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EFFECT OF BIOFERTILIZATION WITH NITROGEN FIXERS ON GRAIN YIELD AND LATE WILT DISEASE OF MAIZE

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ABSTRACT

Greenhouse and field experiments were conducted to study the effect of inoculation with single or mixed of nitrogen fixing bacteria (NFB) as *Azotobacter chroococcum* and *Azospirillum brasilense* on the soil on grain yield of maize(*Zea maize*, L.) and the incidence of late wilt disease. Significant effect on plant grain yield was obtained after inoculation of N_2-fixing bacteria. Application of half dose of nitrogen fertilizer (60 kg N/feddan) with inoculation of N_2 fixing bacteria, increasing grain yield as compared with application of 120 kg N/feddan was obtained after inoculation of N_2-fixing bacteria. In the application of half dose of nitrogen fertilizers (60 Kg N/feddan) with the inoculation of N_2 fixing bacteria. The increasing of grain yield per feddan was more than the application of full dose of N-fertilizer (120 kg N/fed). In the same time the percentage of late wilt disease was decreased. Maize plants grown in inoculated pots showed significant increases in both ability of controlling late wilt disease, and grain yield comparing with uninoculated pots.

Also, 50% of recommended dose of N-fertilizer (60 kg N/fed) with inoculation of N_2-fixers gave the highest values in grain yield / feddan especially with composite (3), which is used as mixture of the two N_2-fixers. Soil used were silty-clay in field while the used in pots were sandy loam. The treatments of experiments uninoculation with N_2-fixers and recommended dose was full o N-fertilizer used as control.

INTRODUCTION

Integrated Fertilizer Management (IFM) is recent concept recommended for the newly reclaimed soil. It implies the rational use of chemical fertilizer together with biofertilization with functional groups of microorganisms, mainly biological nitrogen fixing bacteria (NFB). Many bacterial species including *Azotobacter chroococcum, Azospirllum brasilense* and *Bacillus megatherium* act as plant growth promoting rhizobacteria (PGPR) (Al-Laithy, 1983 and Hegazi *et al.*, 1983) and a pparently s timulate plant growth mainly by modifying root development, which improve micro-elements and water uptake especially in the early stages of plant development (Fages, 1994 and Antoun *et al.*, 1998). Increased plant grain yield through inoculation in the field. Seed inoculation with composite inocula generally, gives more effect on some plant characters, compared with mono-bacterial inoculation (Sadik, 1998). Association between *Azotobacter* and *Azosperillum* with maize plants has been demonstrated to cause significantly increase dry weight and N content (Smith *et al.*, 1984). Amard and Dahdah (1997) and Sabry *et al.* (1999 and 2000) indicated that *Azotobacter, Azospirillum, Pseudomonas* and *Rhizobium* individually alone or in mixtures improved total plant dry weight and yield of wheat compared to uninoculated plants. Also, there are relationship between nitrogen fixer microorganisms and root and stalk rot disease of maize (Iswaran, 1960; Mahmoud, 1981 and

Al-Laithy, 1996) as seen in grain yield affected with *Azotobacter* inoculation in the soil. On the other hand, the N_2-fixation in *Azotobacter spp.* are able to produce phytohormones and hydrogen syanide (Antoun *et al.*, 1998), they also exhibited antagonistic compounds against many plant pathogenic fungi.

The objective of the present study is to evaluate the response of maize plants to inoculation with some nitrogen fixer microorganisms as single inoculum or mixture under half recommended nitrogen fertilizer dose in newly reclaimed soil on the grain yield and incidence of some corn diseases especially late wilt disease caused by *Cephalosporium maydis*. Also, to decrease environmental pollution especially in the soil. The importance of non-symbiotic microorganisms in agriculture was investigated (Mashatin, 1970; Harper *et al.*, 1979 and El-Hosseiny *et al.*, 1979).

MATERIALS AND METHODS

A pot experiment was conducted at the greenhouse of Corn and Sugar Crops Research Dept., Agric. Res. Cent., Giza, Egypt during 1998 season. Field experiment was executed at Tag El-Ezz Research Station during 1999 and 2000 seasons. Maize seeds cv. Balady and hybrid 310 were used in greenhouse experiment, seeds were grown in pots, 30 cm diameter filled with sandy loam soil (10 kg/pot), at a rate of 4 grains / pot, thinned to one seedling / pot after 10 days. In the field trail, maize seeds were broad casted in 6 m^2 plots (2.4 x 2.5 m). Treatments were applied in a complete randomized design with three replicates for each treatment. Mechanical and chemical analysis of the used soil according to Pipper (1950) are presented in Table (1).

In both greenhouse and field experiments, maize plants were fertilized with two applications. The first was treated with 60% of recommended dose of NPK, the second was treated with 100% of recommended dose. Maize grain of cv. Giza Balady and Hybrid 310 were planted on 10^{th} of May in 1999 and 2000 seasons. Grains were sown (in field) in hills on the lower third of the ridge with a distance of 30 cm apart between hills. Plants were thinned to one plant per hill before the first irrigation. Chemical fertilization was applied in two equal doses before the first and second irrigation. respectively.

N_2-fixing bacteria were prepared in liquid culture with the concentrations in the three composites (1, 2 and 3). These composites were used respectively in the greenhouse and field condition with the two application of N fertilization (50% and 100%).

1. Isolation and identification of *Azotobacter chroococcum*:

.They were made by subculturing a representative number of *Azotobacter* isolates differing in their cultural characteristics as near as possible from the characteristic positive tubes. A loop of *Azotobacter growth* (pellicle) was transferred to N-free slant agar. The obtained pure *Azotobacter* isolates were characterized according to the methodology described by Sherman (1967) and Norris & Chapman (1968). The identified on the basis of Buchanan and Gibbons (1974) keys.

Table 1. Mechanical and chemical analysis of the soil used in greenhouse and field experiments

Soil analysis	Soil used in the field	Soil used in the greenhouse
Mechanical analysis:		
Coarse sand (%)	1.60	9.16
Fine sand (%)	7.01	37.50
Silt (%)	39.23	42.00
Clay (%)	44.10	0.90
Soil texture	Silty clay	Sandy loam
Chemical analysis:		
$CO_3 + HCO_3$	0.21	0.17
Soluble anions (mg/100g soil):		
Cl^-	0.93	0.41
SO^-_4	0.06	0.90
Soluble cations (mg/100g soil):		
NO^+	0.37	0.28
K^+	0.03	0.07
Ca^{++}	0.47	0.74
Mg^{++}	0.33	0.39
Organic carbon (%)	2.23	1.85
Total nitrogen (%)	0.11	0.10
C/N ratio	20.13	17.29

2. Isolation and identification of *Azospirillum brasilense*:

Azospirillum brasilense was provided by A.R.C., Giza, Egypt. Broth culture of each strain contained 7 x 10^9 cells/ml was separately carried on vermiculite-based 1:3 v/w as a local carrier material. Once before sowing, different amount of each separate carrier were mixed according to treatments and used in a rate 1000 cm^3/feddan.

3. Isolation of the caused organism (*Cephalosporium maydis*):

The lower internodes (4^{th} to 5^{th} internodes above soil level) of the affected maize materials were used for isolation of the field isolates of *C. maydis*. Materials were thoroughly washed in running water and surface sterilized by alcohol and flamed. Under aseptic conditions, they were peeled. Small pieces of the internal tissues were cut out and plated on potato dextrose agar plus yeast extract (PDA+Y) and incubated at 27°C for 5 days.

The fungal isolates were microscopically examined. *C. maydis* isolates were identified according to their cultural morphological and microscopical characters comparing with description of Samra *et al.* (1962). Stak cultures of all field isolates were kept at law temperature (3-5°C).

4. Preparation of biofertilizers:

Four species of N_2-fixing bacteria, act as plant growth promoting and by modifying of root development, which improve microelements and water uptakes especially in the early stages of plant. The bacteria was grown on special media. Liquid media were used in the composites as (1, 2 and 3).

Composite 1: *Azotobacter chroococcum* + 50% of N fertilization.
Composite 2: *Azospirillum brasilense* + 50% of N fertilization.
Composite 3: *A. chroococcum* + *Azospirillum brasilense* + 50% of NPK fertilization.

These isolates used with the composites were grown in broth culture with the concentration 7 x 10^9 cells/ml and used for greenhouse experiments.

5. Preparation of *(Cephalosporium maydis)*, the causal organism of late wilt disease:

C. maydis was grown in milk bottles of 0.05 kg capacity containing the following medium as per each potatoes extract 40 ml, 1 gm yeast extract, 10 gm wheat bran washed sand 25 gm and 5 gm wheat straw. After three weeks of inoculation at 30°C, the content of the bottles were mixed. A known amount of the inoculum (100 gm/ No. 25 pot of capacity 6 kg soil), this inoculum translate and mixed with the top 7-8 cm layer of the soil. This infested soil was planted with the two varieties under testing (hybrid 10 and hybrid 310) in greenhouse conditions. At the field conditions, we used two maize susceptible varieties to late wilt disease. This grains were collected from different sources in Dakahlia governorate to evaluate the prevalence of such pathogens on maize grains.

RESULTS AND DISCUSSION

Data of greenhouse and field experiments (Table 2) indicated that inoculation of the maize plants in the two varieties (Balady and hybrid 310) under test with composite 1 or 2 or 3 applied with 50% of N fertilization recorded significant increase in grain yields and decreasing the incidence and disease severity compared with uninoculated ones under 50% or full dose of nitrogen fertilization. These results of greenhouse and field experiments were in harmony with those obtained by Al-Laithy (1996).

Data showed that composite inoculum (3), which consists of (*Azotobacter chroococcum* and *Azospirillum brasilense* with 50% of recommended dose of N fertilization) gave the highest grain yield comparing with other composites 1 and 2 (Table 2 and 3). Also, the application of composite 3 reduce the incidence and disease severity of late wilt disease during the two seasons, while the chemical fertilization was 100% of N.

In general. treatments of composite 3 inoculum with 50% dose of chemical fertilization gave the lower value in both greenhouse or field conditions allover the two seasons. Uninoculated treatments with the biofertilizers and application of full dose (100% N) used as control gave the highest values of incidence and disease severity in late wilt disease of the two varieties of maize under test (Tables 4 and 5).

The decrease in the percentage of incidence and disease severity of the late wilt disease may be due to the ability of N_2-fixers (*Azotobacter chroococcum* and other fixers) to synthesize stimulatory compounds such as gibberellins, cytokinines and indole acetic acid that act as plant growth regulators (Al-Laithy, 1996 and Pandy *et al.*, 1998).

Table 2. Effect of inoculation with N_2 fixers bacteria combined with 50% of N-fertilization on grain yield and late wilt disease of two maize varieties under greenhouse conditions during two seasons (1999 and 2000).

Treatments	Grain yield (ton/feddan)				Late will disease caused by *Cephalosporium maydis*							
	1999		2000		1999				2000			
	Baldy	Hybrid 310	Baldy	Hybrid 310	Baldy		Hybrid 310		Baldy		Hybrid 310	
					Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI
Composite 1	2.70	3.60	2.60	3.80	7.00	2	3.00	1	7.60	2	3.60	1
Composite 2	2.00	3.30	2.20	3.20	8.60	2	4.60	1	8.50	2	9.70	1
Composite 3	3.00	3.40	2.60	3.90	6.50	2	4.00	1	7.70	2	5.50	1
Control	2.50	2.90	2.40	3.00	11.00	3	3.00	1	12.00	3	2.70	1
LSD at 0.05	0.24	0.36	0.14	0.29	1.74	0.54	0.31	--	1.94	--	0.22	--

Composite 1: ***Azotobacter chroococcum*** **+ 50% of N fertilization. Composite 2:** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Composite 3: ***A. chroococcum*** **+** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Control = Full dose of NPK without (biofertilizer) N_2-fixers. Perc. (%) = Percentage of late wilt disease. DSI – Disease severity index.

Table 3. Effect of inoculation with N_2 fixers bacteria combined with 100% of N-fertilization on grain yield and late wilt disease of two maize varieties under greenhouse conditions during two seasons (1999 and 2000).

Treatments	Grain yield (ton/feddan)				Late will disease caused by *Cephalosporium maydis*							
	1999		2000		1999				2000			
	Baldy	Hybrid 310	Baldy	Hybrid 310	Baldy		Hybrid 310		Baldy		Hybrid 310	
					Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI
Composite 1	2.00	3.00	1.90	3.20	9.00	3	2.00	1	9.70	3	3.20	1
Composite 2	1.90	3.00	2.00	2.20	10.60	3	3.00	1	9.90	3	4.10	1
Composite 3	2.70	3.60	2.50	2.70	8.30	2	1.70	1	6.70	2	3.00	1
Control	1.60	2.70	1.60	1.70	13.20	3	4.00	1	15.00	3	4.40	1
LSD at 0.05	0.24	0.42	0.07	0.39	2.01	--	0.53	--	2.38	--	0.28	--

Composite 1: ***Azotobacter chroococcum*** **+ 50% of NPK fertilization. Composite 2:** ***Azospirillum brasilense*** **+ 50% of NPK fertilization.**
Composite 3: ***A. chroococcum*** **+** ***Azespirillum brasilense*** **+ 50% of NPK fertilization.**
Control = Full dose of NPK without (biofertilizer) N_2-fixers. Perc. (%) = Percentage of late wilt disease. DSI – Disease severity index.

Table 4. Effect of inoculation with N_2 fixers bacteria combined with 50% of N-fertilization on grain yield and la[redacted]te wilt disease of two maize varieties under field conditions during two seasons (1999 and 2000).

Treatments	Grain yield (ton/feddan)				Late will disease caused by *Cephalosporium maydis*							
	1999		2000		1999				2000			
	Baldy	Hybrid 310	Baldy	Hybrid 310	Baldy		Hybrid 310		Baldy		Hybri[redacted]d 310	
					Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI
Composite 1	2.60	4.00	2.70	3.20	15.00	3	3.00	1	15.70	3	3.60	1
Composite 2	2.00	3.80	2.20	3.00	15.70	3	3.20	1	15.90	3	3.70	1
Composite 3	2.80	3.90	3.70	3.50	13.60	3	2.90	1	12.20	3	3.00	1
Control	1.90	3.20	2.00	2.70	16.70	3	3.70	1	15.30	3	4.60	1
LSD at 0.05	0.12	--	0.24	0.35	1.28	--	0.19	--	0.98	--	0.29	--

Composite 1: ***Azotobacter chroococcum*** **+ 50% of N fertilization. Composite 2:** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Composite 3: ***A. chroococcum*** **+** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Control = Full dose of NPK without (biofertilizer) N_2-fixers. Perc. (%) = Percentage of late wilt disease. DSI – Disease severity [redacted]ndex.

Table 5. Effect of inoculation with N_2 fixers bacteria combined with 100% of N-fertilization on grain yield and [redacted]ate wilt disease of two maize varieties under field conditions during two seasons (1999 and 2000).

Treatments	Grain yield (ton/feddan)				Late will disease caused by *Cephalosporium maydi[redacted]*							
	1999		2000		1999				2000			
	Baldy	Hybrid 310	Baldy	Hybrid 310	Baldy		Hybrid 310		Baldy		Hybri [redacted] d 310	
					Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI	Perc (%)	DSI
Composite 1	1.70	3.70	1.60	3.00	16.00	3	2.90	1	15.30	3	2.60	1
Composite 2	1.50	3.70	1.60	3.30	15.00	3	3.00	1	15.20	3	2.40	1
Composite 3	1.80	4.00	1.80	3.60	12.00	3	2.00	1	11.70	3	2.00	1
Control	2.00	2.90	1.30	2.20	17.00	3	3.90	1	16.70	3	3.00	1
LSD at 0.05	0.74	0.31	0.11	0.35	1.23	--	0.08	--	1.84	--	0.31	--

Composite 1: ***Azotobacter chroococcum*** **+ 50% of N fertilization. Composite 2:** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Composite 3: ***A. chroococcum*** **+** ***Azospirillum brasilense*** **+ 50% of N fertilization.**
Control = Full dose of NPK without (biofertilizer) N_2-fixers. Perc. (%) = Percentage of late wilt disease. DSI – Disease severity [redacted]ndex.

Vancura (1961) investigated the effect of N_2 fixer *Azotobacter chroococcum* inoculation in wheat and tomato rhizoshpere and effect of inoculation with *Azospirillum* (Hegazi *et al.*, 1982).

Patel (1969) added that especially while *Azotobacter spp.* become established in rhizosphere of plant roots, N_2 fixers can stimulate the growth. In the same time, N_2-fixers increased the grain yield in both pots or field conditions, and there are relationship of higher grain yield and the total counts of N_2-fixers and microbial activity in soil microbial population densities including free living N_2-fixers around the roots of maize plants may be attributed to its stimulation role (Tables 4 and 5).

Inoculation plants with any of composite resulted successful in controlling or reducing late wilt disease compared with control. Data in Tables (4 and 5) show that inoculated plant with N_2-fixers in rhizosphere were able to actively colonize the rhizosphere particularly in the presence of half dose of nitrogen and the improvement in the viability of tested microorganisms may be due to the biopreparation (Belimov *et al.*, 1995; El-Sawah, 2000 and Hauka, 2000). The highest numbers of total bacteria (15.3 x 10^6 / g soil) in the application of half dose of nitrogen, but in the full dose the total number of the bacteria was 10.6 x 10^6 / g soil.

The increasing of grain yield of maize may be due to producing certain growth promoting substances (Antoun *et al.*, 1998). On the other hand, full dose of N-fertilizer gave the highest value in other characters as N-content or dry weight and highest shoot compared with the 50% dose of N fertilizer.

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أثر التسميد الحيوى بمثبتات النيتروجين على محصول الحبوب ومرض الذبول المتأخر فى الذرة الشامية

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تم إجراء تجارب الأصص تحت ظروف الصوبة بقسم بحوث أمراض الذرة والمحاصيل السكرية وذلك بغرض دراسة تأثير التلقيح بالبكتريا المثبتة للنتروجين مفردة أو مختلطة على محصول الحبوب وكذلك على حدوث مرض الذبول المتأخر فى الذرة الشامية صنفى بلدى وهجين ثلاثى ٣١٠ فى العامين الزراعيين (١٩٩٩ ، ٢٠٠٠)٠

وقد تم التلقيح بميكروبين هما أزوتوباكتر كروكوكم وأزوسباريللم برازيلنس مع إستخدام ٥٠% أو ١٠٠% من المعدل الموصى به من الأسمدة النيتروجينية الكيماوية٠ وقد أثبتت النتائج أن تلقيح الأصص بمعلق البكتريا المستخدمة وبالتركيز ٧ × ١٠ [٩] خلية فى السنتيمتر المكعب قد أعطى زيادة معنوية فى محصول الحبوب ونقصاً واضحاً فى نسبة وشدة الإصابة بمرض الذبول المتأخر تحت ظروف الصوبة٠

وفى حالة تجارب الحقل التى تمت فى عامين متتاليين ١٩٩٩ ، ٢٠٠٠ فى محطة البحوث الزراعية بتاج العز على نفس الصنفين أجرى التلقيح بميكروب واحد مرة وبكليهما مرة أخرى على ٥٠% أو ١٠٠% من معدل السماد الكيميائى النيتروجينى الموصى به٠ أوضحت النتائج تطابقاً مع تلك المأخوذة فى الأصص٠ حيث ظهر أن التلقيح بالمخلوط من الميكروبين تحت الدراسة أو المكون رقم (٣) قد أعطى أعلى محصول حبوب (طن/فدان) خصوصاً مع الصنف هجين ثلاثى ٣١٠ (٣,٥ طن/فدان) و (٣,٦ طن/فدان) بالمقارنة بالكنترول فى الصنفين٠

ومما لا شك فيه أن تكلفة السماد المعدنى المتزايدة والأثر المتبقى لهذه المركبات الكيماوية على التربة والكائنات الحية بها وعلى المحصول وبالتالى على البشر والحيوانات والأسماك يعطى دلالة واضحة على أهمية الإتجاه للتسميد البيولوجى بقدر الإمكان لتساعد على نظافة البيئة التى نعيش فيها٠ بالإضافة إلى زيادة كمية وجودة المحاصيل التى تستخدم فيها مخصبات بيولوجية (من بكتريا أو طحالب أو فطريات)٠

والذى يبشر بالخير أنه مع تزايد إستخدام الكائنات الحية فى المقاومة الحيوية للأمراض النباتية بدرجة معقولة فإن زيادة التسميد بالمخصبات الحيوية سيحقق بإذن الله دفعة هائلة لزيادة المنتج الزراعى النظيف مما يعود على البشرية بالخير والرفاهية٠