RESPONSE OF TWO WHEAT CULTIVARS TO BIOFERTILIZATION AND ORGANIC FARMING SYSTEMS



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ABSTRACT

Two lizemeters experiments in of Bacteriology Lab., Sakha Agricultural Research Station were undertaken to evaluate the effect of biofertilization and organic farming systems on yield components and rhizosphere bacterial counts of wheat plant, cultivars (Misr 1 & Seds 12 through winter seasons of 2013 and 2014). The results indicated that grains yield and weight of 100 grains of wheat cultivars significantly increased due to biofertilization system, while there were no significant differences in grains yield due to application of the organic farming system application. In contrast, organic farming treatment achieved significant increases in weight of 100 grains as compared with traditional control treatment. In respect to protein percentage of wheat grains, biofertilization treatment caused increases over traditional one, while, organic farming system attained significant decreases than those of traditional control, and this decreases were clear for Sids 12 than Misr 1 cultivar.

Mineral concentrations in wheat grains showed different trends regarding their response to biofertilization and organic farming systems. N percentage did not significantly affected by biofertilization; however, there were significant decreases due to application of organic farming system than that of traditional control. Regarding phosphorous concentration, showed different response trends, whereas, biofertilization and organic farming treatments increased wheat grains of both cultivars at the two seasons, while the differences were significant under organic farming treatment only. The studied systems over traditional control, whoever, the differences did not reach to the level of significance.

Total bacterial counts of the rhizosphere soil of wheat plants increased due to biofertilization and organic farming treatments over those for traditional one, the increases were significant. The highest recorded counts achieved in case of organic farming treatment. Otherwise the counts of *Azospirillum* sp. achieved the same trend, but the increases due to biofertilization and organic treatments over that of traditional control were highly significant.

Keyword: Wheat cultivars, biofertilization, organic farming.

INTRODUCTION

Nitrogen, phosphorus and potassium are essential nutrients required by both plant and microorganisms, their major physiological roles are accumulation and release of energy during cellular metabolism (Marchner, 1995).

Attempts to maximize yield under traditional agriculture on small holdings have been made but small scale efforts may not be enough to ensure sustainability in agriculture and food security.

Many problems faced the application of mineral fertilizers were those efficient use of applied nitrogen fertilizers by various crops in Egypt is poor.

(Abd Elmonem and Anderson 1995) reported 28% recovery by wheat cultivar (Sakha 92). Soil fertility is diminished gradually due to soil erosions, loss of nutrients, accumulation of salts and toxic elements, water logging and unbalanced nutrient compensation.

The problems of phosphate fertilizers in our Egyptian soil are related to its fixation or precipitation in a form of insoluble calcium phosphate. However, diverse group of organisms in soil employ variety of solubilization reaction to release soluble phosphorus from insoluble phosphate (Singh and Kapoor, 1994). Also, a continuous decrease in potassium concentration in our soil especially after the construction of high dam

Irrational use of chemical fertilizers especially nitrogenous fertilizers led to high extent of environmental pollution, as nitrates concentration increased in drainage water causing enrichment of wild weeds in water streams. Stahl et al, 2009 recorded high concentrations of nitrates in water of Bahr El-Bakar drain (60- 80 mg.l⁻¹). Eutrophication phenomenon appeared with the increasing nutrient elements in water and biochemical oxygen demand (BOD) level increased, whereas, Farag and Donia (2006) recorded BOD levels in Egyptian drains ranged from 230 to 950 mg.l⁻¹. In this case, anaerobic conditions are predominant causing deleterious changes in water quality and led to fish dyeing. At the same time, there is a direct relationship between increasing NO₃ in soil and evolution of nitrous acid (N₂O) which is a greenhouse goal (Portmnn et al, 2012)

Because of all these determined effect of chemical agriculture, scientist look for alternate strategies. Organic wastes and biofertilizers are the alternate sources to meet the nutrient requirement of crops, to bridge the future gap and rationalized the use of chemical fertilizers.

The most direct hazardous effect is the increasing nitrate concentrations in portable water, edible fruits and vegetables which directly intake by animals and man causing many dangerous diseases like cancer due to formation of the carcinogenic nitrosamine compound, as well as methomeglibonima and Alzahimar diseases (Hubbard, 2015)

The biofertilizers contribute in decreasing the used amounts of chemical fertilizers. From biofertilizers, the nitrogenous biofertilizers such as *Azospirillum* sp., Researches confirms that its application save from 15 to 25 % of chemical nitrogenous fertilizers (Alamri and Mostafa, 2009). The solubilized phosphate bacteria shared also by saving half of chemical phosphatic fertilizer, in addition potassium solubilizing bacteria solubilize the bounded potassium and liberated it as soluble forms for plant use (Zakaria, 2009). Also, these biofertilizers, are useful in releasing plant phytohormones, siderofores, vitamins, antitoxins and antibiotics in the rhizosphere of the plant which positively reflect on health of plants and increasing their productivity (Beneduzi et al., 2012).

To absolutely get of agrochemical pollutants we must return to organic agriculture in order to obtaine clean environment and healthy food material for wellfair of humanity. Thus, extended and developed researches in scope of organic agriculture urgently needed in order to reach by its productivity to maximum levels, therefore, the present research aimed to investigate the potentiality of biofertilization system (save about 50 % of chemical fertilizers)

and organic agriculture system on yield and quality of some wheat cultivars compared to chemical fertilized traditional system.

MATERIALS AND METHODS

Media used:

Medium 1-used for growing of *Azospirillum* sp.:

Nitrogen-free semisolid malate (NFb) Baldani and Dfbereiner. (1980) (grams per liter of distilled water): K_2HPO_4 , 0.5;MgSO₄ – 7H₂0, 0.2; NaCl, 0.1; yeast extract,0.5; FeCl₃ . 6H₂ 0, 0.015; DL-malic acid,5.0; KOH, 4.8; and agar, 1.75. The pH was adjusted to 7.0 with 0.1 N KOH, and the medium was autoclaved at 121°C for 20 min.

Medium 2- used for growing phosphate dissolving bacteria (PDB)

Nutrient solution (g.I⁻¹): composed of K_2SO_4 , o.485; MgSO₄. 7H₂O, 0.2; FeCl₂ 0.01; CaCl₂, 0.376; H₃PO₄, 0.018; ZnSO₄.7H₂O, 0.0028 (Shrdeta et al., 1984). All these contents were dissolved in 1 liter distilled water and the pH of solution was adjusted to pH 6.9 using KOH.

Medium 3-used for growing Potassium dissolving bacteria (KDB) (g.l⁻¹):

Aleksandrov medium (Hu et al., 2006). Glucose, 5.0 g; MgSO₄. $7H_2O$, 0.005 g; FeCl₃, 0.1 g; CaCO₃, 2.0 g; potassium mineral, 2.0 g, calcium phosphate, 2.0 g and distilled water 1 liter.

It is worthy to mention that previous experiments were carried out to select the high efficiency of bacterial isolates in solubilization of phosphorus and potassium to be used in our study.

Compost:

Compost pile was constructed using wastes of rice, cotton and maize by a percentage of 60 %, farmyard manure (35%) and fertile soil (5%). The pile was turned every week and was frequently hydrated with water to make the moisture about 60 %. And chemicals analysis was shown in Table (1). **Wheat cultivars:**

Wheat grain cultivars (Misr 1 and Sids 12) were kindly obtained from field crop institute, Sakha Agricultural Research Station.

The experiment undertaken in lizemeter composed of 20 units each of 70 × 70 cm, 70 g of wheat grains were sown in three rows for each lyzemeter unit. The compost amounts were applied according to type of the treatment. Also, the rock phosphate and feldspar (400 kg/ fed.) for each were added to biofertilization and organic agriculture treatments. In traditional treatment, 15 ppm of P_2O_5 and 15 ppm K_2O were added as superphosphate and potassium sulphate, respectively. The soil was ploughed and be ready for planting (analysis of soil was shown in Table 2). The wheat grains of biofertilized and organic agriculture treatments were inoculated with mixed inoculum composed of *Azospirillum* sp., phosphate dissolving bacteria (PDB) and potassium dissolving bacteria (KDB) with the density of about 1 × 10⁹. ml⁻¹ for each. The grains of control did not inoculated. The wheat grains Misr 1 and Sids 12 cultivars were sown in 3 rows for each lyzemeter unit and irrigated at 60 % of water holding capacity. The treatments which arranged as complete randomized were as the following:

- 1- **Traditional control:** including application of full dose of NPK (100%) and consider as control.
- 2- **Biofertilization treatment:** (*Azospirillum* sp.+ phosphate dissolving bacteria+ potassium dissolving bacteria + 50 % NPK + 400 kg.fed ⁻¹ rock phosphate and 400 kg.fed ⁻¹ feldspar + 2 ton compost / fed.
- 3-**Organic farming treatment:** including biofertilizer + 8 ton compost / fed. + 400 kg .fed ⁻¹ rock phosphate + 400 kg .fed ⁻¹ feldspar.

Statistical analysis:

Data obtained from the two studied seasons were statically analyzed by the methods of Duncan multiple range test (Duncan, 1955)

Table 1: Chemical analysis of compost.

pH in suspension (1:10)	EC in suspension (1:10)	N %	P %	K %	Fe (ppm)	Mn (ppm)	Zn (ppm)	0.C %	0.M %
7.1	3.36	1.86	0.73	0.55	1020	280	30	27.9	48

Table 2: Some physical and chemical analysis of the experimental soil.

Parameter	Value
Some physical properties	
Particle size distribution	
Clay %	54.6
Silt %	22.1
Coarse sand %	5.7
Fine sand %	17.6
Texture grade	Clayey
Some chemical properties	
pH (1:2.5 water suspension)	7.1
EC (ds.m ⁻¹ in soil paste)	3.36
Soluble Cations (meq. L ⁻¹)	
Ca ⁺⁺	4.38
Mg ⁺⁺	8.18
Na⁺	9.24
K⁺	0.2
Soluble Anions (meq. L ⁻¹)	
SO ₄ ⁻	11.53
CI	6.72
HCO ₃	3.75
CO ₃	00
Available macro element (ppm)	
N	45.6
P	12.28
K	230.7

Physical and chemical properties were determined according to the standard methods reported by Black et, al., (1965) and Jackson (1967)

RESULTS AND DISCUSSION

Table 3: Effect of biofertiliz	zation and organi	ic farming systems on spike
weight and grain	yield of wheat	cultivars yield components
(Masr 1 & Sids 12))	

	Spikes weight (g. m ⁻²)					
Treatment	20	13	2014			
	Misr 1	Seds 12	Misr 1	Seds 12		
Chemical fertilization (NPK)	233.07 b	214.39 b	202.79 c	199.60 c		
Biofertilization	281.58 a	237.29 ab	263.12 b	234.12 b		
Organic farming	283.00 a	257.26 a	330.17 a	277.67 a		
F test	**	*	**	**		
L.S.D.(0.05)	21.09	23.57	28.63	21.85		
Grains yield (g. m ⁻²)						
Chemical fertilization (NPK)	164.69 b	138.79 c	148.76b	124.33 b		
Biofertilization	237.24 a	201.23 a	190.48 a	175.17 a		
Organic farming	175.31 b	164.17 b	125.50 b	121.56 b		
F test	**	**	**	**		
L.S.D.(0.05)	25.68	15.09	27.35	21.26		

Means followed by different litters within a column are significantly different at P= 0.05

Data presented in Table 3 indicated that application of organic farming was very clear and showed their beneficial effects on grain yield on both two wheat cultivars at the two experimental seasons than that of chemical fertilization with NPK of control treatment. While, treatment with biofertilizers was superior in achieving the highest values of grain yield being on the average 213.86 g.m² for cultivars Misr 1 (average of the two seasons) and 188.2 g.m² for cultivars Seds 12. While average increase was about 36.46% for cultivars Misr 1 as compared with traditional treatment. The corresponding value for Sids 12 was about 30%. On another hand chemical fertilization with NPK and organic farming did not significantly different except that of Sids 12.

In respect to spike weight $(g.m^2)$ Table 3 showed that application of organic farm led to further increase of both wheat cultivars at any of investigated periods with total average 287 g.m² fallowed by biofertilization treatment (234.0 g.m²). While the control treatment fertilized with NPK was the least (212.46 g.m²).

	Grain index (g)					
Treatment	2	013	2014			
	Misr 1	Seds 12	Misr 1	Seds 12		
Chemical fertilization (NPK)	5.46 c	5.68 b	4.88 c	4.68 b		
Biofertilization	8.78 a	6.83 a	8.18 a	6.47 a		
Organic farming	6.27 b	5.59 b	6.06 b	5.79 a		
F test	**	**	**	**		
L.S.D.(0.05)	0.63	0.81	0.37	0.75		

 Table 4:
 Grain index of wheat (Misr 1 & Seds 12) as affected by biofertilizers and organic farming systems.

Means followed by different litters within a column are significantly different at P= 0.05

In general, the application of biofertilization and organic farming systems improved the average weight of 100 grains for the two cultivars (Table 4). The treatment of biofertilization for both studied cultivars (Mis1 and Sids 12) gave average weight of 100 grains about 8.78 and 6.83g at season 2013 compared to control treatment which achieved 5.46 and 5.68 g respectively. Also, the treatment of organic farming had a positive effect as the average weight of 100 grains increased for both cultivars at both seasons except for Sids 12 cultivars at season of 2013 which did not exhibit significant difference than traditional control treatment.

N % in grains						
Treatment	2	2013	2014			
	Misr 1	Seds 12	Misr 1	Seds 12		
Chemical fertilization (NPK)	2.53 a	2.79 a	2.59 b	2.63 a		
Biofertilization	2.71a	2.76 a	2.84 a	2.69 a		
Organic farming	2.10 b	1.96 b	2.28 c	2.06 b		
F test	**	**	**	*		
L.S.D.(0.05)	0.2	0.35	0.17	0.43		
	Pro	tein % in grains	S			
Chemical fertilization (NPK)	14.44 a	15.90 a	14.75 b	14.96 a		
Biofertilization	15.45 a	15.75 a	16.16 a	15.33 a		
Organic farming	11.97 b	11.17 b	12.97 c	11.76 b		
F test	**	**	**	*		
L.S.D.(0.05)	1.16	2.01	0.96	2.47		

Table 5: Effect of biofertilization and organic farming systems against
chemical fertilization one on nitrogen and protein percentage
of wheat grains through winter seasons 2013 and 2014.

Means followed by different litters within a column are significantly different at P= 0.05

Nitrogen concentration in wheat grains were recorded in Table 5, the biofertilization treatment did not significantly affect nitrogen concentration in wheat grains of both cultivars. While organic farming treatment caused significant decreases than both those of traditional control and biofertilization treatment. The traditional treatment for Sids 12 cultivar at season 2013 gave the highest N% in wheat grains (2.79%) and the lowest percentage recorded for treatment of organic farming, Sids 12 cultivar at season of 2013, which attained 1.96%.

Similarly, data of Table 6 showed that biofertilization treatment caused insignificant increases in protein percentage of wheat grains over traditional one. While, organic farming treatment caused highly significant decreases than those of traditional control treatment, ranged from 15.90 % for Sids 12 cultivar at season 2013 to 15.45% for Misr 1 cultivar at season 2013.

Nitrogen and protein percentage in wheat grains did not significantly affected by biofertilization system did help the wheat plants to accumulate the maximum amount of nitrogen and achieved the requirements to plants. This may be attributed to the role of two sources of nitrogen afforded by bacterial

inoculants and the compost fertilizer that contain 50% of the recommended dose of mineral nitrogen Adesemoye and Kloepper (2009) found that biological fertilization compensate a large amount of the plant requirement from nitrogen, also Nguyen et al., (2013) reported that addition of compost supply the plant with part of nutrients requirement. While, in case of organic farming systems in the present study caused significant decrease in grains nitrogen and protein percentage, this may regard to biofertilization which supply the plant with a part of their needing through nitrogen fixation and the applied compost (8 ton / fed.) which contains 80 % of its nitrogen in the first season (about 25 % only) Binh et al., (2015). For these reasons, the organic farming system applied in the present study may not achieve all requirement of the plant from nitrogen.

	P % in grains					
Treatment	20	13	2014			
	Misr 1	Seds 12	Misr 1	Seds 12		
Chemical fertilization (NPK)	0.64 b	0.62 b	0.63 b	0.59		
Biofertilization	0.68 ab	0.65 ab	0.69 ab	0.65		
Organic farming	0.74 a	0.71 a	0.75 a	0.66		
F test	*	*	*	n.s		
L.S.D.(0.05)	0.07	0.09	0.07			
K % in grains						
Chemical fertilization (NPK)	0.37	0.35	0.39 b	0.36		
Biofertilization	0.37	0.38	0.39 b	0.36		
Organic farming	0.42	0.43	0.45 a	0.36		
F test	n.s	n.s	*	n.s		
L.S.D.(0.05)			0.04			

 Table 6: P and K % in wheat grains (Misr 1 & Seds 12) as affected by biofertilizers and organic farming systems.

Means followed by different litters within a column are significantly different at P= 0.05

Phosphorus concentration in wheat grains exhibited different trend, whereas both the biofertilization and organic farming treatments attained increases in phosphorus percentages over those of traditional one (Table 6). However, these increases did not reach to significant levels under application of biofertilization system, while the differences regarding organic farming treatment were significant.

Otherwise the results recorded slight increases for potassium percentage in wheat grains due to application of biofertilization and organic farming systems (Table 5), and these increases over control treatment mostly were not significant except for the organic farming treatment, Misr 1 cultivar at season 2014 which attained K % about 0.45 due to organic farming system compared to 0.39% due to control treatment for Misr 1 cultivar at season 2014.

On the other hand, the increase of phosphorus and potassium percentages due to biofertilization and organic systems may be due to the

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soluble forms of P and K present in compost, whereas Binh et al., (2015) reported that about 80% of P and K in compost are in soluble forms and may be adequate for plant needing, therefor the addition of compost may correct the plant deficiency from these elements. Also, the phosphate and potassium solubilizing bacteria present in the biofertilizer inoculant gave the plants a large amount of available P and K through their ability for releasing mineral and organic acids which changed the insoluble element to soluble forms.

Table 7: Variation in log total count of bacteria (T.C.B) and Azospirillum
sp. (cfu. ml ⁻¹) in the rhizosphere of wheat plants as affected
by biofertilization and organic farming systems compared to
traditional one.

	Log T.C.B. cfu.ml ⁻¹					
Treatment	20	13	2014			
	Misr 1	Seds 12	Misr 1	Seds 12		
Chemical fertilization (NPK)	7.28 b	7.21 b	7.18 b	7.23		
Biofertilization	7.44 a	7.38 a	7.39 a	7.38		
Organic farming	7.43 a	7.41 a	7.43 a	7.37		
F test	*	*	*	n.s		
L.S.D.(0.05)	.130	0.13	0.15			
Log	Azospirillun	n sp. cfu.ml	1			
Chemical fertilization (NPK)	5.54 c	5.56 c	5.53 c	5.58 c		
Biofertilization	5.92 a	5.96 a	5.99 a	5.97 a		
Organic farming	5.68 b	5.78 b	5.80 b	5.74 b		
F test	**	**	**	**		
L.S.D.(0.05)	0.13	0.15	0.12	0.16		

Means followed by different litters within a column are significantly different at P= 0.05

Table 6 showed log of total count of bacteria in the rhizosphere of wheat plants cultivar Misr 1 and Sids 12 under systems of biofertilization and organic farming compared to traditional system. Generally, bacterial counts showed increases (≥ 0.05) in the rhizosphere of wheat plant under both biofertilization and organic farming systems over those of traditional control treatment. The highest records resulted from application of organic farming system, which gave 7.43 and 7.43for Misr 1 and 7.41 and 7.38 for Sids 12 at two seasons respectively. Similarly the log of *Azospirillum* sp. counts presented in rhizosphere of wheat plants attained increases under biofertilization and organic farming systems compared to those of traditional system and the variations were significant. The highest count of *Azospirillum* sp. was 5.99 for cultivar Misr 1 at season of 2014 compared to 5.54 and 5.53 for cultivar of Misr 1 at seasons of 2013 and 2014 under traditional control system.

Log of total bacterial and *Azospirillum* sp. counts in the rhizosphere of wheat plants increased under application of biofertilizer and organic farming systems over traditional systems. This may resulted from improving physical and chemical characteristics of soil due to long term application of compost and biofertilizers in the experimental Lyzemeter unit (Nour El-Din and Talha, 2011).

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It is recommended to replace the traditional fertilization system by biofertilization one for its effectiveness for saving chemical fertilizers, ensuring soil fertility and increasing plant yield. Organic farming system, on the other hand must be taken into consideration for its absolute safety for environment and human health.

CONCLUSION

The increase in grains yield resulted from biofertilization system over traditional system was due to the effective role of bacterial inoculant applied in this treatment, which achieved availability for nutrient elements (nitrogen, phosphorous and potassium). This availability resulted from the biofertilizer bacteria contained in the inoculant (Azospirillum sp., PDB and KDB). Azospirillum sp. fix a reasonable amount of atmospheric nitrogen be available for plant use (Okon et al, 1994). The phosphate dissolving bacteria solubilize a large amount of insoluble phosphate present in rhizosphere soil of the plant. Also, potassium dissolving bacteria are beneficial in solubilization of pounded unavailable potassium in soil. These elements become available for plant absorption (Kesaulya et al, 2015). Nour El-Din et al (2010) formulated an inoculum composed of these applied microorganisms Azotobacter choroococcum and Bacillus megatherium and Bacillus circulans and they found a positive effect on yield of sugar beet, the increase over un inoculated control, these microorganisms had other important effect on the plant ,summarized the mechanism of bacterial biofertilizers on the following:

- 1-Release of plant phytohormones which increase root area of plant
- 2-Release of sidrophores that chelate ferric ions to be available for plant roots3-Production and release of bactericide, fungicide and antibiotics giving a protection to plant against soil born disease
- 4-Release of amino acids and vitamins in rhizosphere
- 5-Release of antiethyllen compounds which attain protection to plant against biotic and abiotic stresses
- 6-Enrichment of beneficial microbial counts in plant rhizosphere as found in table 7 of the presented study.

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أقيمت تجربتين بالليزيمتر الحاص بالمعمل البكتيرى فى محطة البحوث الزراعية بسخا لموسمى 2013 و 2014 شتاء لتقييم تأثير نظامى الزراعة العضوية والتسميد الحيوى على مكونات انتاج صنفين من القمح (مصر 1و سدس 12). اظهرت النتائج استجابة صنفى القمح مصر 1و سدس12 لنظام التسميد الحيوى وكانت هناك زيادة معنوية فى وزن 100 حبة ولكن لم تظهر اختلافات معنوية فى انتاجية الحبوب بسبب نظام الزراعة العضوية والتى حققت زيادة معنوية عن الكنترول لوزن 100 حبة. وبالنسبة لنسبة البروتين فى حبوب القمح سببت معاملة التسميد الحيوى زيادات عن معاملة الكنترول ولكن اظهر نظام الزراعة العضوية نقصا معنويا عن وكان هذا النقص اكثر وضوحا لصنفى سدس 12 عن مصر 1.

بالنسبة لمحتوى الحبوب من العناصر الكبرى مثل النيتروجين والفوسفور والبوتاسيوم . اثبتت النتائج عدم وجود تأثير معنوى على نسبة النيتروجين بالحبة لمعاملات التسميد الحيوى ومن ناحية اخرى كان هناك تغير طفيف نتيجة نظام الزراعة العضوية مقارنة بالتسميد الكيماوى(الكنترول). اما بخصوص نسبة الفوسفور فى الحبوب اظهرت النتائج ان هناك زيادة معنوية نتيجة تطبيق نظامى التسميد الحيوى والزراعة العضوية فى كلا الصنفين. واوضحت نسبة البوتاسيوم بالحبوب ان هناك زيادة طفيفة نتيجة نظامي الزراعة العضوية والتسميد الحيوى مقارنة بالتسميد الكرماوى (الكنترول).

اثر تطبيق نظامي ألزراعة العضوية والتسميد الحيوي على الأعداد الكلية للبكتريا بمحيط جذور نبات القمح وادي الي زيادة معنوية مقارنة بالتسميد الكيماوي وكانت افضل النتائج عند تطبيق نظام الزراعة العضوية حيث حققت اعداد جنس الأزوسبيرليم نفس الأتجاه مع وجود فروق عالية المعنوية عن معاملة الكنترول.

واعتمادا علي نتائج البحث فإننا نوصي بإستخدام التسميد الحيوي بدلا من التسميد الكيماوي وذلك لزيادة انتاجية وتحسين جودة محصول القمح صنفي مصر 1 وسدس 12 وكذلك زيادة خصوبة التربة مقارنة بالتسميد الكيماوي مع الأخذ في الأعتبار ان تطبيق نظام الزراعة العضوية امن للبيئة وصحة الأنسان.