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Estimate Different Bioagent as A Biofertilizer with Two Level From Chemical Fertilizer on Wheat Crop Improvement

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Abstract

Different formula of bioagents (*Rhizobium cicceri* cp-93, *Azospirillum sp., Pseudomonas fluorescence*, *Trichoderma harzianum*) used in this study as a biofertilizer on wheat crop with two level of chemical fertilizer (0 and 12.5 kg/donm Dap) compared to 50kg/donm Dap (standard amount).the study carried out in Iraq/Diyala –Alkhales during November 2014,results showed significant increase in no. of spikes, no. of spikelet's, length of spike ,Weight of 1000 seed and yield of one m^2 when adding (*Rhizobium cicceri* cp-93,*Azospirillumsp+ Trichoderma harzianum* +12.5 kg/donm Dap) in comparison with the 50kg/donm Dap. Other formulas recorded same results with the treatment 50kg/Donm Dap with not significant differences except Wight of 1000 seed which recorded significant increased in all formula, while (*Rhizobium cicceri*cp-93,*Azospirillumsp+* zero Dap)treatment recorded significant decrease from 50 kg Dap in the most parameters in study.

Keywords: biofertilizer , wheat ,bioagent ,improvement.

تقييم عدد من العوامل الاحيائية باستخدامها كسماد احيائي مع مستويين من التسميد الكيميائي لتحسين محصول الحنطة

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الخلاصة

استخدمت في هذه الدراسة توليفات مختلفة من العوامل الاحيائية cp-(*Rhizobium cicceri* 93,*Azospirillum sp., Pseudomonas fluorescence* and *Trichoderma harzianum*) كأسمدة احيائية على محصول الحنطة مع مستويين من التسميد الكيميائي (صفر و 12.5كغم / دونم من الداب) ومقارنتها مع 50 كغم للدونم من الداب وهي التوصية السمادية القياسية المستخدمة في حقول الحنطة. تم اجراء التجربة في العراق محافظة ديالى ناحية الخالص في تشرين الثاني 2014 وأظهرت النتائج زيادة معنوية في عدد السنابل وعدد السنيبلات وطول السنيبلة ووزن 1000حبة وزيادة في حاصل المتر المربع الواحد عند استخدام التوليفة رايزوبيا + ازوسبيرلم+ ترايكوديرما +12.5 كغم للدونم من الداب مقارنة مع 50 كغم من الداب .اما بقية المعاملات فكانت مقاربة في النتائج لمعاملة التسميد الكيميائي ولكن من دون فروق معنوية باستثناء وزن 1000 حبة حيث تفوقت معنويا بقية التوليفات على معاملة التسميد بـ 50 كغم للدونم من

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الداب بينما التوليفة المكونة من الرايزوبيا والازوسبيرلم +صفر من الداب حيث أظهرت انخفاضا معنويا عن معاملة التسميد الكيميائي في اغلب الصفات التي تناولتها الدراسة.

Introduction

World major source of food for human nutrition comes from the most important grain crops such as wheat, rice and maize[1]. Wheat is the most important plant that grown in a wide range of climate condition[2]. Its importance has risen even more due to frequently experienced food shortage and its role in world trade[3]. As the food demand is increasing world population, so the growers need to use additional nutrient inputs to increase their yields. Mineral fertilizer are the traditional source of nutrients [1]. Strong use of the chemical composition for promoting production level and protecting plant, disturbs ecological balance of the soil and ventilates some nutrients according to other researchers view, although chemical fertilizer had an important role in green revolution, their unstable application caused reduction of soil fertility and pollution of environment. Stable agriculture based on using biofertilizer and with the aim of reducing or removing chemical problems of mineral fertilizer [4] .The term biofertilizer or microbial inoculant can be defined as preparation containing strains of microorganisms, which can augment the biological process such as nitrogen fixation, phosphate solubilization or mineralization, excretion of plant growth promoting substances of cellulose or lignin biodegradation in soil, compost or other environments[5]. Biological nitrogen fixation play an important and positive role in the maintenance of N_2 in the soil. In the past the use of nitrogen fertilizer, green revolution, mono- cropping system use to obtain maximum yield in less time. but now days there is judicious use of chemical fertilizer with nitrogen fixing inoculants [6] . Biofertilizer play an important role in the growth of plant as well as they bring down the cost of chemical fertilizer [7] So it is the most safe alternative to the use of chemical fertilizer. *Azospirillum* has shown high nitrogen fixation attributes. The association of *Spirillum lipoperum* , *Azospirillum brasilense* was reported. As *Azospirillum* sp. fixes appreciable amount of atmospheric nitrogen and remain loose symbiosis with tropical plant root, experiments were conducted to use *Azospirillum* as inoculants to supplement the nitrogen need of cereal and leguminous plants [8].Smith, R. etal [9] were the first to use nitrogen fixing *Azospirillum* sp. as inoculants in commercial scale to test whether these organisms supply the nitrogen need of field crops or not.

Rhizobia are capable of nitrogen fixing symbiosis with more than 1130 species of leguminous plants. Most rhizobia are specific in their association with legume. Rhizobia have been taxonomically grouped and designated to a particular species based on cross inoculation group [5].

Rhizobium is known to survive in soil unaffected by continuous use of nitrogenous fertilizer .they can survive at low Temp. as well as tolerate worm temp. up to 50 C for more than few hours. The bacterium survived for several years in dry stored soil although the mechanism of survival was not reported [8].Naturally-occurring rhizobia, isolated from nodules of legume have also been shown to infect root of many agricultural species such as rise, wheat and maize via cracks made by merging lateral roots, Rhizobial infection of these non-legume crop can be genetically manipulated for increased plant growth and possibly grain yield [10]. Because rhizobia produce phytohormones such as auxins , cytokinins,gibberllins and absesic acids it is likely that their release into cropping systems promotes plant growth and possibly increase yield even though no nitrogen fixation by rhizobia has been detected in these non-legumes. This is in addition to the role of microbial metabolites in making nutrients available to plant [11].

Trichoderma spp. are fungi that are present in nearly all soils and other divers habits. In soil they frequently are the most prevalent cultivable fungi [12]. *Trichoderma spp.* are most popular research tools as inoculates which have been largely used against several plant pathogenic fungi .In recent years they have become popular as plant growth promoter [13]. *Trichoderma spp*. was found to colonize the root epidermis and outer cortical layer and release bioactive molecules that cause walling off the *Trichoderma* thallus, So *Trichoderma* increase root development, crop yield ,seedling fresh weight and foliar area .In addition to induction of pathogen resistance in plant, increased plant growth and nutrient uptake also occur [14].

Pseudomonas sp. are gram negative, motile, rod shape bacteria and have various phytobenificial traits which include production of hydrogen cyanide, sidrophores, protease, antimicrobial and phosphate solubilizing enzymes [15]. The strain of *Pseudomonas sp*. were found to induce resistance

against different pathogens these bacteria are more and more used as biological agents against fungi such as *Rhizoctonia solani, P. fluorescens.* is wide spread in agricultural soil and has many traits that make them sound contenders as PGPR[16]. Murthy, [17] found that, the application of *P. fluorescens* as seed treatment could prove to be a beneficial component of integrated pest managements, these *P. fluorescens* isolates also are a good growth promoters, and able to induce systemic resistance in tomato plant .

The use of mixed biofertilizers is advocated to get the maximum benefits due to additive and synergistic effect[18].Beneficial rizosphere organisms are two groups (i) microorganisms with direct effects on plant growth promotion [plant growth promoting rhizosphere] and biological control agent that indirectly with plant productivity through the control of plant pathogens. In addition to their primary effects on plant productivity and health [19].

The present study based on adding a mixed of beneficial microorganisms to the wheat crop as seed coating treatment in the field experiment to study the effect of these organisms on improving the crop and soil, also their ability to reduce using of chemical fertilizer .

Material and Methods

1. preparation of inocula:

The bioagents that used in this study were obtained from biofertilizer laboratories of plant protection directorate ministry of agriculture/Baghdad-Abu-Ghraeb (*Rhizobium ciccere, Pseudomonas fluorescens*) each one was grown and activated in 1000 ml nutrient broth by incubating at 28C˚ in cooled shaking incubator for 2 days to attain uniform cell density10^7-10^8cfu/ml [20] ,bacteria were carried on the specific carrier containing (charcoal and peat 3:1)and incubated for two days at 28C˚.

Trichoderma harzianum was purshased from plant pathology lab. in plant protection directory.(local isolate10^8 spore\ml powdered [21].

2. field experiment:

Field experiment was carried out in Al-khalis region \ Diyala governorate in November 2014 using Rashid cultivar to study the effect of biofertilizer in improving and increasing of wheat crop with two levels (0- 1/4) of standard amount of chemical fertilizer ,the standared recommended amount used in wheat fields of this area is 50 Kg/donm (2500)m^2 of Dap.

Treatments:

1. T1 seed with 50kg/donm Dap (control).
2. T2- *rhizobium* CP-93+*Azospirillum sp.* + 12.5 kg/donm Dap.
3. T3- *rhizobium* CP-93 +*Azospirillum sp.* + 0 Dap)
4. T4-*rhizobium* CP-93 +*Azospirillum sp.* +*P. fluorescence* +12.5 kg/donm Dap.
5. T5-*rhizobium* CP-93 +*Azospirillum sp.* +*P .fluorescence*+ 0 Dap.
6. T6-*rhizobium* CP-93+*Azospirillum sp.*+*T. harzianum*. +12.5 kg/donm Dap
7. T7- *rhizobium* CP-93 +*Azospirillum sp.*+ *T. harzianum*.)+ 0 Dap
8. T8-*rhizobium* CP-93+*Azospirillumsp*+*P. fluorescence.*+*T. harzianum*)+12.5 kg/donm Dap.
9. T9-*rhizobium*CP-93 +*Azospirillum sp.* +*P. fluorescence* .+*T. harzianum*)+ 0 Dap.

Each treatment was done in one donm (2500m^2) devided into blocks within dimensions 10m width and 250m length, with CRD experiment design, planted with 35 kg of wheat seeds per donm.

Seed coating was done by mixing the seed with bio fertilizer ,sugar solution (10%)was added to the seeds before mixing to get perfect coating with carrier.

Inoculated seeds were air dried under shade for 1-2 hr., coated seeds were sowed by sowing machine. Each experiment unit separated by 1m as unplanted distance .the field was immediately irrigated after sowing to ensure uniform germination.

Plants harvested at maturity stage in may 2015 number of spikes, length of the spike, number of spikelets in the spike ,weight of 1000 seed and yield of 1m^2, were measured within 6m^2 for each treatment.

Analysis of variance was carried out using Genstate computer software packages. The comparison of mean was investigation using L.S.D.at 0.05% probability.

Results and discussion:

The result show that using 12.5 kg dap/2500 m^2 combined with biofertilizer gave best effect on the crop and in some treatments recorded significant increased in all parameters compared with the (50 kg) dap chemical fertilizer.

Table 1- results of use different formula of bioagint as biofertilizer on wheat crop.

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**	**L.S.D**
number of spikes	366.7	376.7	390	353.3	349	450	383.3	393.3	345	54.3
Length Of spikes/cm	14.47	13.73	13.03	13.67	14.25	15.03	14.67	13.2	13	0.85
number of spiklets	18.93	18.27	18.2	18.37	19.87	19.57	20.63	19.9	19	1.59

Table-1, revealed that T6 gave the best results and showed significant increasing in all studied traits except in number of spikelets Table-1, this go one better than other treatments is due to adding microorganisms as biofertilizer combined with 12.5kg/donm Dap to the soil .The positive effect of these microorganisms (*Azospirilum* and *Rhizobium*) such as nitrogen fixation, exudation of plant growth regulators like auxins, gibberellins and cytokinen, with increase of nutrient availability that caused this increasing in grain yield[22].

Table 2- The effect of different treatments of biofertilizer with 12.5 kg/donm Dap on wheat yield

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**	**L.S.D**
Weight of 1000 seed/gm	33.97	39.12	34	40.2	38.36	46.5	42.97	39.73	38.87	2.42
Yield gm / m^2	450	467	367	500	450	683	533	450	400	55.4

T_6 also has *Trichoderma sp*. Which Reported to release secondary metabolites that besides inhibiting the pathogen population also help growth enhancement of the crop. The colonization of *T. harizianum* in the root resulted increase in growth of root thus providing enough strength for more nutrients uptake by roots[21]. Shelton, A.[22] indicated that corn root are colonized by *Trichoderma* strain (T- 22) require about 40% less nitrogen fertilizer than corn whose root lack the fungus.

Our results was similar to the finding of Hussain, T. *et al.* [23]. when they used an isolates of *Azospirillum sp*. In the inoculation of wheat seed and showed that *Azospirillum sp.* alone increased wheat grain yield by 9.47% over the control. However, where *Azospirillum sp.* was applied in combination with NP, grain yield increased by 14.2% compared with application of NP alone.
Mehboob, I. *et al.* [24] found when they treated wheat seeds with *Mesorhizobium cicceri* isolates (20 isolates) for improving root and shoot, length of wheat ,seedling, 12 isolates increased root and shoot leangth up to 47.69%, and that is may be due to the ability of rhizobia to introduce different metabolites like phytohormons, organic acids, sidrophores, enzymes and exopolysaccharides in the rhizosphers.

The grain yield of wheat improved when wheat plants were grown with combination of chemical nitrogen and biofertilizer inoculation, this interaction effects were found significant for grain yield of wheat in many nitrogen application levels. The grain yield of inoculation plant with biofertilizer was higher than of the non-inoculated plant at the same rate of nitrogen application [25, 26].Our study was in the same side with Sharma, *et al.* [21] that the *T. harizianum* application on wheat crop significantly increased the growth promoting in term of number of tillers, rootiets, number of grain per spike and weight of 100 seeds in comparison to the crop without *T. harizianum* treatment.

T_2, T_4 , T_5 T_8 and **T_9** also recorded positive results in many studied traits with non-significant differed from **T_1** Tables- (1 and 2) such as number of spikes , number of spiklets/spike and yield /m^2 .While the (**T_2,T_4,T_5, T_8** and **T_9**) recorded significant increase from **T_1** in the weight of 1000 seed, (Table-2). That results belong to the positive effect off adding biofertilizer(*Azospirillium* + *Rhizobium*)in **T_2** and adding(*Azospirillium* + *Rhizobium* and *P. fluorescence*)in **T_4 , T_5 ,T_8** and **T_9** *P. fluorescence* suppress the soil borne pathogens through rhizosphere colonization ,antibiosis and iron chelation by siderophore production .Certain fluorescent *Pseudomonads* are also found to promote

plant growth by production of plant growth promoting substances and solubilization of minerals such as phosphorus [27], Our result is in harmony with Rokhzadi and Toashih [28]they reported using combined of(*Azospirillium* + *Rhizobium* + *P. fluorescence*) increase the grain yield in chickpea 864 kg/ha comparison with 543.9 kg/ha uninoculated seed, and nodule dry weight of the inoculated seed was 54.49 mg/plant while 33.37 mg/plant un inoculated plant,1705.6 kg/ha for inoculated in biomass comparison with 1082.3 kg/ha uninoculated . Nanri, *et al.* [27] reported, potato production was increased 3 time with the biofertilizer containing *P. fluorescence* Eyvazian [29] found, application of different strains of *Pseudomonas.* bacteria, especially strains 136 & 138 not only can lead to increase yield and performance in planting barely , but also lower utilization of chemical fertilizers.

From recent study we can notes adding mixed of bioagent as biofertilizer to the wheat crop by seed coating combined with amount of chemical fertilizer(12.5)kg/donm leading to increase the yield and many other traits such as number of spikes , number of spikelets, weight of 1000 seed.

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