.70	3.80	5.63	6.73	0.33	0.84	1.43	1.76
	P 2	3.30	4.20	6.56	7.60	0.35	0.88	1.48	1.89
	P3	4.10	5.30	8.00	8.70	0.44	1.01	1.72	2.11
P.putida	Control	1.40	1.77	2.78	3.34	0.12	0.35	0.66	0.73
	P 1	4.00	4.93	7.50	8.80	0.35	0.96	1.78	1.91
	P 2	4.50	5.40	8.00	9.10	0.45	0.98	1.83	2.01
	P3	5.10	6.00	8.93	10.30	0.52	1.22	1.91	2.70
Mix	Control	1.90	1.95	3.03	3.38	0.22	0.43	0.70	1.08
	P 1	5.42	5.43	8.20	8.90	0.63	1.20	1.89	2.85
	P 2	6.11	6.42	9.32	9.90	0.74	1.28	2.01	3.08
	P3	6.96	7.56	10.86	12.40	0.98	1.48	2.77	3.23
LSD 0.05 Bio-fertilizer		0.065	0.067	0.097	0.106	0.009	0.013	0.022	0.030
LSD 0.05 P fertilizer		0.080	0.082	0.118	0.130	0.011	0.016	0.028	0.036
LSD0.05 2 factors		0.113	0.117	0.167	0.184	0.015	0.023	0.039	0.052

Table (9): Effect of bio and mineral P levels on number of nodules /4 plants and dry weight of nodules (g /4 plants) of *Melilotus elegans* plants .

Treatment		Number of nodules				Dry weight of nodules			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	13	15	10	12	0.006	0.015	0.009	0.017
	P 1	38	43	27	31	0.016	0.042	0.024	0.046
	P 2	49	54	36	40	0.052	0.055	0.056	0.058
	P3	65	72	50	61	0.056	0.059	0.058	0.067
P.putida	Control	28	32	22	25	0.006	0.018	0.010	0.020
	P 1	80	88	60	65	0.018	0.050	0.028	0.052
	P 2	104	116	87	74	0.056	0.057	0.058	0.068
	P3	152	163	28	134	0.060	0.064	0.065	0.070
Mix	Control	35	43	34	37	0.007	0.024	0.011	0.026
	P 1	100	120	91	98	0.020	0.066	0.030	0.068
	P 2	141	157	105	117	0.060	0.069	0.070	0.074
	P3	165	183	139	142	0.070	0.089	0.084	0.093
LSD 0.05 Bio-fertilizer		1.77	1.94	1.36	1.52	0.0009	0.0008	0.0011	0.0008
LSD 0.05 P fertilizer		2.17	2.38	1.67	1.86	0.0011	0.0009	0.0013	0.0010
LSD0.05 2 factors		3.07	3.36	2.36	2.63	0.0015	0.0013	0.0019	0.0014

Table (10): Effect of bio and mineral P levels on nodules nitrogen % and nodules / hr/M LC2 H4 / g dry of *Melilotus elegans* plants.

Treatment		Nodules Nitrogen %				Nodules / hr/M LC2 H4 / g $^{-1}$ dry			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.46	0.49	0.39	0.42	119	129	93	102
	P 1	1.31	1.36	1.06	1.11	295	359	250	269
	P 2	1.61	1.69	1.20	1.26	460	526	340	372
	P3	1.91	1.96	1.55	1.59	775	810	458	481
P.putida	Control	0.53	0.58	0.41	0.43	316	332	134	146
	P 1	1.52	1.60	1.10	1.14	860	922	363	384
	P 2	1.85	1.90	1.45	1,58	1305	1435	415	489
	P3	1.99	2.05	1.63	1.73	1613	1768	612	645
Mix	Control	0.66	0.70	0.53	0.56	443	494	215	232
	P 1	1.89	1.95	1.42	1.47	1250	1372	580	610
	P 2	1.98	2.04	1.75	1.87	1710	1836	692	745
	P3	2.03	2.41	1.78	1.99	2302	2418	810	870
LSD 0.05 Bio-fertilizer		0.021	0.022	0.017	0.019	23	25	8	8
LSD 0.05 P fertilizer		0.025	0.027	0.021	0.023	29	30	10	10
LSD0.05 2 factors		0.036	0.038	0.030	0.033	41	43	13	14

Table (11): Effect of bio and mineral P levels on plant height (cm) of *Melilotus elegans* plants.

Treatment		Time			
		C 1 S 1	C 1 S 2	C 2 S 1	C 2 S 2
PDB	Control	22	30	18	29
	P 1	62	83	49	76
	P 2	69	85	59	78
	P3	73	87	63	80
P.putida	Control	22	32	19	31
	P 1	63	90	50	82
	P 2	73	92	60	85
	P3	78	44	64	86
Mix	Control	25	34	19	32
	P 1	72	96	52	84
	P 2	76	97	65	87
	P3	83	101	67	87
LSD 0.05 Bio-fertilizer		0.79	0.97	0.66	0.82
LSD 0.05 P fertilizer		0.97	1.19	0.80	1.00
LSD0.05 2 factors		1.37	1.69	1.14	1.42

Table (12): Effect of bio and mineral P levels on shoot fresh and dry weight (kg / 4 plants) of *Melilotus elegans* plants.

Treatment		Fresh weight				Dry weight			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.543	0.727	1.295	1.414	0.075	0.098	0.310	0.360
	P 1	1.550	2.020	3.500	3.720	0.215	0.273	0.838	0.947
	P 2	2.100	2.870	4.080	4.410	0.233	0.291	0.851	0.993
	P3	2.950	3.410	4.830	5.220	0.289	0.317	0.992	1.720
P.putida	Control	1.001	1.162	1.573	1.756	0.083	0.098	0.346	0.545
	P 1	2.680	2.950	4.250	4.620	0.238	0.273	0.935	1.435
	P 2	3.110	3.820	4.970	5.410	0.259	0.291	0.962	1.475
	P3	4.320	5.690	6.040	6.500	0.342	0.389	1.201	1.710
Mix	Control	1.208	1.472	1.972	2.208	0.127	0.141	0.409	0.631
	P 1	3.450	3.810	5.330	5.810	0.363	0.393	1.105	1.660
	P 2	4.720	5.970	6.430	6.890	0.423	0.472	1.355	1.844
	P3	5.530	6.460	7.210	7.940	0.615	0.680	1.710	2.210
LSD 0.05 Bio-fertilizer		0.054	0.065	0.066	0.071	0.005	0.006	0.014	0.020
LSD 0.05 P fertilizer		0.066	0.080	0.081	0.087	0.006	0.007	0.018	0.025
LSD0.05 2 factors		0.093	0.113	0.114	0.124	0.009	0.010	0.025	0.035

Table (13): Effect of bio and mineral P levels on leaves fresh and dry weight (kg / 4 plants) of *Melilotus elegans* plants.

Treatment		Fresh weight				Dry weight			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.455	0.648	0.858	1.018	0.049	0.063	0.226	0.317
	P 1	1.300	1.800	2.320	2.680	0.141	0.176	0.611	0.834
	P 2	1.650	1.990	2.630	3.090	0.160	0.195	0.642	0.842
	P3	1.980	2.510	3.110	3.570	0.189	0.227	0.803	0.925
P.putida	Control	0.581	0.774	1.214	1.414	0.060	0.070	0.289	0.354
	P 1	1.660	2.150	3.280	3.720	0.172	0.194	0.781	0.931
	P 2	2.150	2.760	3.840	4.310	0.205	0.268	0.798	0.959
	P3	2.700	2.980	4.010	4.550	0.242	0.296	0.933	0.998
Mix	Control	0.728	0.904	1.354	1.486	0.069	0.084	0.326	0.379
	P 1	2.080	2.510	3.660	3.910	0.196	0.232	0.881	0.997
	P 2	2.790	3.110	3.910	4.600	0.258	0.310	0.989	1.100
	P3	3.220	3.670	4.180	4.950	0.297	0.357	0.132	1.430
LSD 0.05 Bio-fertilizer		0.031	0.033	0.041	0.047	0.0027	0.0033	0.0103	0.0115
LSD 0.05 P fertilizer		0.038	0.041	0.050	0.057	0.0033	0.0041	0.0126	0.0140
LSD0.05 2 factors		0.053	0.058	0.070	0.081	0.0047	0.0057	0.0178	0.0198

As for bio-fertilization effect on yield components, the sequences are as follows: mixed biofertilization was the first power followed by *P.putida* then PDB and the main effect of P fertilization was P3>P2>P1 and found true for the two seasons. The treatment of mixed of (*P.putida* + PDB) gave the highest value of yield components under study. The present results are in agreement with those obtained by **Evans and Kearney (2003) , Nichols *et al.*, (2007) and Dear and Ewing (2008).**

Effect of bio and mineral P fertilizers on nutrients contents and oil% of Melilotus plants

Results in Tables (14, 15 and 16) for nutrients contents of (N and P) for shoot and leaves of Melilotus as well as oil percentage are shown in Tables (14, 15 and 16).

Table (14): Effect of bio and mineral P fertilizers on shoot and leaves nitrogen % of *Melilotus elegans* plants.

Treatment		Shoot Nitrogen %				Leaves Nitrogen %			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.42	0.59	0.54	0.69	0.48	0.53	0.64	0.69
	P 1	1.2	1.65	1.47	1.82	1.38	1.47	1.74	1.82
	P 2	1.38	1.7	1.7	1.88	1.52	1.61	1.88	1.94
	P3	1.43	1.97	1.74	2.06	1.61	1.73	1.89	12.15
P.putida	Control	0.43	0.65	0.58	0.75	0.55	0.59	0.71	0.85
	P 1	1.24	1.8	1.56	1.97	1.56	1.65	1.92	2.24
	P 2	1.52	1.92	1.61	2.15	1.68	1.82	2.01	2.37
	P3	1.85	2.01	1.82	2.32	1.79	1.91	2.19	2.68
Mix	Control	0.53	0.62	0.62	0.76	0.56	0.62	0.80	0.88
	P 1	1.5	1.73	1.67	2.01	1.61	1.73	2.16	2.32
	P 2	1.61	1.88	1.74	2.46	1.82	1.88	2.59	2.47
	P3	1.74	1.97	1.92	2.82	1.94	2.01	2.68	2.77
LSD 0.05 Bio-fertilizer		0.017	0.019	0.018	0.024	0.018	0.019	0.024	0.105
LSD 0.05 P fertilizer		0.021	0.024	0.022	0.030	0.022	0.023	0.029	0.128
LSD0.05 2 factors		0.030	0.034	0.031	0.042	0.032	0.033	0.041	0.181

Table (15): Effect of bio and mineral P fertilizers on shoot and leaves Phosphorus %. of *Melilotus elegans* plants

Treatment		Shoot Phosphorus %				Leaves Phosphorus %			
		Time							
		C1 S1	C1 S 2	C2 S1	C2 S2	C1 S 1	C1 S 2	C2 S 1	C2 S 2
PDB	Control	0.057	0.063	0.078	0.095	0.080	0.090	0.093	0.100
	P 1	0.164	0.174	0.211	0.250	0.228	0.250	0.250	0.262
	P 2	0.172	0.184	0.240	0.262	0.240	0.258	0.262	0.273
	P3	0.186	0.192	0.262	0.271	0.250	0.262	0.271	0.281
Azoto.	Control	0.061	0.069	0.101	0.110	0.083	0.092	0.102	0.109
	P 1	0.174	0.192	0.273	0.289	0.236	0.255	0.275	0.288
	P 2	0.188	0.196	0.292	0.302	0.250	0.266	0.280	0.296
	P3	0.195	0.210	0.292	0.302	0.258	0.273	0.291	0.307
Mix	Control	0.064	0.086	0.106	0.114	0.084	0.094	0.110	0.111
	P 1	0.182	0.240	0.287	0.301	0.240	0.261	0.296	0.291
	P 2	0.196	0.258	0.291	0.310	0.258	0.275	0.301	0.313
	P3	0.211	0.262	0.302	0.321	0.262	0.281	0.313	0.324
LSD 0.05 Bio-fertilizer		0.0020	0.0024	0.0029	0.0029	0.0026	0.0027	0.0029	0.0030
LSD 0.05 P fertilizer		0.0024	0.0029	0.0036	0.0036	0.0032	0.0033	0.0035	0.0036
LSD0.05 2 factors		0.0034	0.0041	0.0050	0.0051	0.0021	0.0047	0.0050	0.0051

Table (16): Effect of bio and mineral P fertilizers on shoot and leaves oil % of *Melilotus elegans* plants .

Treatment		Time			
		C 1 S 1	C 1 S 2	C 2 S 1	C 2 S 2
PDB	Control				
	P 1	0.295	0.325	1.825	2.050
	P 2	0.365	0.445	2.040	2.320
	P3	0.580	0.959	2.220	2.645
P.putida	Control	0.231	0.441	0.796	1.018
	P 1	0.660	1.225	2.150	2.680
	P 2	0.750	1.305	2.250	2.885
	P3	0.980	1.595	2.625	3.635
Mix	Control	0.361	0.511	1.190	1.609
	P 1	1.030	1.420	3.215	4.235
	P 2	1.390	1.540	3.660	4.365
	P3	1.470	1.855	4.020	4.555
LSD 0.05 Bio-fertilizer		0.0155	0.0202	0.0362	0.0437
LSD 0.05 P fertilizer		0.0190	0.0247	0.0443	0.0535
LSD0.05 2 factors		0.0269	0.0350	0.0626	0.0757

The main effect of bio-fertilizers on N, P and oil% content in shoot and leaves of Meliletus plants followed the trend of mix >*P.putida* >PDB . The nutrients increased with increasing P rates up to P3. The integration between bio and mineral fertilizers were achieved the highest nutrients content of Meliletus plants. The superior treatment was Bio-Mixed (*P.putida* +PDB) which achieved the highest values of nutrients content during two seasons. The results agree with those obtained by **Cherif *et al.*, (2015) ; Yaish (2016) and El-Sharabasy *et al.*, (2018).**

CONCLUSION

The mixed of bio fertilizer treatment had the highest effect on total counts microbial activity in soil, the sequence of mixed biofertilization treatment>*P.putida* > PDB and increased with increasing P addition rates up to P3. The highest values were obtained due to the addition treatment of (*P.putida* + PDB) + P3 which was the superior treatment as compared with the other treatments and that found true for the two plant cuts and during the two successive growing seasons. The mineral nutrients increase with increase P rates especially P mineral treatments. The integration between bio and mineral fertilizers were achieved the highest nutrients content of *Melilotus* plants. The superior treatment was Bio-Mixed (*P.putida* +PDB) which achieved the highest values of nutrients content during two seasons.

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تأثير الريزوباكتريا والتسميد المعدني على المحصول ومكوناته لنبات الحندقوق تحت ظروف منطقة الحمام- مصر

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أجريت تجربة حقلية لمدة سنتين متتاليتين (2018 و 2019) باستخدام تجارب حقلية عشوائية كاملة بواقع ثلاث مكررات لكل معاملة في منطقة الحمام محافظة مرسي مطروح – مصر. (بين تقاطع خط الطول 30° 34' 51" شمالاً والارتفاع 30° 15' 40" شرقاً). كان نبات الحندقوق *Melilotus elegans* هو المحصول الذي تم زرعه في قطع (3×4م) على شكل صفوف. أما معاملات التسميد الحيوي فهي الريزوبيا والبكتيريا المذيبة للفوسفات الباسيلس ميجاتيريم *Bacillus megatherium* والسيدوموناس بيوتيدا *Pseudomonas putida* تم التسميد المعدني كمعاملة عامة بثلاث معدلات 15 و 30 و 45 كجم P_2O_5 / فدان. حيث يخلط سوبر فوسفات الكالسيوم (15.5 P_2O_5%) مع التربة أثناء تحضير التربة للزراعة .

تمت إضافة الأسمدة النيتروجينية والبوتاسيوم بمعدل واحد وهو 80 كجم نيتروجين /فدان. علي صورة نترات امونيوم و40 كجم على هيئة كبريتات البوتاسيوم مقسمة على جرعتين متساويتين عند الشتلات وبعد الحش في مرحلة واحدة. تمت إضافة 10م^3 من السماد العضوي لكل المعاملات تحت الدراسة. استهدفت هذه الدراسة تحقيق أقصى إنتاجية لنباتات الحندقوق من خلال التكامل بين الأسمدة الحيوية والمعدنية وخاصة الأسمدة الفوسفاتية.

أظهرت النتائج التي تم الحصول عليها بوضوح أن معاملة التسميد الحيوي المختلط سجلت أعلى القيم للمحصول ومكوناته يليه *P.putida* وكانت المعاملة Bio- mixed (*P.putida*+PDB) التي حققت أعلى قيم لمكونات المحصول وخلال الموسمين خاصة مع المعدل الاعلى P3 والتي حققت اعلي استفادة وتكامل بين الاسمدة الحيوية والمعدنية و أعطت أعلي قيم لمكونات المحصول ومحتواه من عناصر النيتروجين والفوسفور وكذلك نسبة الزيت به ووضح ذلك لكلا الحشتين الاولي والثانية وكذلك خلال موسمى الزراعة وتوصي الدراسة باستخدام مخلوط الاسمدة الحيوية + المعدل الاعلي من التسميد الفوسفاتي P3 تحت ظروف منطقة الدراسة بمنطقة الحمام – محافظة مرسي مطروح – مصر