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SIGNIFICANCE OF BIOFERTILIZATION FOR IMPROVING YIELD, CHEMICAL AND TECHNOLOGICAL PROPERTIES OF WHEAT PLANTS GROWN IN SALINE SOIL

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ABSTRACT: Two field experiments were conducted at El-Hamoul region, Kafr El Sheikh Governorate in the seasons 2003 and 2004 to study the effect of compost application and inoculation with a mixture of some N_2-fixing and phosphate-dissolving microorganisms in addition to A-mycorrhizae on yield, chemical and technological characteristics of grains of wheat plant.

Compost addition and microbial inoculation increased grains and straw yield of wheat plant through the two studying seasons. The interaction between compost addition and microbial inoculation increased grains and straw yield by values much higher than the effect of each factor alone, and the best influence was achieved by using microbial inoculation+5 tons compost/fed. Studying treatments did not affect grains nitrogen %, while the same treatments increased phosphorus %. Protein % did not significantly increase due to the above mentioned treatments. Compost addition decreased proline concentrations (mg/g) in wheat grains. In contrast, microbial inoculation and interaction between compost addition and microbial inoculation raised grains proline concentrations, and the interactions attained significant differences than un-treated control. The technological characteristics of resulted grains influenced in different manner, whereas, all treatments increased germination %, weight of 1000 grains and seedling dry weight over un-treated control, while radical and root lengths were not significantly influenced. Compost addition as well as microbial inoculation achieved a positive net return (L.E./fed.). The treatment (inoculated + 5 tons compost/ fed.) attained the highest net return average (3942 L. E./ fed.) compared to 1856.4 (L. E./fed.) for un-treated control.

Key words: Compost, N_2-fixers, phosphate - dissolvers, A-mycorrhizae, salinity, wheat

INTRODUCTION

Saline soils are world wide problem. Approximatly 40 x 10^6 hectares of irrigated soils allover the world are affected with salinity, and about 40% of world's land may be susceptible to salinity (Raid and Snigh, 1999).

Increase of salinity leads to increasing osmoses of soil solution, thus the plants and soil microorganisms need big efforts for uptake water and nutrients from soil, consequently this result in a decrease in crop yield according to type of plant species and tolerance to salinity. This, also, leads to deterioration of microbial density and activity, soil characteristics and crop yield (Richards, 1954).

To overcome salinity problem, it is of importance to choose the tolerant plant species and varieties planted in this type of soil. In addition, this soil profile must be frequently washed with good quality water. On the other hand, using the salinity-tolerant micro-organisms for inoculation of plants sown in this type of soil must be considered in order to increase its fertility and improves its characteristics. These soil micro-organisms as reported by Younes *et al.* (2000) secrete many organic compounds that increase soil aggregation and aeration. Fungal hyphae, also, increased soil aggregates, as A-mycorrhizal fungal which, in addition, absorb amounts of salts in their hyphae and consequently decrease the effect of salinity on plants. Kim *et al.* (1998), Osundina (1998) and Singh and Kapoor (1999) stated that N_2-fixing and phosphatc-dissolving microorganisms incre-ased levels of nitrogen and phosphorus in soil and plants, which increase its fertility and productivity.

Addition of organic matter to soil is very important in improving soil fertility, microbial density and productivity. Elmholt and Kjoller (1989) found that the organic matter in the form of animal manures, crop residues, and green manure composts have been known for longer time to improve soil tilth, nutrient status and crop production. In addition, Zebarth *et al.* (1999) and Garcia *et al.* (2000) stated that the beneficial effects of organic amendements include decreased soil bulk density and increased water holding capacity, aggregate stability, saturated hydrolic conductivity, water infeltration rate and microbiolo-gical activity.

Therefore, the aim of the present study is to investigate the response of wheat plants grown in salt-affected soil to application of different levels of compost and inoculation with combined inoculum containing a mixture of some N2-fixing, phosphate-dissolving microorganisms and A-mycorrhizae isolated from saline soils.

MATERIALS AND METHODS

Compost: is manufactured using rice hay (60%), farmyard manure (25%), poultry manure (10%) and fertile soil (5%). Farmyard manure, poultry manure and soil were mixed thoroully (mixture). A layer of rice hay with 5m length, 2m width and 25cm hight was formed and sprayed with water, followed by a layer of the aforementioned mixture (fertilizer+soil) with 20 cm hight. These layers was repeated alternatively till the pile reached to about 1.5m hight. Water was sprayed after each formed layer and after that all pile was covered with soil. The pile was mixed each week till maturation, the moisture maintained at 60% throughout the decomposition time. It's chemical and biological characterestics shown in Table 1b.

Microbial inoculum composed of:
Azotobacter: isolated by using Vancura and Mucura (1960) medium which composed of: Sucrose , 30.0g; $MgSo_4$-$7H_2O$, 0.2g; $CaCo_3$, 2.0g; $Fe_2(So_4)_3$, 0.005g; $NaMo_4$, 0.005g; $NaBo_3$, 0.005g; distilled water, 1L. The cells suspension density was 8.5 x 10^8 CFU/ml.
Azospirillum: isolated by using medium of Dobereiner (1978) Which composed of : KOH, 4.0g; Malic acid, 5.0g; $MgSo_4$-$7H_2O$, 0.2g; NaCl, 0.1g; $MnSo_4$-H_2O, 0.1g; $CaCl_2$, 0.2g; $FeSo_4$-$7H_2O$, 0.5g; $NaMoO_4$, 0.002g; distilled water, 1L. The cells suspension averaged 9 x 10^8 CFU/ml.
A-mycorrhizae: consists of spores and plant roots obtained from Faculty of Agriculture, Kafr El-Sheikh, Tanta Univ. The inoculum cultured by inoculation maize plants, grown in pots, with 100 g of micorrhizal inoculum spread at 3cm under soil surface. After 3 months of growth, mycorrhizae isolated by wet sieving using 53-µm sieve (Daniels and Skipper, 1994). The final micorrhizal inoculum contains hyphae, vesicles, arbuscules and spores of mycorrhizae. All micobial types were isolated from rhizosphere of wheat plants grown in saline soil (El-Hamoul region). About 50 ml from cultures of *Azotobacter* and *Azospirillum* were mixed with 200 g of peat moss prior to inoculation of seeds, in addition, 100 g of soil contained A-mycorrhizal inoculum was mixed with the bacterial mixture at the time of sowing. Inoculation process undertaken by mixing the aforementioned combined inoculum (300g/fed.) with wheat grains using Arabic gum as a stacking material.

Complete randomized block design with four replicates was used. Plots area was 3 x 3 m^2.

Compost was applied with the levels of zero, 2.5 and 5 tons/feddan. Un-inoculated plots were dressed with 70 kg N/fed. as ammonium nitrate and with 30 kg P_2O_5/fed. as calcium super phosphate, but inoculated plots received 50 kg N/fed. and 15 kg P_2O_5/feddan. Potassium was added to all plots at 15 kg KO/fed. as potassium sulphate. Wheat grains were broadcasted in each plot with the rate of 70 kg/feddan. Some chemical charateristics of compost and experimental soil are shown in Tables 1a and 1b.

Table 1a: Chemical characteristics of the experimental soil

Ec dS^{-1}	pH	O.C.%	O.M%	Cations ($meq\ L^{-1}$)				Anions ($meq\ L^{-1}$)				Available macroelements (ppm)		
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3^{-}	HCo_3^{-}	Cl^{-}	So_4^{-}	N	P	K
10.8	7.94	1.68	1.89	26.0	30.0	51.6	2.90	4.0	9.0	39.2	56.3	28.3	1.76	209

Plants were collected at harvest for estimation of grains yield (tons/fed.), weight of 1000 grains (g), density of grains (g/cm^3), germination %, radical length (cm), shoot length (cm) and seedling dry weight (g). Germination % was carried out under optimum conditions according to International rules (I.S.T.A., 1993). Radical length, shoot length and seedling dry weight were measured according to the procedures reported in the seeds vigor testing handbook (A.O.S.A, 1991). Weight and volume of 1000 grains were estimated by counting manually and weighted, then their volume was measured by absolute displacement method (Kramer and Twigg, 1962).

Chemical Analysis

Grains were ground to fine powder and sieved through 2 mm sieve, then packed in paper bags until analysis. Nitrogen % was determined using micro-Kjeldahl method reported by (A.O.A.C., 1975). Phosphorus was measured colorimetrically according to Snell and Snell (1967).

All experimental data were statistically analyzed by using technique of analysis of variance (ANOVA) as mentioned by Steel and Torrie (1980) using IRRISTAT software version 3/93 Biometric unit. International Rice Research Institute, Mannila, Philippine. Duncan (1955) multiple range test was used to compare means at 0.05 level of probability.

Table 1b: Chemical characteristics of applied compost

Character	Value
Weight of m^3 (kg)	510 kg
Moisture %	18.2
pH	7.6
Ec	6.65
Organic carbon %	24.6
Organic matter %	42.41
Total nitrogen %	1.85
C/N ratio	1:13.3
Amoniomic- nitrogen (ppm)	580
Nitrate-nitrogen (ppm)	250
Total phosphorus %	1.6
Available phosphorus (ppm)	410
Total potassium %	2.3
Available potassium (ppm)	620
Humic value	5
C.E.C. (meq/ 100g)	83.7
Fe (ppm)	960
Zn (ppm)	280
Mn (ppm)	320
Cu (ppm)	140
Growth of herbs	Zero
Nematods	Zero
Microbial parasites	Zero

RESULTS AND DISCUSSION

Grains and Straw Yield

Data presented in Table 2 indicated that compost application had a positive influence on grain and straw yields of wheat plants. In the first season, the treatments with un-inoculated + zero compost (control), un-inoculated + 2.5 tons compost/fed. and un-inoculated + 5.0 tons compost/fed. attained grain yield evaluated 0.99, 1.55 and 3.27 tons/fed. respectively. These treatments, also, achieved straw yield evaluated 1.68, 1.83 and 2.41 tons/fed., at the same order. The difference between control and treatment with un-inoculated + 5.0 tons compost/fed. was significant. Results of the second seasons were similar to those of the first season.

Data of Table 2, also, showed that microbial inoculation notably increased grains and straw yields of wheat plants. In this connect, the treatment of inoculated + zero compost attained grains and straw yields up to 1.81 and 1.70 tons/fed. compared to 0.991 and 1.68 tons/fed. at the same order for control treatment (un-inoculated + zero compost). The differences were significant for grain yield only. The results of the second season were similar to those of first one.

The interaction between compost application and microbial inoculation improved productivity much higher than each one alone. The best treatment was inoculated + 5.0 tons compost/fed. which achieved grain yield of 3.99 and 3.83 tons / fed. for the first and second seasons respectively, and gave straw yield of 4.15 and 2.60 tons/fed. at the same order. The differences between treatments and controls were significant except for straw yield of the second season.

Organic fertilization improving soil characteristics, fertility and plant productivity (Zebarth *et al.*, 1999 and Garcia *et a.*, 2000). The organic manure used in the present investigation is the compost which application is plentiful to get rid of agricultural wastes which cause environmental pollution. This compost is of high nutritional value as observed from compost composition presented in Table (1b). Galal and Thabet (1999) reported that compost application positively increased yield of wheat plants. These results are completely in harmony with those of the present study, that indicated remarkable increase in grain and straw yield of wheat plants due to compost application up to 5 tons/ feddan. Recently, Speir *et al.* (2004) reported that yield of silver beet (*Beta vulgaris* L.) increased with increasing compost application.

As well, biological fertilization with N_2-fixing and phosphate-dissolving microorganisms are of great importance in increasing crop production (Rao *et al.* 1990; Kundu and Sharma, 1994 and Toro *et al.*, 1997), and saving mineral fertilizers (Zein *et al.*, 2000). Moreover, inoculation of plants grown in salt-affected soils with salt- tolerant microorganisms offered them tolerance against salinity, thereby increased their productivity (Zahran, 1999). In the present study, wheat plants grown in saline soil (EC,10.8 dSm^{-1}) were inoculated with microorganisms isolated from saline soil. The inoculation remarkably increased grains and straw yields of the plant. In addition, inoculation of wheat plant, in the present investigation, with the mixed inoculum saved about 100 kg super phosphate/feddan and 75 kg urea/ feddan. Thereby, the use of the present inoculum could be valuable in increasing plant yield, saving mineral fertilizer and decreasing environmental pollution.

Table 2: Effect of microbial inoculation and compost addition on grains and straw yields of wheat plants

Treatments	Grains yield (tons/fed.)		Straw yield (tons/fed.)	
	2003	2004	2003	2004
Un-inoculated, without compost (control)	0.99a	2.75a	1.68a	2.31a
Un-inoculated + 2.5 tons compost/fed.	1.55ab	3.01ab	1.83ab	2.51a
Un-inoculated+ 5.0 tons compost/fed.	3.27c	3.40b	2.41ab	2.47a
Inoculated, without compost	1.81b	3.29ab	1.70ab	2.76a
Inoculated+2.5 tons compost/fed.	2.99c	3.35ab	3.50bc	2.85a
Inoculated+ 5.0 tons compost/fed.	3.99d	3.83b	4.15c	2.60a

Un-inoculated treatments received 70 kg N/fed. as ammonium nitrate and 30 kg P_2O_5 as calcium super phosphate.

Inoculated treatments received 50 kg N/fed. as ammonium nitrate and 15 kg P_2O_5 as calcium super phosphate.

Chemical Constituents of Wheat Grains

Data recorded in Table 3 indicated that nitrogen percentages of wheat grains did not significantly affected by treatments of inoculation and/or compost addition. Similarly, data of Table 3 showed that all applied treatments did not significantly increase protein % in wheat grains, however, the studied treatments caused increases in phosphorus % over control (un-inoculated+ zero compost) reached in most cases to significance. The highest value of P % was recorded due to treatment of inoculated + 2.5 tons compost/fed. reached 0.422 % compared to 0.204 % for control (un-inoculated+ zero compost). Data of total fats % (Table, 3) indicated that the aforementioned treatments did not significantly affect total fats and ash % in wheat grains as compared with the control treatment (un-inoculated+ zero compost). On the other hand, data of proline concentration (Table, 3) showed significant variations among applied treatments and control, whereas, compost application in absence of microbial inoculation significantly decreased proline concentrations. Meanwhile, inoculation treatments increased proline concentrations over control treatment which, in most cases, were significance such as treatments of inoculated + 2.5 tons compost/fed. and inoculated +

5.0 tons compost/fed. which attained 1.356 and 1.179 mg/g respectively compared to 0.807 mg/g for control one.

Nitrogen and protein percentages of wheat grains were not affected due to application of compost and/or microbial inoculation, this may be attributed to dilution of the element throughout growth activation induced by the studied treatments. However, phosphorus % increased due to compost addition and /or microbial inoculation, these results were strengthened by reports of Azcon *et al.* (1978), Kim *et al.* (1998) and Singh and Kapoor (1999). The inoculation with phosphate-solubilizing bacteria (PSB) and A-mycorrhizae may be the main reason for increasing plant P%, whereas, phosphate-solubilizing bacteria solubilize un-available phosphate in soil, which became available for plant uptake. In addition, A-mycorrhizae absorb available phosphate dissolved by PSB and suplement the plant with it (Kim *et al.*, 1998). Abdelgani *et al.*(1999) found that inoculation of fenugreek plants with rhizobia increased their fats content. In contrast, our results did not show significant differences in fats % between control and studied treatments.This may be related to the difference in the plant species.

Table 3: Effect of microbial inoculation and compost addition on some chemical compositions of wheat grains

Treatments	N%	P%	Protein %	Total fats %	Ash %	Free proline (mg/g)
Un-inoculated, without compost (control)	2.217a	0.204a	11.29b	1.417a	2.06a	0.807c
Un-inoculated+ 2.5 tons compost/fed.	1.867a	0.392b	11.90b	1.313a	2.06a	0.727b
Un-inoculated+ 5.0 tons compost/fed.	2.150a	0.307ab	11.22b	1.343a	1.93a	0.711b
Inoculated, without compost	2.050a	0.321b	12.58b	1.380a	2.08a	0.865c
Inoculated+2.5 tons compost/fed.	2.117a	0.422b	11.68b	1.437a	1.96a	1.356e
Inoculated+ 5.0 tons/fed.	1.817a	0.350b	13.01b	1.533a	1.97a	1.179d

Un-inoculated treatments received 70 kg N/fed. as ammonium nitrate and 30 kg P_2O_5 as calcium super phosphate.
Inoculated treatments received 50 kg N/fed. as ammonium nitrate and 15 kg P_2O_5 as calcium super phosphate.

The present study indicates that proline concentration sharply increased due to inoculated treatments over untreated control. Proline accumulation is a common metabolic responses of higher plants to water deficit and salinity stress (Samaras *et al.*, 1995, Taylor, 1996 and Rhodes *et al.*, 1999). Proline may functions as protein compatible hydro trope (Srinivas and Balasubramanian, 1995) and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989) or as nitrogen storage and pH regulation (Delauney and Verma, 1993). Selection for hydroxyproline-resistant mutants of barley and winter wheat has succeeded in identifying lines that accumulate greater quantities of proline than wild types. Salt tolerant and polyethylene glycol resistant mutants of *Nicotiana plumbaginifolia* have been derived from protoplast culture and appear to have enhanced proline accumulation in comparison to wild-type (Sumaryati *et al.*, 1992). So, inoculation treatments in the present study may be caused increase in salinity resistance of the plant, which may be reflected on the yield.

Technological Characteristics

Data recorded in Table 4 showed germination % and some technological characteristics of wheat grains as affected by inoculation and/or compost application. Results indicated that all applied treatments increased laboratory germination % than control treatment, the increases mostly were significant. So far, all applied treatments except treatment of un-inoculated +2.5 tons compost /fed. gave 100% germination compared to control treatment which gave 91%.

The studied grains vigor characteristics are: weight of 1000 grains, radical length, shoots length and weight of seedling. Most of applied treatments increased weight of 1000 grains over control, but the only significant increase over control was recorded due to the treatment of inoculated+2.5 tons compost /fed. which attained 61.53 g compared to 52.83 g for control treatment. The applied treatments did not significantly influence radical and shoot lengths of the germinated grains, but weight of seedling remarkably influenced, whereas, all studied treatments significantly increased seedling dry weight (g) over control treatment. The highest value was recorded by the treatment of un-inoculated + 5 tons compost/fed., followed by inoculated+5 tons compost /fed. (0.045 and 0.036 g) compared to 0.023g for control treatment (un-inoculated + zero compost).

Inoculation and/or compost addition slightly increased weight of 1000 grains and significantly increased germination % and weight of seedling. This may be attributed to the effective role of microbial inoculation together with organic manure on plant nutrition. Bahatia *et al.*, (1998) and Younes *et al.* (2000) indicated that inoculation with N_2-fixing and phosphate-dissolving microorganisms may improve soil fertility and productivity. Also, the present data recorded in Table 3 indicated that inoculation and/or compost addition increased plant phosphorus percentage. In addition, study of Nour El-Dein *et al.* (2005, in press) indicated that inoculation of wheat` plants with *Azotobacter* and/or farmyard manure addition increased weight of 100 grains and seedling dry weight.

Table 4: Effect of microbial inoculation and compost addition on laboratory germination %, and some seedling vigor characteristics of wheat grains

Treatments	Laboratory germination %	Seedling vigor			
		Weight of 1000 grains	Radical length (cm)	Shoot length (cm)	Seedling dry weight (g)
Un-inoculated, without compost (control)	91a	52.83a	14.7a	11.8a	0.023a
Un-inoculated + 2.5 tons compost/fed.	96ab	54.87ab	16.3a	13.2a	0.034b
Un-inoculated+ 5.0 tons compost/fed.	100b	54.47ab	16.6a	15.5b	0.045c
Inoculated, without compost	100b	51.40a	16.5a	12.1a	0.033b
Inoculated+2.5 tons compost/fed.	100b	61.53b	17.2a	12.3a	0.033b
Inoculated+ 5.0 tons/fed.	100b	56.47ab	16.2a	12.3a	0.036b

Un-inoculated treatments received 70 kg N/fed. as ammonium nitrate and 30 kg P_2O_5 as calcium super phosphate.
Inoculated treatments received 50 kg N/fed. as ammonium nitrate and 15 kg P_2O_5 as calcium super phosphate.

Economic Evaluation

Data of Table 5 showed economic evaluation of the studied treatments. Data includes costs, value of grain and straw yield (L.E./fed.) and the net return (L.E./fed.). Net return is the important factor considered here. It is obvious that all applied treatments notably increased net return (L.E./fed.), and the highest average of net return was attained by the treatment of inoculated+5 tons compost/fed. which achieved 3942 L.E./fed. in comparison to 1856.4 L.E./fed. for un-treated control. Thereby, we recommend the application of the treatment which had good return under (inoculated + 5 tons compost/fed.), salinity circumstances.

Table 5: Economic evaluation of average of the two study seasons for wheat crop treated by compost levels and/or microbial inoculation

Treatments	Fixed costs	Changed costs	Total costs	Value (L.E./fed.) Grians value	Straw value	Total value	Net return (L.E. / fed.)
Un-inoculated, without compost (control)	300	400	700	2057.6	498.8	2556.4	1856.4
Un-inoculated + 2.5 tons compost/fed.	300	650	950	2506.4	542.5	3048.9	2098.9
Un-inoculated+ 5.0 tons compost/fed.	300	900	1200	3669.0	643.5	4312.8	3112.8
Inoculated, without compost	300	405	705	2802.3	557.5	3359.8	2554.8
Inoculated+2.5 tons compost/fed.	300	655	955	3488.1	793.8	4291.9	3336.9
Inoculated+ 5.0 tons/fed.	300	905	1205	4303.2	843.8	5147.0	3942.0

Fixed costs (L.E./fed.) includes: costs of seeds (100), irrigation (50), herbicides (50) and harvest (100).
Changed costs (L.E./fed.) includes: compost (100 L.E./ton), chemical fertilizers (300 for uninoculated treatments and 150 for inoculated ones), labor (100) and inoculum (5).
Value of wheat hay is 250 L.E./ton and value of grains is 1100 L.E./ton.

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أهمية التسميد الحيوي و إضافة الكومبوست في تحسين الإنتاجية وبعض الخواص الكيماوية والتكنولوجية لنبات القمح المنزرع في أرض ملحية

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أجريت تجربتين حقليتين بمنطقة الحامول - محافظة كفر الشيخ موسمي ٢٠٠٣ و ٢٠٠٤ بهدف دراسة تأثير التلقيح بمخلوط من بعض الميكروبات المثبتة للأزوت الجوي و المزيبه للفوسفات مع الميكورهيزا الداخلية التي عزلت من أراضي متأثرة بالملوحة (منطقة الحامول) و ذلك على إنتاجية نبات القمح (سخا ٩٣) و بعض الصفات الكيماوية و التكنولوجية للحبوب الناتجة.

زادت معاملات إضافة الكومبوست و التلقيح الميكروبي من محصول الحبوب و القش لنبات القمح خلال موسمي الدراسة. و أعطي التداخل بين التلقيح الميكروبي و إضافة الكومبوست زيادة في الإنتاجية اعلي من كل معاملة علي حدة و كانت أفضل معاملة هي (التلقيح+ ٥ طن كومبوست للفدان). لم تؤثر معاملات الدراسة معنويا علي نسبة النيتروجين بالحبوب، في حين زادت من نسبة الفسفور، كما لم تتأثر نسبة البروتين بدرجة معنوية. قللت إضافة الكومبوست من كمية البرولين بالحبوب (ملليجرام/جرام) ، في حين زاد التلقيح الميكروبي و التداخل بين التلقيح الميكروبي و الكومبوست من كميته و كانت الزيادة معنوية في حالات التداخل بين التلقيح الميكروبي و الكومبوست. تأثرت الخواص التكنولوجية بطريقة مختلفة، حيث زادت كل المعاملات من نسبة إنبات الحبوب ووزن ١٠٠٠ حبة ووزن البارادات الجاف و ذلك عن معاملة المقارنة، في حين لم يتأثر طول الجزير و الريشة بدرجة معنوية. كان لإضافة الكومبوست صافي عائد (بالجنيه المصري) ايجابي، كما زاد التلقيح الميكروبي من صافي العائد، و أعطت المعاملة (تلقيح+ ٥ طن كومبوست/للفدان) اعلي صافي عائد، حيث حققت متوسط صافي عائد قدره ٣٩٤٢ جنيها مصريا في مقابل ١٨٥٦,٤ جنيها لمعاملة المقارنة.