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EFFECT OF (HALEX-2) BIOFERTILIZER INOCULATION ON COWPEA YIELD AND MINERAL FERTILIZER-N OPTIMIZATION

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ABSTRACT

A field experiment was conduced during the summer seasons of 2005 and 2006 at Disuq district, Kafr El-Sheikh Governorate to study the combined effect of inoculation with Halex-2 (as biofertilizer) and mineral nitrogen levels on cowpea yield and their net return.

Split plot design was used with four replicates. The main plots were assigned by two treatments of uninoculated (control), and inoculated with Halex-2. Whereas, the sub-plots were assigned by five N levels (0, 10, 20, 30 and 40 kg N fed^{-1}).

Four polynomial quadratic equations were established to show the following results:

1. The maximum and optimum N rates were decreased as Halex-2 used in the two seasons.
2. The maximum and optimum cowpea yields were increased as Halex-2 used in the two seasons.
3. The highest maximum yield (1413.2 kg fed^{-1}), the highest total value of yield (7065.0 L.E fed^{-1}) and the highest return of bio and N fertilizer (2936.6 LE fed^{-1}) were obtained as Halex-2 used in the first season.
4. The average of efficiency and the efficiency of nitrogen fertilizer at optimum rate were increased as Halex-2 used.
5. The soil nitrogen content during plant growth (X_s) was increased as Halex-2 added.
6. The contribution of soil N was increased as Halex-2 used in the two seasons.
7. The contribution of N fertilizer was increased as N levels increased in the two seasons.

Keywords: Biofertilizer inoculation-cowpea the quadratic polynomial equation-nitrogen levels.

INTRODUCTION

Recently, great efforts are expanded to increase the legumes production in Egypt. Legumes production can help in solving the problem of fodder shortage as it is considered one of the most important sources of protein for human and their livestock. Cowpea (*Vigna unquiculata* L. Walp) is one of the most important vegetable legumes due to its high protein content, heat tolerant, low fertilizer requirements and it can grow easily in the new reclaimed lands (El-Waraky and Kasem, 2007).

In the semi-arid regions, soil is inherently poor in nitrogen due to fast degradation of organic matter. Thus, nitrogen fertilization is usually required. Cowpea, like other legumes, have a symbiotic relationship with a specific soil bacteria (*Rhizobium* spp.), which fixes atmospheric nitrogen. However, under low available nitrogen in the soil starter of nitrogen fertilizer is required before

the fixation begins, either from the soil reserve or through N application (Hussaini *et al.,* 2004). Since N_2-fixation usually initiated after nodule formation. Thus mineral N, especially nitrate (Fathy *et al.,* 2000) or/and biological fertilization (Hassanein and El-Shebiny, 2000) may be a critical source of nitrogen for legume plants. Several investigators (Hassouna and Abou-Nasr, 1992 on soybean; Hassanein and El-Shebiny, 2000 on sugar beet and Bin Ishaq, 2002 on pea) indicated that application of biofertilizer Halex-2 at 10 g. kg^{-1} seeds significantly resulted in taller plants with more N concentrations in leaves, higher protein contents in seeds, and greater total yields than in the case of untreated control.

The excessive use of nitrogen fertilizers represents the major cost of crop production and creates pollution of agroecosystem (Fisher and Richter, 1984). Therefore many investigators have given more attention to the quantitative expression of the response of crops to fertilizer application based on changes in cultural practices. Thabet and Balba (1994), Hassanein and El-Shebiny (2000), Atia (2005) and Atia *et al.* (2007) were used the polynomial quadratic equations to calculate the net return from optimum rates of nitrogen applied and the contribution of soil and fertilizer nutrients to the yield.

The objective of the present study is to estimate the more economic use of bio and mineral fertilizers and determine the returns from optimum rates of nitrogen applied to cowpea.

MATERIALS AND METHODS

A field experiment was carried out at Disuq district Kafr El-Sheikh Governorate, during the summer seasons of 2005 and 2006 using cowpea (*Vigna unquiculata* L. Walp) seeds variety Kafr El-Sheikh-1. The physical and chemical soil properties of the experimental sites were determined according to Jackson (1958) and presented in Table 1.

Table 1: Some properties of the experimental soils.

Season	Mechanical analysis			Texture	pH*	EC** dSm^{-1}	O.M. %	Available elements ppm		
	Sand %	Silt %	Clay %					N	P	K
1st	9	51	40	Loamy clay	7.9	1.2	1.67	29	5.5	440
2nd	8.7	52	39.3	Loamy clay	7.9	1.1	1.70	22	5.5	380

***1: 25 soil: water suspension**
**** Soil paste extract**

Halex-2; is a biofertilizer containing a mixture of non symbiotic N_2-fixing bacteria of the general Azospirillum, Azotobacter and Klebsilla, was used. The biofertilizer was supplied by the biofertilization Unit. Plant Pathology Department, Alex. Univ. The biofertilizer was used at the rate of 10 g. kg^{-1} seeds. Seed inoculation was performed by adding an adequate amount of distilled water to the biofertilizer and mixed with the seeds just before sowing. Uninoculated seeds (control treatment) were mixed with distilled water. In all treatments cowpea seeds were inoculated by an

effective strain of rhizobium bacteria just before sowing. The sowing dates were 15 and 18 of April in the first and second seasons, respectively.

Split-plot design was used with four replicates. The main plots were assigned by two treatments (uninoculated and inoculated with Halex-2), whereas the sub-plots were assigned by five N levels (0, 10, 20, 30 and 40 kg N/fed^{-1}). Each sub-plot contained 4 rows, 4 m in long and 0.6 m in width, comprising an area of 9.6 m^2. Spacing between plans within rows was 20 cm and sowing was done on one side of the row. Plants were thinned to two plants per hill after three weeks from the planting. Nitrogen fertilizer, in the form of ammonium sulphate (20.5% N), was added to the soil as one dose before the first irrigation. The other recommend agriculture practices were used.

After harvest seed yields were determined. All obtained data were statically analyzed using COSTAT Software (1985).

Quantitative analysis:

The quadratic polynomial equation has been used to describe the cowpea seed yield response to nitrogen levels and biofertilizer (Halex-2), its general form is:

$$Y = B_0 + B_1 X_i \pm B_2 X^2_i$$

Where, the term, (Y) is the yield corresponding to nutrient rates X_i. The term B_0 is the intercept, and B_1 and B_2 are the linear and quadratic coefficients, respectively. The constraints B_0, B_1 and B_2 were calculated using the least squares method.

The maximum addition of fertilizer (X_m), the maximum yield (Y_m), the optimum rate of fertilizer (X_{opt}), the optimum yield (Y_{opt}), the average of efficiency ($e\overline{X}$) of the fertilizer application rate (x) along the range from x = 0 to x = i, the efficiency of fertilizer at optimum rate (ex_{opt}), the efficiency of soil nitrogen (ex_s) and the soil nitrogen content (x_s) can be calculated from the following equations, respectively.

1. $X_m = -\frac{B_1}{2B_2}$ — Balba (1961)

2. $Y_m = B_0 - \frac{B_1^2}{4B_2}$ — Capurro and Voss (1981)

3. $X_{opt} = \frac{P_r - B_1}{2B_2}$ — Balba (1964)

4. $Y_{opt} = B_0 + \frac{Pr^2 - B_1^2}{4B_2}$ — Balba (1964)

Where the (Pr) = $\frac{\text{Price of fertilizer unit}}{\text{Price of one kg of crop}}$

5. $e\overline{X} = B_1 + B_2 X_i$ ... at X_i = 5 units — Thabet and Balba (1994).

6. $eX_{opt} = B_1 + B_2X_{opt}$ ... at X = optimum rate
Hassanein and El-Shebiny (2000)

7. $eX_s = \frac{B_0}{X_s}$ Thabet and Balba (1994)

8. $X_s = \frac{-B \pm \sqrt{B_1^2 - 4B_0B_2}}{2B_2}$ at y = 0

9. $SE = \sqrt{\frac{(Observed - Calcualted)^2}{n-2}}$

10. The contribution of soil N = $\frac{X_s}{X_f + X_s}$ x calculated yield

11. The contribution of fertilizer = $\frac{X_f}{X_f + X_s}$ x calculated yield

RESULTS AND DISCUSSION

In the present study, cowpea seed yields were increased successively and significantly with N increments. The polynomial quadratic equations were established to express the cowpea seeds response to N application with or without the biofertilizer (Halex-2) are presented in Table 2.

Table 2: The polynomial equations expressing yield of cowpea seed-rates of nitrogen with and without the biofertilizer (Halex-2) in the two seasons (2005-2006).

Season	Treatment	The polynomial equations	X_s
2005	N	$Y = 711.23 + 351.68 X - 52.807 X^2$	1.63
	N + Halex	$Y = 825.68 + 357.71 X - 54.450 X^2$	1.81
2006	N	$Y = 643.31 + 358.95 X - 51.557 X^2$	1.48
	N + Halex	$Y = 789.40 + 332.92 X - 48.49 X^2$	1.86
Average	N	$Y = 677.27 + 355.31 X - 52.182 X^2$	1.55
	N + Halex	$Y = 807.54 + 345.32 X - 51.46 X^2$	1.84

The experimental and calculated cowpea seed yields values obtained from the polynomial equations 1-6 are presented in Table 3. The calculated yields closely approximate experimental yield as shown form the values of standard error (SE) of estimates and determination coefficient (R^2). The chi square test showed that the calculated yield values from each equations do not significantly differ from the experimental values for each treatment (Table 3).

Table 3: Observed and calculated cowpea seed yield kg fed.$^{-1}$ under levels of nitrogen fertilizer and addition of biofertilizer (Halex-2) in the two seasons (2005 and 2006).

Inoculation	N levels	Season 2005		Season 2006		Average of 2005 and 2006	
		Observe	Calculate	Observe	Calculate	Observe	Calculate
Without	0	708.30	711.23	654.00	643.31	681.15	677.27
	10	1022.20	1010.10	931.30	950.70	976.75	980.40
	20	1184.60	1203.35	1149.00	1154.97	1166.80	1179.16
	30	1303.90	1291.00	1283.50	1256.14	1293.70	1273.57
	40	1269.70	1273.03	1241.50	1254.19	1255.60	1263.61
With inoculation	0	819.90	825.68	797.90	789.40	808.90	807.54
	10	1152.50	1128.94	1063.50	1073.85	1108.00	1101.39
	20	1287.30	1323.30	1241.40	1261.34	1264.35	1292.32
	30	1433.20	1408.76	1388.80	1351.87	1411.00	1380.31
	40	1379.10	1385.32	1330.30	1345.44	1354.70	1365.38

Maximum and optimum rates:

The values of maximum and optimum N rates for each treatment were calculated and presented in Table 4. The maximum N rates (X_m) decreased from 3.33 unit N fed^{-1} to 3.28 unit N fed^{-1} and from 3.48 unit N fed^{-1} to 3.43 unit N fed^{-1} as biofertilizer Halex-2 added in the first and second seasons, respectively. The mean values of the two seasons decreased from 3.40 unit N fed^{-1} to 3.36 unit N fed^{-1} as biofertilizer Halex-2 was used. The values of the optimum N rates (X_{opt}) show the same trend, where it decreased as biofertilizer Halex-2 was used in the first and second seasons. On the other hand, the values of X_{opt} were less than the values of X_m, whereas the X_{opt} were calculated by differentiating (y) in the polynomial equations from 1-6 with regard to X (dy/dx) and equating with the ratio (Pr) of the price of fertilizer unit and the price of cowpea unit (kg). The decrease of X_m and X_{opt} as biofertilizer Halex-2 added may be attributed to two reasons. The first is the effect of non symbiotic N_2-fixing bacteria on soil nitrogen, where the soil nitrogen increased from 1.63 unit N fed^{-1} to 1.81 unit N fed^{-1} and from 1.48 unit N fed^{-1} to 1.86 unit N fed^{-1} in the first and second seasons, respectively (Table 2). The second is the increase of the surface area per unit root length and enhanced root hair branching with an eventual increase in the uptake of nutrients from the soil (Jagnow *et al.*, 1991). The results are in agreement with those obtained by Shiboob (2000) and Hassanein and El-Shebiny (2000).

The maximum and optimum yield:

Data tabulated in Table 4 show that the maximum and optimum cowpea yields were increased as Halex-2 used. The Y_m increased from 1296.8 kg fed^{-1} to 1413.2 kg fed^{-1} and from 1268.1 kg fed^{-1} to 1361.0 kg fed^{-1} as Halex-2 used in the first and second seasons, respectively. The average of the increase in the two seasons was 8.2%

Table 4: The maximum N rate (X_m), optimum N rate (X_{opt}), maximum yield (Y_m), optimum yield (X_{opt}) and the returns of cowpea under (Halex-2) biofertilizer inoculation and mineral fertilizer-N.

Season	Treat.	X_m unit N fed^{-1}	X_{opt} unit N fed^{-1}	Y_m kg fed^{-1}	Y_{opt} kg fed^{-1}	Total values of yield L.E kg^{-1}	Total values of yield at control L.E. kg^{-1}	Return of biofer. L.E fed.$^{-1}$	Return of N fert. L.E fed.$^{-1}$	Return of bio and N fert. L.E. fed.$^{-1}$	Fert. cost L.E fed.$^{-1}$	Net return of fert. L.E fed.$^{-1}$	L.E./L.E.
2005	N	3.33	3.26	1296.8	1296.5	6482.5	3556.2	-	2926.4	2926.4	110.8	2815.0	25.41
	N + H	3.28	3.22	1413.2	1413.0	7065.0	4128.4	572.2	2364.4	2936.6	129.5	2807.1	21.68
2006	N	3.48	3.41	1268.1	1267.8	6339.0	3216.5	-	3122.5	3122.5	116.0	3006.5	25.92
	N + H	3.43	3.36	1361.0	1360.7	6803.5	3947.0	730.5	2126.0	2856.5	134.2	2722.3	20.29
Average	N	3.40	3.34	1282.1	1281.9	6409.5	3386.4	-	3023.1	3023.1	113.6	2909.5	25.61
	N + H	3.36	3.29	1386.9	1389.6	6933.0	4037.7	651.3	2244.0	2895.3	131.9	2763.4	20.95

Price of cowpea = 5 L.E. kg^{-1}
Fertilizer price = 34 L.E unit^{-1}
Halex Price = 20 L.E fed.$^{-1}$
Fertilizer unit 10 kg N

The returns from applied optimum rates:

The returns per feddan from the applied nitrogen (optimum rates) with and without Halex-2 inoculation were presented in Table 4. The results show that the total values of yield increased from 6582.5 L.E fed^{-1} to 7065.0 L.E fed^{-1} and from 6339.0 L.E. fed^{-1} to 6803.5 L.E. fed^{-1} in the first and second season, respectively. Also, the total values of the control was yield at control increased as Halex-2 added to soil in the two seasons. The average of the two seasons was increased from 3386.4 L.E fed^{-1} to 4037.7 L.E fed^{-1} as Halex-2 used. This result means that addition of one unit of Halex-2, (its price was 20 L.E.) gave us an increase of yield equal to 651.3 L.E. fed^{-1}. On contrast the return of nitrogen fertilizer decreased as Halex-2 added to soil, where the average of the two seasons decreased from 3023.1 L.E. fed^{-1} to 2244.0 L.E fed^{-1}. Again the net return of fertilizer decreased from 2815.6 LE fed^{-1} to 2807.1 L.E. fed^{-1} and from 3006.5 L.E. fed^{-1} to 2722.3 L.E. fed^{-1} in the first and second seasons, respectively. Also, the L.E/L.E decreased from 25.61 L.E/L.E to 20.95 L.E/L.E. as Halex-2 used in the two seasons. Generally, the addition of Halex-2 increased the total values of yield, the total values of yield at control and save money and fertilizer.

Efficiencies of nitrogen fertilizer and soil nitrogen:

The efficacies of soil nitrogen (eX_s), the average efficiency ($e\overline{X}$) and the efficiency of nitrogen fertilizer at optimum rate (eX_{opt}) are present in Table 5. The data illustrate the effect of Halex-2, where the eX_s increased from 436.34 kg unit^{-1} fed^{-1} to 456.18 kg unit^{-1} fed^{-1} in the first season, but it decreased in the second one. The average of the two seasons increased from 436.95 to 438.88 kg unit^{-1} fed^{-1}. The increase of soil nitrogen efficiency means that the nitrogen introduced by N_2-fixing bacteria was available to cowpea plants. Also, the average efficiency and the efficiency of nitrogen fertilizer at optimum rate increased as Halex-2 added to soil in the two seasons (Table 5).

Table 5: Efficiencies of soil nitrogen (eX_s), the average efficiency ($e\overline{X}$) and the efficiency of nitrogen fertilizer at optimum rate (eX_{opt}) and the soil nitrogen (X_s).

Season	Treatment	(X_s) unit N fed.$^{-1}$	eX_s	$e\overline{X}$	EX_{opt}
			Kg unit^{-1} fed^{-1}		
2005	N	1.63	436.34	500.00	539.08
	N + H	1.81	456.18	607.88	650.35
2006	N	1.48	434.67	437.08	467.50
	N + H	1.86	424.41	595.48	626.51
Average	N	1.55	436.95	468.54	502.98
	N + H	1.84	438.88	601.70	638.24

Contribution of soil and fertilizer N:

The results in Table 6 show that the contribution of N fertilizer were increased as N rates increased from N_0 up to N_4 in the two seasons. Generally, the contribution fraction of soil N were decreased as N rates increased in the two seasons (Table 7). The contribution fraction of soil nitrogen was higher as Halex added than it without addition of biofertilizer. Data in Tables 6 and 7 show three trends. The first is the contribution of soil N increased as Halex added to cowpea plants in the two seasons. The second is the contribution fraction and the contribution of soil N was decreased as N rates increased. The third one is the contribution fraction and the contribution of fertilizer N take the negative trend compared with the soil N. This may be due to the fixed-N by the non symbiotic N_2-fixing bacteria in the biofertilizer (Halex-2), which increased soil-N. Thus it decreased the fertilizer contribution.

Table 6: The contribution of soil N and added N fertilizer to cowpea yield in the two seasons (2005 and 2006).

Inoculation	N-level	2005		2006		Average of 2005 and 2006	
		Soil N	Fert. N	Soil N	Fert. N	Soil N	Fert. N
Without inoculation	N_0	711.23	0.00	643.31	0.00	677.27	0.00
	N_1	626.26	383.84	570.42	380.28	598.04	382.36
	N_2	541.51	661.84	496.64	658.33	518.83	660.33
	N_3	450.85	839.15	414.53	841.61	433.01	240.56
	N_4	369.18	903.82	338.63	915.56	353.81	909.80
	N_{opt}	427.85	868.65	380.34	887.46	410.21	871.69
With inoculation	N_0	825.68	0.00	789.4	0.00	807.54	0.00
	N_1	722.52	406.42	698.0	375.85	715.90	385.49
	N_2	635.18	688.12	605.44	655.90	620.31	672.01
	N_3	535.33	873.43	513.71	838.16	524.52	855.79
	N_4	429.44	955.88	430.54	914.90	436.92	928.46
	N_{opt}	508.68	904.32	489.85	870.85	499.18	887.42

Table 7: The contribution fraction of soil N and added N fertilizer to cowpea plants at two seasons (2005 and 2006).

Inoculation	N-level	2005		2006		Average of 2005 and 2006	
		Soil N	Fert. N	Soil N	Fert. N	Soil N	Fert. N
Without inoculation	N_0	1.00	0.00	1.00	0.00	1.00	0.00
	N_1	0.62	0.38	0.60	0.40	0.61	0.39
	N_2	0.45	0.55	0.43	0.57	0.44	0.56
	N_3	0.35	0.65	0.33	0.67	0.34	0.66
	N_4	0.29	0.71	0.27	0.73	0.28	0.72
	N_{opt}	0.33	0.67	0.30	0.70	0.32	0.68
With inoculation	N_0	1.00	0.00	1.00	0.00	1.00	0.00
	N_1	0.64	0.36	0.65	0.35	0.65	0.35
	N_2	0.48	0.52	0.48	0.52	0.48	0.52
	N_3	0.38	0.62	0.38	0.62	0.38	0.62
	N_4	0.31	0.69	0.32	0.68	0.32	0.68
	N_{opt}	0.36	0.64	0.36	0.64	0.36	0.64

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تأثير التلقيح بالسماد الحيوى (هالكس -٢) على محصول اللوبيا وترشيد استخدام الاسمدة المعدنية النيتروجينيه

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**قسم بحوث الخضر ــ معهد بحوث البساتين ــ مركز البحوث الزراعية ــ الجيزه ــ مصر

أقيمت تجربه حقلية خلال الموسم الصيفى لعامى ٢٠٠٥ ، ٢٠٠٦م تحت ظروف اراضى مركز دسوق بمحافظة كفرالشيخ لدراسة أثر التلقيح بالسماد الحيوى هالكس-٢ تحت مستويات مختلفة من السماد النيتروجينى.

استخدم تصميم القطع المنشقة مرة واحدة فى أربع مكررات وكانت القطع الرئيسية لخمسة مستويات نيتروجينية (صفر ، ١٠ ، ٢٠ ، ٣٠ ، ٤٠كجم نيتروجين لكل فدان). وكانت القطع الشقية لمعاملتين (بدون تلقيح والتلقيح بالسماد الحيوى هالكس-٢).

استخدمت معادلات الدرجة الثانية للحصول على النتائج التالية:

١- تناقص المعدل السمادى الأعظم والأمثل عند استخدام السماد الحيوى هالكس-٢.

٢- إزداد المحصول الأعظم والأمثل من اللوبيا عند استخدم السماد الحيوى هالكس-٢ فى العامين.

٣- كان أعلى محصول أعظم (١٤١٣,٢كجم/فدان) وأعلى عائد كلى (٧٠٦٥,٠ جنيه للفدان وأعلى عائد صافى (٢٩٣٦,٣ جنيه/فدان) من السماد الحيوى والمعدنى معا عند استخدام الهالكس-٢ فى العام الأول.

٤- إزداد متوسط كفاءة السماد وكذا كفاءة السماد عند المحصول الأمثل فى العامين مع استخدام السماد الحيوى هالكس-٢.

٥- إزداد محتوى الأرض من النيتروجين مع إضافة السماد الحيوى هالكس-٢.

٦- إزدادت مساهمة النيتروجين الموجود بالارض فى المحصول الناتج مع إضافة السماد الحيوى.

٧- إزدادت مساهمة السماد النيتروجينى فى المحصول الناتج مع زيادة معدلات السماد المضاف.