INFLUENCE OF FOLIAR APPLICATION WITH SOME MICRONUTRIENTS AND SOME MICROORGANISMS ON GROWTH OF PEANUT PLANT

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ABSTRACT

Two field experiments were conducted in 2003 and 2004 seasons at El-Boston district (El-Hoseen village) to study the influence of foliar application with a mixture of micronutrients: Mn, Zn, Fe, Cu and B (MN), a suspension of Azotobacter (Az), a suspension of packing yeast (Sa) and the mixture of them (Mix) on nodulation, yield and some chemical constituents of peanut (Arachis hypogea) plant (Giza, 5).

Data indicated that Mix treatment significantly increased number and dry weight of nodules. The dry weight of plant, also, positively responded to this treatment. All treatments attained remarkable increase in nitrogen % of seeds and the Azotobacter treatment gave the highest value. In addition, most of applied treatments exhibited increase in phosphorus % of seeds. While, potassium % did not significantly changed. Most of treatments increased Cu, Mn and Fe elements in plant seeds and the treatments of Azotobacter and Mix recorded the highest values. The studied treatments increased seeds yield of the plant. The spray with micronutrients and Azotobacter achieved the highest increases over un-sprayed control. All the applied treatments increased the net return (L. E./fed.). The highest net return was observed for spray with micronutrients and Azotobacter. However, this point needs more extension studies for confirmation and development of the crop. Key words: Peanut, foliar application, micronutrients, Azotobacter, packing yeast

INTRODUCTION

eanut plant is an important crop extensively agricultured in Egypt, especially in new reclaimed soils. Roots of the plants symbiotically associated with nodule-forming N2-fixing bacteria (rhizobia) causing enrichment to the soil with nitrogen. Since 1961, Ruinen introduced the vital role of foliar epiphytes, and in 1971 she reported that phyllosphere microorganisms supported dinitrogen-fixation. Numerous microflora responsible for N2-fixation in soybean either symbiotically (Bessems, 1973) or asymbiotically (Freire, 1973) inhabitant phyllosphere. Fokkema (1976) and El-Hosseiny and Rabie (1979) concluded that phyllosphere microflora play an important role in plant diseases and nutrition.

Mahmoud et al (1987) found that phyllosphere of some medicinal plants contains large numbers of different microorganisms. Their numbers increased in summer than in winter. They found some N₂-fixing microorganisms such as Azotobacter and Closteridia.

A number of yeasts were found also in phyllosphere of Triticum vulgar like Rodotorulla glutinis and Cryptococcus magnus (Foda et al. 1988). Subba Rao (1984) and Ahmed et al. (1995) mentioned that active dry yeast as a foliar fertilizer enhanced growth and trees nutritional status. Shadia et al. (1998) attributed the positive role of foliar application of active dry yeast to its contents of different nutrients, high percentage of protein, larger amounts of vitamin B and natural plant growth regulators such as cytokinins.

In recent years the application of fertilizer fluids has become more common. Liquid fertilizers are generally easier to transport than solids ones and cause less labor problem in handling and application (Mengel and Kirkby, 1987). They reported also that application is particularly useful under conditions

where nutrients uptake from the soil is restricted such as Fe, Mn Zn and Cu.

Foliar application with micronutrients has been extensively studied, although, the studies on peanut crop still few. On the other hand, the previous literature on foliar application with microorganisms and their combination with micro-nutrients were scarce. Therefore, the present investigation aimed to study the effect of foliar application with Saccharomyces cervisiae solution (Sa), Azotobacter spp. culture (Az), a mixture of micronutrients: Mn, Zn, Fe, Cu and B (MN) and their combinations (Mix) on yield components of Arachis hypogea and some chemical constituents of the plant seeds.

MATERIALS AND METHODS

Two field experiments were conducted in El-Bostan district, El-Hoseen village, Behara Governorate, in winter seasons of 2003 and 2004.

The used microorganisms:

1- Saccharomyces cervisiae(Sa): was used as a concentration of 1 g active dry yeast/L with the rate of 200 liter/feddan (Shadia et al., 1998). Viable counts of active dry yeast was estimated using glucose nutrient broth medium which averaged about

4 x 10¹⁰ cells/g yeast.

2-Azotobacter spp.(Az): was isolated and cultured in laboratory of Microbiology, Sakha Agricultural Research Station, Kafr-El-Sheikh, Sakha, using Vancura and Mucura medium (1960): sucrose, 30.0 g, K₂HPO₄, 0.16 g, NaCl, 0.2g, MgSO₄-7H₂O, 0.2g, CaCO₃, 2.0g, FeSO₄, 0.005g, NaMO₄, 0.005, NaBO₄, 0.005, distilled water, 1 liter. The viable counts of cells were 9.2 x 10⁸ cell/ml of culture diluted to 9.2 x 10⁶ at application time. The Azotobacter (Az) was applied by the rate of 200 liter of diluted culture/feddan.

3-Micromutrients (MN): Mn, Zn and Fe as chelated compounds, Cu as CuSo₄ and B as boric acid. All elements were mixed and applied as solution contains 0.5 g of each element in one liter of tap water. The nutrients solution was applied at the rate of 200 liter/feddan.

Rhizobium spp. (Arachis): inoculum was prepared in Microbiology Lab., Sakha Agricultural Research Station which cultured in yeast mannitol medium:

Mannitol, 10g, K2HPO₄, 0.5g, MgSO₄-7H₂O, 0.2g, NaCl, 0.1g, yeast extract, 0.5g and distilled water, 1 liter (Somasegaran and Hoben, 1985). The viable counts were about 1x 10° cells/ml of culture.

Seeds: variety of (Giza, 5).

Experimental soil: is a sandy soil and some physical and chemical characteristics of it are presented in Table 1.

Table 1: Some physical and chemical properties of experimental soil.

Sand%	Sitt?	6	Clay%	Textu	ne	O.M.%	,	рH	EC, dSm
93.44	6.56		-	Sandy		0.42	7	7.92	0.33
Cations	(mg/L)		Anion	s (mg/L)			Mac	ro-nutrient	a (ppm)
Na	Ca ⁺	Mg [↔]	CO ₃	HCO ₃	Cl ⁻	So ₄	N	P	K
0.62	0.86	0.17	-	1.24	0.16	0.72	9	16	122

The experiment was statistically designed as complete randomized plot design with four replicates. Plot area was 3x5 m². The treatments were as follow:-1-Control, un-sprayed,

- 2-foliar application with Saccharomyces cervasiae (Sa),
- 3- foliar application with Azotobacter spp. (Az),
- 4-foliar application with micronutrients mixture (MN),
- 5- foliar application with a mixture of them (Mix).

Seeds of all treatments including control were inoculated with peat-based inoculum contains Rhizobium spp. (Arachis), the viable counts of rhizobia in the inoculum was about 3.3x108 CFU/g. Peanut seeds were planted on ridges at 70 cm distance. Seeds were sown in hills at 30 cm between plants. Experimental plot contains 7 ridges, the two border ridges were excluded from sampling. Irrigation was carried out weekly with amount of water about 150 m³ by sprinkler method. All plots were fertilized with N, P, K as ammonium sulphate, calcium super phosphate and potassium sulphate at rates of 20 kg N/fed., 30 kg P₂O₅/fed. and 15 kg K₂O/fed. respectively. The calcium super phosphate was added to soil before tillage, while N and K fertilizers were divided into five doses (15 days between each one) and applied just before irrigation. Weeds were controlled twice by howing between each of them one month. Plants sprayed with the experimented materials at 45 days of growth and at flowering.

Plant samples were collected after 60 days from sowing to estimate dry weight of plant (g/plant), number of nodules per plant and dry weight of nodules (g/plant). At harvest, seeds were collected for yield estimation (tons/fed.). Seed samples were collected for chemical determinations. Seeds were oven dried at 70° C till weight stability, then ground and packed in paper bags prior to chemical analysis. Nitrogen was estimated in seeds by micro-Kjeldahl method reported by (A.O.A.C., 1975). Phosphorus was measured

calorimetrically according to (Snell and Snell, 1967). Potassium was measured by flame-photometer as described by Richard *et al.* (1945).

All experimental data were statistically analyzed by estimating analysis of variance (ANOVA) as reported by Steel and Torrie (1980) using IRRISTAT software version 3/93 Biometric unit. Duncan (1955) multiple range test was used to compare means at 0.05 level of probability.

RESULTS AND DISCUSSION Number and dry weight of nodules, and dry weight of plants:

Data presented in Table 2 indicated that foliar spray with Az and MN decreased number of nodules compared to control. However the Mix treatment exhibited consistent significant increases (408 and 444) compared to (349 and 314) for controls in first and second seasons respectively. Dry weight of nodules also increased by application of Mix and MN treatments. But, spray with Sa and Az showed decreases which mostly were significant compared to controls in both studied seasons. Dry weight of plant increased due to all studied treatments. The highest values attained by the treatments of Mix (171.8 g/plant) in the first season and Sa (134 g/plant) in the second season. The decreases in number and dry weight of nodules caused by Sa and Az were unexpected and could be attributed to the role of both microorganisms in the phyllosphere as supplying the plants with some of their requirements from nitrogen and other elements which enhanced plant growth but still inadequate for nodulation process. This explanation was supported by the data of Ruinen (1971) and Bessems (1973) who stated that the phyllosphere microflora support dinitogen fixation. Active dry yeast was found by Ahmed et al. (1997) to contain different nutrients, large amounts of vitamin B and natural plant growth regulators such as cytokinins. While, the positive influence of MN and Mix treatments could be related to the activated role of micro-nutrients to nodulation process as well as nitrogenase activity. In this connect, Rao et al. (1978) and Haque and Bunda (1980) reported that biological nitrogen fixation might not be effectively preceded in the absence of micro-nutrients. The absence of Cu markedly depressed nodule development and N2-fixation, because of the essential role of Cu in carbohydrate biosynthesis, thus it has an indirect effect in N2-fixation, since N2-fixing nodules have a very high demand for carbohydrates. In the present study,

the enhanced effect of foliar application with Sa, Az, MN and Mix treatments on dry weight of plant were in complete harmony with studies of many authors (Abd-El-Maksoud et al., 1984, on micro-nutrients), (Khalafalla et al., 1984, on Azotobacter) and (Ahmed et al. 1995, on active dry yeast). The reasons of this enhancement my be due to nutrients and plant growth hormones released from yeast, N₂-fixation of Azotobacter and activated role of micro-nutrients to most of the plant enzymes.

Table 2: Number of nodules, dry weight of nodules (g/plant) and dry weight of peanut plant (g/plant) as affected by foliar application with Azotobacter app., Saccharomyces cervisiae, micromytrients and their combinations through seasons 2003 and 2004.

Treatment	Number of nodules per plant		Dry weight of nodules (g/plant)		Dry weight	of plant (g/plant)
	2003	2004	2003	2004	2003	2004
Control	349Ъ	314b	1.27bc	0.80b	80.87a	104.70Ь
Sa	333b	433c	0.95d	0.67b	104.00cde	134.0 a
Az.	179f	241ab	0.73e	0.57c	91.30abc	107.41b
MN	312c	247ab	1.60a	0.84b	125.90f	126.70ab
Mix	408a	444c	1.37b	1.13a	171.80g	123.59ab

Sa: Saccharomyces cervasia, Az: Azotobacter spp., MN: micronutrients Mix: a mixture of them Means followed by the same letters are not significantly different according to Duncan's multiple test at 0.5 % level

N. P and K concentrations:

Data presented in Table 3 showed that all treatments exhibited remarkable significant increases in nitrogen percentages over the control. Foliar application with Azotobacter (Az) attained the highest values in the two growing seasons (4.83 and 5.43%). The differences mostly were significant especially in the first season of growth. These results were in harmony with those of Abd-El-Maksoud et al. (1984). They found that foliar application with micronutrients or micronutrients+ Azotobacter increased protein percentage in soyabean seeds. Khalafalla et al. (1984) showed that phyllosphere enrichment with Azotobacter . micronutrients and Azotobacter + micronutrients raised nitrogen and protein contents of soyabean seeds. This positive effect could be due to the beneficial effect of Azotobacter as N2-fixer and phytohormone producing (Ruinen, 1971). In addition, micronutrients enhance function of most plant enzymes and improved nodulation and N2-fixation (Rao, et al. 1978 and Mengle and Kirkby, 1987). While Saccharomyces cervasiae contains a large amount of protein (34.87%) as well as release of cytokinines (Shadia et al., 1998).

Data of Table 3 showed that most of foliar application treatments with microorganisms and/or micronutrients gave slight increases in phosphorus % of peanut seeds. However most of these increases were not significant. In contrast, different studied foliar application treatments, mostly, exhibited slight decreases in potassium % of peanut seeds. Mix treatment gave insignificant increases throughout the two studied seasons in potassium % giving 1.217 and 1.250 % compared to 1.170 and 1.221 % for control treatments of first and second seasons, respectively. Pazzar et al. (1965) concluded that the better growth of plant and increase in seed yield by spraying micronutrients might be due to increase in nucleic acid phosphorus, phosphoprotein and phospholipids. While the role of active dry yeast in enhancing P and K may related to its own contents of different macro- and micro-nutrients, high percent of protein, large amounts of vitamin B and natural plant growth hormones such as cytokinins (Ahmed et al., 1997). In addition, rhizosphere and phyllosphere microflora play an important role in plant nutrition (El-Hoseiny and Rabie, 1979).

Table 3: Nitrogen, phosphorus and potassium percentages as affected by foliar application with Azotobacter spp., Saccharomyces cervisiae, micro- nutrients and their combinations through seasons 2003 and 2004.

Treatment	Nitrogen%		Phosphoru	s %	Potassium%	
	2003	2004	2003	2004	2003	2004
Control	4.20e	4.38a	0.182abc	0.182a	1.170ab	1.221at
Sa	5.04a	4.32a	0.200a	0.200a	1.210a	1.163ab
Az.	4.83b	5.43e	0.139c	0.245a	1.127ab	1.235a
MN	4.38d	4.90bcd	0.150bc	0.200a	1.077b	1.120ab
Mix	4.62c	4.56ab	0.225a	0.213a	1.217a	1.250a

Sa: Saccharomyces cervasia,

Az: Azotobacter spp.,

MN: micro-nutrients.

Mix: mixture of them

Means followed by the same letters are not significantly different according to Duncan's multiple test at 0.5 % level.

Concentrations of Cu. Mn. and Fe:

Data in Table 4 illustrated that most of microbial and /or micro-nutrients treatments increased Cu concentrations in peanut seeds, especially in the second season. The highest concentrations were recorded due to application of Azotobacter and mixture treatments, hence they attained 7.26 and 5.42 mg/g in the first season and 8.78 and 12.79 ppm of seeds in the second season respectively. Mn concentration followed a similar trend, whereas most treatments especially second season of the significantly increased Mn concentration. Spray with Azotobacter and mixture achieved the highest records, they attained 54.35 and 44.35 ppm in the first season and 52.50 and 76.50 ppm of seeds in the second season respectively. In addition, Fe concentrations followed a similar trend, as the applied treatments generally increased Fe concentrations in the seeds and the differences were significant in the second experimental season. Spray with Azotobacter attained consistent high results (127.6 and 166.18) compared to (111.05 and 115.35 ppm) for un-treated controls in the first and second seasons, respectively.

Zeinab (1996) indicated that foliar application with chelated Fe and Zn gave corresponding increase

of these elements in soybean plants. In similar study, Kabeel et al. (1998) reported that foliar spray with Mg+Fe+Zn+Mn increased these micronutrients in leaves of pear trees. They attributed this to quick absorption of sprayed elements via leaves. As well, Amin et al. (1998) reported similar results on maize plant. However Ali et al. (1995) found a negative relationship between foliar application with Fe. Mn and Zn and their concentrations in lunine seeds, they attributed this influence to the increase in plant growth and thereby, dilution of these elements in the plant tissues. On the other hand, the enhancing effect of foliar application with yeast may be due to its high contents of minerals particularly N.P and K, and certain natural hormones as well as some vitamins especially vitamin B. While the role of foliar application with Azotobacter in increasing micronutrients in peanut plants could be attributed to the importance of Azotobacter as N₂-fixer in N₂fixation as mentioned by Ruinen (1971) that phyllosphere microflora supported N2-fixation which reflected on plant growth, in addition to providing plant with a part of its nutritional requirements.

Table 4: Copper, manganese and iron concentrations of seeds (ppm) as affected by foliar application with Azotobacter spp., Saccharomyces cervisiae, micro-nutrients and their combinations through seasons 2003 and 2004.

Treatment	Cu (ppm)		Mn (g	pm)	Fe (рр m)	
	2003	2004	2003	2004	2003	2004
Control	5.07a	5.66b	37.35a	24.13b	111.05a	115.35ab
Sa	4.76a	6.89b	35.98a	28.1a	102.1a	109.88a
Az.	7.26dbc	8.78c	54.35abc	52.5b	127.6a	166.18c
MN	4.185a	6.97c	36.18a	31.6c	153.5a	157.48c
Mix	5.42a	12.79d	44.35a	76.5d	100.35a	183,85cd

Sa: Saccharomyces cervasia,

Az: Azotobacter spp.,

MN: micro-nutrients,

Mix: mixture of them

Means followed by the same letters are not significantly different according to Duncan's multiple test at 0.5 % level

Seeds yield and net return (L.E./fed.):

The foliar application with different studied treatments significantly increased seed yields of peanut plants over un-treated control (Table, 5). These data also, indicated that there were no significant variations among seeds yield of all different spray treatments, except MN treatment in the second season only, which surpassed their corresponding treatments. In general, MN treatment consistently attained higher seeds yield (23.4 and 11.2% over the control) in first and second seasons, respectively.

Data of Table 6 illustrated average of some economic evaluation parameters of the two studying seasons. Data includes costs, value of seeds and net return (L.E./fed.). Net return is the most important parameter considered here. Average of net return achieved by all studied treatments obviously increased over untreated control. Foliar spray with MN attained the highest net return (2110 L.E./fed.) followed by spray with Az (1705 L.E./fed.) then spray with Sa (1682.5 L.E./fed.) compared to 1367.5 L.E./fed. for un-inoculated control.

Table 5: Seeds yield (tons/fed.) as affected by foliar application with Azotobacter spp., Saccharomyces cervisiae, micro-nutrients and their combinations through seasons 2003 and 2004.

Treatment	Seeds yi	ield (tons/fed)	ed) The difference than contro			
	2003	2003	2002	2003		
Control	2.82b	3.67bcd		_		
Sa	3.50c	3.49bc	24.1	-4.9		
Az.	3.24bc	3.78bcd	14.9	3.0		
MN	3.48c	4.08d	23.4	11.2		
Mix	3.11bc	3,84cd	10.3	4.6		

Sa: Saccharomyces cervasia,

Az: Azotobacter spp.,

MN: micro-nutrients,

Mix: a mixture of them

Means followed by the same letters are not significantly different according to Duncan's multiple test at 0.5 % level.

Table 6: Average of some economic evaluation parameters for peanut plant sprayed with some micronutrients and/or microorganisms.

Treatment	Fixed costs (L.E./fed.)	Changed costs (L.E./fed.)	Total costs (L.E./fed.)	Total income (L.E./fed.)	Net return (L.E./fed.)	Increase than control (L.E./fed.)
Control	3500	0.0	3500	4867.5	1367,5	
Sa	3500	60.0	3560	5242.5	1682.5	315.0
Az	3500	60.0	3560	5265.0	1705.0	337.5
MN	3500	60.0	3560	5670.0	2110.0	742.5
Mix	3500	60.0	3560	5212.5	1652.5	285.0

Fixed costs (L.E./fed.) include: field rent (500), irrigation (250), tillage (150), seeds (400), fertilizers (300), herbicide control (900), harvesting (400), transporting (100), and yield preparation (100).

Changed costs (L.E./fed.) include: Sa (20), Az (20), MN (20) and charge of spray (40)

Crop charge was 1500 L.E./fed.

The literatures on peanut plant are scarce, thus we will relate our discussion on other plant species. The significance of foliar application with Azotobacter micronutrients. (as N₂-fixing microorganisms) and/or packing yeast on seeds yield has been confirmed by previous studies such as those of Abd-El-Maksoud et al. (1984), Mengel and Kirkby (1987), Dale and Krystyana (1988), Ghosh et al. (1995), Mukhopadhyay and Sen (1997) and Ali (2001). Whereas, Abd-El-Maksoud et al. (1984) stated that inoculation of soybean with Rhizobium + micronutrients (as foliar application) gave high yield. Shadia et al. (1998), also, reported that 2 g/liter of active dry yeast applied as foliar spray during vegetative, flowering and fruiting stages improved yield of calyxes of Roselle plant. The spraying with N₂-fixing microorganisms (Klebsiella) increased yield

components of Nigela sativa and Trigonella focuum graecum (Muckopadhyay and sen, 1997). The role of micronutrients in plant metabolism was discussed by Mengel and Kirkby (1987) as they interacting in enzymes functions and configuration. Fe functions in enzymes systems in which haem or haemin function as prosthetic groups, Mn2+ sharing in bridge ATP with enzyme complex, Zn2+ resemble Mn2+, it bridges about the binding and conformation between enzyme and substrate, while Cu is a constituent of many enzymes such as phenolase, superoxide dismutase, carboxylase, oxygenase, cytocrom oxidase and amine oxidase. It was suggested that there is a specific requirement for Cu in symbiotic N2-fixation, whereas, N2-fixing nodules have a very high demand for carbohydrates and Cu introduced in activating carbohydrate metabolism. Unlike many of essential plant nutrients.

B is not a component of an enzyme. B has a direct effect on sucrose synthesis rather than in transport of it (Mengl and Kirkby, 1987). In recent study, El-Set et al. (2005) found that productivity of soybean improved by inoculation with rhizobia in combination with 1000 ppm citric acid + a mixture of micronutrients.

The positive role of active dry yeast was suggested by Shadia et al. (1998) which could be due to its high contents with minerals particularly N, P, K and certain natural hormones (cytokinins). The increase of CO₂ release through fermentation process effectively stimulates photosynthesis and accelerates biosynthesis of carbohydrates and facilitating stomata opening as well as serving as wetting agent for the spraying solution. As far, Subba Rao (1984) showed that active dry yeast contains high amounts of vitamins especially B which playing an important role in improving growth and controlling incidence of fungidiseases.

The positive role of spray with Azotobacter was supported by studies of many investigators, whereas, Ghosh et al. (1995) found that spraying Brassica juncea cv. plants with Kelbsiella three times increased yield (29.4%) compared to control. The positive effect of foliar application with Azotobacter as N₂-fixing microorganisms might be due to N₂-fixed as mentioned by Ruinen (1971) that phyllosphere microflora supported dinitrogen fixation. In addition, Azotobacter produces phytohormones such as cytokinins and gibberellins (Sprenat, 1990) thus promoted plant growth and yield.

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الملخص العربي

تأثير الرش ألورقي ببعض العناصر ألصغري ويعض الكائنات الدقيقة على نمو نبات الفول السودائي

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أقيمت تجربتين حقايتين موسمي ٢٠٠٣ و ٢٠٠٤ بمنطقه النوبا ريه - قرية الحسين بغرض دراسة تأثير الرش ألورقي بمخلوط من المغناصر الصغري (منجنيز - زنك - حديد - نحاس - بورون) و معلق من موكروب الازوتوباكتر و معلق من خميره الخبيز و كذلك مخلوط منهم علي عمليه التعقيد و الانتاجيه و بعض الخواص الكيماوية لنبات الفول السوداني.

أظهرت النتائج أن معامله المخلوط زادت و بدرجه معنوية من عدد و وزن العقد الجز ريه النبات. كما استجاب الوزن الجاف النبات بصوره ايجابية بسبب تطبيق جميع المعاملات. حققت كل المعاملات زيادة في نسبه الليتروجين بالبنور و كان اعلمي تسأثير بسبب السرش بميكروب الازوتوباكتر. كما أعطت معظم المعاملات زيادة في نسبه الفسفور في حين لم تظهر نسبه البوتاسيوم فروقا معنوية بسبب الرش بهذه المماملات. زادت معظم المعاملات من نسبه المنحاس و المنجنيز و الحديد في البنور و أعطت معاملتي الازوتوباكتر و المخلوط اعلى نسب في نلك. حققت معاملات الرش زيادة في إنتاجيه البنور و كانت اعلى زيادة بسبب الرش بالمناصر الصغري ثم الازوتوباكتر، أوضح متوسط صافي عائد عند الرش بالمناصر الصغري يليه الرش بالازوتوباكتر، و نحن نقرح تكثيف البحوث في هذا الاتجاء التأكيد هذه النتائج و لتحقيق تنميه أفضل لمحصول الفول السوداني.