EFFECT OF BIOFILM WITH BIOFERTILIZER OF *Pseudomonas fluorescens* AND *Rhizobium leguminosarum* ,CHEMICAL FERTILIZER LEVEL AND ADDATION TECHNIQUE ON SOME GROWTH AND YIELD TRAITS OF WHEAT*(Triticum asetivum L.)*

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ABSTRACT

This Research was performed to evaluate the efficiency of Biofilm Biofertilizer produced from local isolates of *Pseudomonas fluorescens, Rhizobium leguminosarum*, with two addition technique include with bentonite as carrier and with liquid inoculant(without carrier) and three levels of chemical fertilizers (zero,25,50)% of recommendation on some growth and yield traits of wheat variety Rashid Biofilm Biofertilizer and chemical fertilizer affect significantly on all growth characters of wheat plant, Bentonite gave the best result compare with liquid inoculant, use of Biofilm Biofertilizer with 50% of the chemical fertilizer recommendation gave highest growth and yield of wheat .The duple Biofilm Biofertilizer of *P. fluorescens* plus *R. leguminosarum* was superior in enhancing growth and yield of wheat plant .The treatment of Biofilm Biofertilizer of *P. fluorescens* and *R. leguminosarum* with Bentonite and 50 chemical fertilizer recommendation gave45.89cm^2, 47.77 spad,70.00 gm,39.81mgN g^{-1} 4.931mgP g^{-1}35.471mgK g^{-1} of leaf area, chlorophyll content, weight of 1000 grains, Nitrogen, Phosphorus, Potassium uptake in the vegetative part respectively.of plant

Key words: Biofilm Biofertilizer, carrier, chemical fertilizer and wheat
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مجلة العلوم الزراعية العراقية -2018 :49(4):646-654 عبدالرضا وجاسم

تأثير الغشاء الحيوي مع السماد الحيوي لبكتريا *Pseudomonas fluorescens* و *Rhizobium leguminosarum* ومستويات السماد الكيميائي وطريقة اضافة اللقاح في بعض صفات النمو و الحاصل للحنطة

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المستخلص

نفذت هذه التجربة لتقييم كفاءة السماد الحيوي المدعم بالغشاء الحيوي المنتج من عزلات محلية لبكتريا *Pseudomonas fluorescens* و *Rhizobium leguminosarum* مع طريقتين للإضافة هما استعمال البنتونايت كحامل للقاح او اللقاح السائل مباشرة (بدون حامل) وثلاث مستويات من السماد الكيميائي هي (صفرو25و50) %من التوصية السمادية في بعض صفات النمو والحاصل لمحصول الحنطة صنف رشيد . ادى استعمال السماد الحيوي المدعم بالغشاء الحيوي الى التأثير معنويا في جميع صفات النمو المدروسة لنبات الحنطة ،ادى استعمال البنتونايت لإعطاء نتائج افضل عند استعماله كحامل للقاح مقارنة باللقاح السائل، ان استعمال السماد الحيوي مع غشاء الحيوي مع 50% من التوصية السمادية كان قد اعطى نتائج افضل لصفات النمو المدروسة، ادت اضافة السماد الحيوي المزدوج والغشاء الحيوي المتكون من *P. fluorescens* و*R. leguminosarum* لتفوق هذه المعاملة في تحفيزه لنمو نبات الحنطة. اعطت معاملة السماد الحيوي ذي الغشاء الحيوي لبكتريا *P. fluorescens* و *R.leguminosarum* باستعمال البنتونايت مع 50% من التوصية السمادية 45.89سم^2 و47.77 سباد و70.00غم و39.81 ملغم N غم$^{-1}$ و4.93 ملغم Pغم$^{-1}$و35.47 ملغم Kغم$^{-1}$للمساحة الورقية ومحتوى الكلوروفيل ووزن الف حبة و النتروجين و الفسفور و البوتاسيوم الممتص في الجزء الخضري للنبات بالتتابع.

كلمات مفتاحية: سماد حيوي، الغشاء الحيوي، حامل، اسمدة معدنية، حنطة.
البحث مستل من اطروحة الدكتوراه للباحث الثاني.

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INTRODUCTION

Agricultural development require optimum use of microorganisms activities to increase the available of nutrient for plants and this technique known as biofertilization which are an alternative, inexpensive and appropriate source for environment in comparison with chemical fertilizer the difficulty Lies in the spread of this technology is in enabling these microorganisms to survive effectively in the plant rhizosphere and soil (20). To solve this problem ,researchers carried out many attempts to extend the survival of microorganisms in soil through use different carriers but despite the remarkable success of this method they sought to find other possibilities to add biofertilizer by focusing on the importance of Biofilm Biofertilizer and developing strategies for the survival of microorganisms alive longer duration (16).Biofilm spread in different environments including soil and their presence, varies between nutrient rich rhizosphere soil to soil poor with nitrogen ,phosphorus , water and other nutrients (28). Different genera and species of bacteria are able to form biofilm on the surface of roots like *Rhizobium* and *Pseudomonas fluorescens* and the later play other important role in biocontrol and plant growth promoting Rhizobacteria (11). *Pseudomonas fluorescen*s bacteria present in various plants rhizosphere and act as PGPR that lead to increasing nutrients (19). Rhizobia are important symbiotic nitrogen fixers which produce different hormones, growth factors and vitamins which stimulate seeds germination and increase photosynthesis pathways in addition to protect plant against diseases (15).The aim of this research was to evaluate the efficiency of Biofilm Biofertilization of local isolates of *Pseudomonas fluorescens* and *Rhizobium leguminosarum* with three levels of chemical fertilizer and two addation technique include with bentonite as carrier and liquid inoculant on some growth and yield traits of wheat (*Triticum asetivum L*).

MATERIALS AND METHODS

Bacterial Biofilm Collection

Pseudomonas fluorescens and *Rhizobium leguminosarum* isolates, were tested previously it form biofilm and were grow in test tube contain 50ml of Tryptic soya broth and incubate at 28c°.Biofilm collected under control conditions and put in scrow tubes phosphate Buffer saline(PBS).

Modification of culture media

Different modification were carried out in the current study for KingB and Yeast Extract Manitol Agar medium in order to make it more suitable for the above bacterial isolates when they grow together.

Inoculation of Bentonite

Bentonite slurry suspension was prepared (20%w:v)using Distilled water in flasks and sterilized at 121c°,15b/in^{2}for 20 minutes ,1ml of *P. fluorescens* inoculum grow in kingB broth medium was added to the first flask and other 1ml of each *P.fluorescens* and *R. leguminosarum* inoculum grow in the modified broth medium was added to the second flask ,then 100ml of 10%arabic gum solution was added to all flasks. wheat seeds were sterilized by ethyl alcohol 95% and soudium hypochloride 15% then washed repeatedly by distilled water ,seeds were added to the flasks and incubated 72hr. at 28c°then it spreaded over cardboard, collected biofilm power was added to the inoculated seeds and lay aside for half an hour befor planting

Biological Experiment

The biological experiment include the following factors. The first Factor : Biofilm Biofertilizer, include three level denote as follow ,P1 Biofilm Biofertilizer of *Pseudomonas fluorescens*,P3 Biofilm Biofertilizer of both *P.fluorescens* and *R. leguminosarum* ,P0:control (without inoculation). The second factor :addation technique ,include two types denote M1: Biofertilizer with Bentonite as carrier and M0:

denoteto liquid inoculation without carrier. The Third Factor: chemical fertilizer ,include three levels denote F0: without addation, F1 addation of 25%of the recommendation and F2 addation of 50%of the recommendation. The biological factorial experiment was conducted using Complete Randomized Design with three replicates ,the experiment unit =3×2×3×3=54 plus three experiment units (complete chemical fertilizer recommendation without biofertilizer) so the total experiment units =57,soil were air dryad, 10kg of 4mm diameter of dry sieved soil were put in pots which sterilized previously from inside by ethyl alcohol Eight inoculated wheat seeds were planting in each pot at 24-11-2015,urea (46%N)was used as nitrogen fertilizer and added by two doses ,the first at planting and the other at elongation period with note that the recommendation was 160kgNh^{-1},one dose of triple super phosphate (20%P)was added at planting note that the recommendation was 100kg$Ph^{-1}$and one dose of potassium sulphate (50%k_2o)was added also at planting period note that it's recommendation was 100kgKh^{-1}.After one week of germination five plant only were leave for each pot ,plants were harvested at 28-4-2016. Chlorophyll content (spad) ,leaf area (cm^2)weigh of 1000grian (g),Nitrogen, Phosphorus and Potassium uptake (mgg^{-1})were estimate after harvesting .chemical ,physical and biological properties of pots soil were analyzed in the laboratory of soil Department .College of Agriculture – University of Al-Muthna. Data were analyzed as analysis of variance using a statistical analysis program Genstat while the means were compared using LSD0.05.

Table1. Some chemical, physical and biological characteristics of soil before planting

Adjective		The value
pH In the extract 1:1		7.2
EC	dsm^{-1}	2.6
Ca^{+2}		1.15
Mg^{+2}		0.57
$CO3^{2-}$		Nil
$HCO3^{-}$	Centimolle L^{-1}	0.49
CL^{-}		0.98
$SO4^{2-}$		1.27
Organic matter O.M(gm kg -1		1.5
N available mg kg^{-1}		24.2
P available mg kg^{-1}		11
K available mg kg^{-1}		165
sand		110
silt	gm kg^{-1}soil	480
Clay		410
Soil texture		Silty Clay
Total bacteria		10^7 x1.4
Total fungi	CFUg^{-1}dry soil	10^5 x0.32

RESULTES AND DISCCUATION

Flag leaf area

Results in Table 2 shows that inoculation with Biofilm Biofertilizer affect significantly in increasing leaf area in comparison with control uninoculuted treatment with the superiority of P3 treatment (37.69cm^2)which represent increasing percentage 48.85%compare to control treatment , significant differences were obtained between types of Biofilm Biofertilizer ,P3gave the superiority with increasing %29.64%over P1 treatment ,these results agree with other Researchers (29) that they found significant effect of Biofilm Biofertilizer in increasing leaf area of tea plant and this increasing may due to the increasing in the availability of nutrients like nitrogen which play important role in protoplasm synthesis ,Enzymes ,co enzymes growth regulators and chlorophyll content(2).

According to Table 2 results which, indicates the effect of chemical fertilizer on leaf area ,F2 treatment gave increasing% of 27.74% in compare with control treatment ,these results conform with the results as Al–Haidere (6) when he obtained increasing in wheat leaf area with the increasing of nitrogen levels and this could be due to the role of nitrogen in activation of meristem cells and Auxins (17). Results also shows the superior of Bentonite carrier (M1 treatment) with 28.60cm^{2}leaf area in compare with liquid inoculant(M0 treatment) that gave 27.66cm^2. The second order interaction shows the superiority of P3F2 treatment when compare with all others of leaf area 44.51cm^{2}and this agree with the results Seneviratne et al (29) , they found increase in leaf area after addation of 50% of chemical fertilizer recommendation and this may due to energy source and available phosphorus that increase the proliferation of microorganisms that form biofilm which produce more organic acids reflect in increasing the available nutrients and all these reflect on increasing leaf area (12). On the other hand P3M1 treatment gave leaf area mean of 38.83cm^{-1}compare with the lowest value of F0M1 treatment 22.83cm^{2}while the statistical results shown in table 2 indicate that the triple interaction treatment P3F2M1 gave the highest value 45.89cm^{2}of leaf area and this may due not to the increasing of nutrient only but to the protection of plant against different diseases (10).

Table 2. Effect of biofilm with biofertilizer, type of carrier and level of chemical fertilizer in the area of the flag leaf area (cm^2)for wheat plant

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
	M0	17.43	18.87	20.35	18.88	
P0	M1	18.22	20.11	20.72	19.68	
P× F		17.83	19.49	20.54		19.28
P1	M0	21.28	25.94	29.99	25.74	
P. fluorescens	M1	21.75	26.27	33.89	27.30	
P×F		21.52	26.11	31.94		26.52
P3	M0	29.78	36.72	43.13	36.54	
P. fluorescens Rhizobium	M1	32.37	38.23	45.89	38.83	
P× F		31.08	37.48	44.51		37.69
RateF		23.48	27.69	32.33	RateM	
F+M	M0	22.83	27.18	31.16	27.06	
	M1	24.11	28.20	33.50	28.60	
Fertilizer standard treatment 100%		50.71				

P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
0.614	0.614	0.501	1.0632	0.868	0.868	1.504	

Chlorophyll content

The results were shows that addation of Biofilm Biofertilizer cause significant increase in Chlorophyll content of wheat leafs compare with control treatment.P3 treatment gave Chlorophyll content 46.68 spad (Table 3), with increasing %of 35.07%from control that gave 30.31 spad and this could be due to gibberellins that produce by microorganisms (33),furthermore the double Biofilm Biofertilizer cause increase in nitrogen in particular $NH_4$and increase root system (13). Table 3 indicates also the role of chemical fertilizer levels especially F2 treatment in obtainment the highest value for chlorophyll content, on the other hand P3F2 treatment gave the highest value 52.40spad and these results reflect the role of Biofilm Biofertilizer support growth regulators that enhance root growth (9)and increasing cells divisition and elongation (5). Concerning the interaction between chemical and carriers, F2M1 treatment was superiored in it's chlorophyll content that was 42.49spad while the third order interaction treatment P3F2M1 gave the highest value 53.50spad of chlorophyll content and this could be due to the role of Biofilm in enhancing the population of microorganisms in soil and rhizosphere more than the liquid inoculum alone (24),(32).

Table 3. Effect of biofilm with biofertilizer, type of carrier and level of chemical fertilizer in chlorophyl content of wheat plant(spad).

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
P0	M0	28.56	30.30	31.37	30.08	
	M1	28.84	31.02	31.77	30.54	
P× F		28.7	30.66	31.57		30.31
P1 P. fluorescens	M0	36.07	38.00	40.61	38.23	
	M1	36.76	38.47	42.21	39.15	
P×F		36.42	38.24	41.41		38.69
P3 P. fluorescens Rhizobium	M0	40.05	45.42	51.30	45.59	
	M1	41.49	48.33	53.50	47.77	
P× F		40.77	46.88	52.40		46.68
RateF		35.30	38.59	41.79	RateM	
F+M	M0	34.89	37.91	41.09	37.96	
	M1	35.70	39.27	42.49	39.15	
Fertilizer standard treatment 100%		59.38				

P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
0.598	0.598	0.488	1.036	0.846	0.846	1.465	

Weight of 1000 grains(gm).

Results in the Table 4 indicates that P3 treatment gave the highest value of 1000grains (55.88g) with increasing %59.36%compare with control treatment that gave 22.71g and these could be due to the role of Biofilm in increasing the efficiency of microorganisms to produce growth hormones (18)which effect on cell division and elongation that increase the surface area of roots and this reflect on more uptake of nutrients which gave highest weight of grains (14). Results showed the superiority of F2 level of chemical fertilizer in increasing 29.57% compare to control and this may be due to the role fertilizer in the balance of nutrients and carbohydrate ,protein content of cells (21). Table 4 indicates that M1 treatment gave 39.67g of the Weight of 1000 grain over control and this may due to the high cation exchange capacity of Bentonite(16)which help to survive of bacteria for long time in rhizosphere (4).On the other hand results showed the superiority of P3M1 treatment when it record the highest value 58.90g and this could be due to the efficiency of microorganisms in this carrier that reflect to more profelration and production of growth promoting substance (7)while the triple interaction treatment P3F2M1 gave the best results and the Weight achieved 70.00g and this may be due to the contribution of this treatment in acceleration of female flowering as a result of increasing in synthesis of metabolite that affect on more nutrients uptake and full grain (3,)(30),(34).

Table 4. Effect of biofilm with biofertilizer, type of carrier and level of chemical fertilizer in Weight of 1000 grain for plant wheat

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
P0	M0	17.16	21.86	28.00	22.34	
	M1	17.44	23.57	28.21	23.07	
P× F		17.30	22.72	28.11		22.71
P1 P. fluorescens	M0	29.77	31.17	37.00	32.65	
	M1	32.50	35.60	43.00	37.03	
P×F		31.14	33.39	40.00		34.84
P3 P. fluorescens Rhizobium	M0	43.30	53.30	62.00	52.82	
	M1	48.70	58.00	70.00	58.90	
P× F		46.00	55.65	66.00		55.88
RateF		31.48	37.25	44.70	RateM	
F+M	M0	30.08	35.44	42.33	35.95	
	M1	32.88	39.06	47.07	39.67	
Fertilizer standard treatment 100%		77.12				

P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
1.111	1.111	0.907	1.925	1.571	1.571	2.722	

Nitrogen uptake

Results in Table 5 shows that Biofilm Biofertilizer cause significant effect on the amount of nutrient uptake by wheat plant ,The P3 treatment record the value 29.20 mg N $g^{-1}$with increasing %61.17%over control which was 11.18mg $Ng^{-1}$and this could be due to the role of biofilm in regulation of growth promoter production like IAA and gibberelins which contributed in increase the growth of root system (9)and this in turn the increasing of nitrogen uptake and other nutrient ,on the other hand addation of chemical fertilizer increase N uptake with the superioty of F2 treatment that gave 24.77mg $Ng^{-1}$with increasing %of 38.99%over control treatment while M1 treatment record 20.58mgNg^{-1}at an increasing rate 9.86%compare to M0 treatment. The Third order(P×M×F) interaction treatment shows that the treatment P3F2M1 gave the highest nitrogen uptake to 39.81mg $Ng^{-1}$and this may due to the role of the combination of biofilm with Bentonite

Table 5. Effect of biofilm with biofertilizer, type of carrier and level of chemical fertilizer in Weight amount of nitrogen uptake mg Ng^{-1} by wheat plant

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
	M0	10.35	11.11	11.79	11.08	
P0	M1	10.39	11.28	12.13	11.27	
P× F		10.37	11.20	11.96		11.18
P1	M0	12.41	16.04	22.00	16.82	
P. fluorescens	M1	13.59	18.90	26.99	19.83	
P×F		13.00	17.47	24.50		18.32
P3	M0	21.25	26.14	35.86	27.75	
P. fluorescens Rhizobium	M1	22.65	29.47	39.81	30.66	
P× F		21.95	27.81	37.84		29.20
RateF		15.11	18.83	24.77	RateM	
F+M	M0	14.67	17.76	23.22	18.55	
	M1	15.54	19.88	26.31	20.58	
Fertilizer standard treatment 100%		53.874				
P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
2.387	2.387	1.949	4.135	3.376	3.376	5.847	

Phosphorus uptake

Results in Table 6 shows the effect of Biofilm Biofertilizer on Phosphorus uptake by plant , P3 treatment gave 3.44mg $Pg^{-1}$with increasing %75.58%compare to control treatment and this could be due to the role of Biofilm on increasing plant hormons that promote plant and root growth which contributed in Phosphorus uptake (9). The second level F2 of chemical fertilizer gave 2.94 mg $Pg^{-1}$with increasing %52.04%compare to control F0 treatment. Bentonite carrier (M1 treatment) gave 2.28mg $Pg^{-1}$with increasing %12.60% over M0 treatment. Results showed that Biofilm Biofertilizer participate in reduction of chemical fertilizer to 50%,so P3F2 treatment gave 4.68 mg $Pg^{-1}$and these results agree with Seneviatne et al(29)whom found that Biofilm Biofertilizer reduce using of chemical fertilizer to half and this results lead to the true that integration in fertilization by addition of 50% Of recommendation with biofertilizer gave better results in comparison with addition of total chemical fertilizer recommendation with low economic costs(7). Results showed that the third interaction treatment P3F2M1 gave the highest phosphorus uptake 4.93 mgPg-1 and this may due to the role of Biofilm acidity as a results of IAA and in increasing Organic acid that increase the availability of phosphorus(18),and these results come in agreement with lgual et al(23),found that using of microorganisms with chemical fertilizer reduce the amount need to be add to 50% during dissolving the orecipitate(25).

Table 6 . Effect of biofilm with biofertilizer, type of carrier and level of chemicalfertilizer in amount of Phosphorus uptake mg Pg^{-1} by wheat plant

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
	M0	0.52	0.79	1.14	0.82	
P0	M1	0.56	0.87	1.17	0.87	
P× F		0.54	0.83	1.16		0.84
P1	M0	1.21	1.81	2.54	1.85	
P. fluorescens	M1	1.39	2.13	3.44	2.32	
P×F		1.30	1.97	2.99		2.09
P3	M0	2.25	3.05	4.43	3.24	
P. fluorescens Rhizobium	M1	2.53	3.47	4.93	3.64	
P× F		2.39	3.26	4.68		3.44
RateF		1.41	2.02	2.94	RateM	
F+M	M0	1.33	1.88	2.70	1.97	
	M1	1.49	2.16	3.18	2.28	
Fertilizer standard treatment 100%		7.18				

P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
0.246	0.246	0.201	0.426	0.348	0.348	0.628	

Potassium uptake

Results showed the superiority of the treatment that received Biofilm Biofertilizer in Potassium uptake compare to non-inoculated seeds P3 treatment gave 24.33mg $Kg^{-1}$and this may due to the role of Biofilm in increasing nitrogen which caused the synthesis of various compounds in the green part of plant that need Potassium for different metabolism pathway which lead to adsorb more Potassium from the soil. On the other hand addition of 50% of chemical fertilizer recommendation increase the K uptake to 70.65 $mgKg^{-1}$and this could be due to the increasing of it's availiablity in the soil (1)in addition inoculated of Bentonite as carrier increase K uptake to 14.35 $mgKg^{-1}$with increasing%14.42%. The results showed also that the interaction treatment P3F 2 gave 32.98 $mgKg^{-1}$ and this superiority may due to the role of Biofilm Biofertilizer to enhance the Potassium from its unavailable source due to the production of different organic acids (22).

The Third order interaction treatment P3F2M1 showed the superiority in comparison with all other treatment when it gave 35.47 $mgKg^{-1}$. This results shows the role of this treatment to prepare good environment for microorganisms in addition to protect its against different ecological stress (27),(28) and enhance the root system to absorb more Potassium from fertilizer or unavailable source (10).

Table7 . Effect of biofilm with biofertilizer, type of carrier and level of chemical fertilizer in Weight amount of Potassium uptake mg Kg^{-1} by wheat plant

P Type of bacterial pollen	M Download method	F Levels of chemical fertilization kg h^{-1} F1	F2	F3	P×M	Rate p
	M0	4.56	5.07	5.95	5.93	
P0	M1	4.71	5.32	6.12	5.38	
P× F		4.64	5.20	6.04		5.29
P1	M0	6.48	8.60	12.01	9.03	
P. fluorescens	M1	8.17	10.84	15.83	11.61	
P×F		7.33	9.72	13.92		10.32
P3	M0	15.45	21.93	30.49	22.62	
P. fluorescens Rhizobium	M1	17.95	24.69	35.47	26.04	
P× F		16.70	23.31	32.98		24.33
RateF		9.56	12.74	17.65	RateM	
F+M	M0	8.83	11.87	16.15	12.28	
	M1	10.28	13.62	19.14	14.35	
Fertilizer standard treatment 100%		42.83				

P	F	M	P×F	P×M	F×M	P×F×M	L.S.D=0.05
1.578	1.578	1.288	2.733	2.231	2.231	3.865	

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