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BIOCONTROLLING OF BACTERIAL STALK ROT DISEASE OF MAIZE

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ABSTRACT

Three biocontrol agents were used in this study against *Erwinia carotovora* var. *Zea* caused soft-rot disease of maize stalks.
Three biocontrol agents revealed inhibitory effect against this pathogen.

The biocontrol agent namely (*Pseudomonas fluorescens*, *Streptomyces griseus* and *Azotobacter chroococcum* showed different reduction in the pathogen growth rate in laboratory experiments. In green-house tests all bioagents were effective comparing with control. Application of bioagents as seed dressing and injection in the stalk of maize plants were used as suspension density of ($7x10^9$ cells per ml) to reduce soft-rot disease.

This work aimed to study the effect of some of biocontrol agents against soft-rot disease caused by (*E. carotovora* var *Zea*).

Keywords : Biocontrol agents; *Pseudomonas sp.*, *Streptomyces sp.* and *Azotobacter sp.*, *Zea maize*.

INTRODUCTION

Corn (*Zea mayz* L.) is one of the most important cereal crops in Egypt and all over the world for its economic and essential uses for human consumption and also in animal feeding. Egypt was a great need to increase grain production because it is a staple food for the majority of Egyptians. There are great effects has been paid to increase maize productivity according to statistical data of Ministry of Agriculture, A.R.E in 2005, were the area of maize was 1.927000 feddan that produce 6.540000 ton. Among this area, EL-Dakahlia governorate cultivated with 64.483 feddan produced (27.15 ardab/feddan) (Anon, 2005).

In Egypt, maize is seriously affected by some plant pathogens inducing some fungal and bacterial diseases that attacked plants from seedling till maturity (Mcheen, 1953). Bacterial stalk-rot disease has been reported to be severity attack the crop causing serious damage and high reduction in grain yield. (Kelman *et al.*, 1957) described a bacterial stalk-rot of maize in Illinois U.S.A.

Many researchers reported that the application of biocontrol agents (EL-Sheshtawy and Dawood, 1998; Gnanamanickam, 2002) such as *Pseudomonas spp.*, *Azotobacter spp.* and *Streptomyces spp.* (AL-Laithy, 1996-2006) were very important bacteria against fungal and bacterial diseases.

The objective of this investigation, three biocontrol agents were used, two from the bacteria (*Pseudomonas fluorescens* and *Azotobacter chroococcum*) and the third was from actinomyces (*Streptomyces griseus*).

MATERIALS AND METHODS

I. Isolation of biocontrol agents :

(1) Isolation of *Pseudomonas spp.* :

Different soil samples were collected from different location in Dakahlia governorate, through 2005 season. Soil samples were homogenized, serial dilution up to 7 x 10^9 were prepared, in which 0.1 ml of each dilution was planted in four plate agar replicates. This was done following the standard dilution planting technique. Different types of bacteria colonies were transferred to a nutrient agar (NA) slants as pure cultures for further studies.

(1.a) Characterization and identification of testing isolates under testing :

Gram stain reaction and cell morphology were observed from colony grown on nutrient agar slants for 24 hours at 28 °C. The identification tests were done following the procedures given by **Schaad (1980)**. Cells dimension were measured using the ocular micrometer attacked to the eye piece of the microscope.

Bacterial colonies isolated from different soil samples taken from maize varieties rhizosphere were screened for their abilities to inhibit the bacteria growth of *Erwinia carotovora* at 28 °C.

(2) Isolation and identification of *Streptomyces spp.* from soil :

Isolation was performed by soil dilution plate technique using starch nitrate agar (S.N.A) medium. Ten gm of dried soil was taken in 90 ml of sterilized water, to make soil suspension. Serial dilutions were prepared and the different dilutions were applied in plates and 20 ml of melted starch nitrate agar medium at around 45 °C was added. After gently rotation, the plates were incubated at 28 °C for 5-7 days. Selected colonies (rough & chalky) of actinomycetes were transferred from plates mixed culture into respective agar medium plates and incubated at 28 °C for 5-7 days. A single colony of each pure culture was transferred to starch nitrate agar slant for further examination.

2.a- Identification of *Streptomyces sp.* :

The isolated physical and morphological characteristics were compared depending on the observation appeared after 7-14 days on S.N.A medium **(Waksman, 1959)**. Also, physiological characterization (Utilization of carbohydrates) were investigated **Waksman, (1967)**, and Enzymatic activity was performed according to **Shirling and Gottlieb (1976a)** and antimicrobial activity according to **Wu, (1984)**.

3- Isolation of *Azotobacter spp.* :

This was carried out by the dilution method in selective liquid medium **(Abd EL-Malek and Ishac, 1968)**, using 5 tubes for each dilution. Incubation period was 15 days at 30 °C. *Azotobacter* positive tubes were distinguished

by the presence of the characteristic pellick and by examining stained preparations. The most probable number was obtained by the use of **Hoskins tables (1934).**

3.a- Purification of *Azotobacter* strains :

This was done according **Gibbson (1974).**

3.b- Identification of *Azotobacters* :

This was carried out according to **Sherman (1967).**

II. Isolation of (*Erwinia carotovora* var. *Zea*) the causal organism of bacterial stalk-rot disease in maize :

For isolation of the pathogenic bacteria, middly affected internodes were thoroughly washed, surface sterilized in alcohol for a few seconds and flamed. Small portions of the inner slightly rotting tissues were transferred into sterile petri dishes.

Bacterial suspension was prepared by teasing such tissues in sterile water, streked into nutrient glucose agar medium and incubated at 30 °C. For isolation of spore-forming bacteria from plants affected with sot-rot, the bacterial suspension was either streaked onto neutral red agar or transferred onto potato slices. These bacteria frequently produced a limited rot in potato slices, and it was able to infect healthy maize plants.

RESULTS AND DISCUSSION

Using bacterial biocontrol agents namely; *Pseudomonas fluorescens*, *Azotobacter chroococcum* and *Streptomyces griseus* from actinomycetes gave promising results in protection maize (*Zea maize* L.) from the soil borne pathogenic bacteria which causes stalk-rot disease of *Erwinia carotovora* var *Zea*. These bioagents affected the pathogen through production of the antibacterial metabolite. All the studied bioagents used in this study appeared growth inhibition of the bacterial pathogen.

Table 1: Antagonistic effect between pathogenic bacteria *E.carotovora* var *Zea* and biocontrol agents under laboratory conditions.

Pathogenic bacteria / Bio-control agents	*Erwinia carotovora* var *Zea*	
	Growth diameter (mm)	Diameter of inhibition zone (mm)
Pseudomonas fluorescens	35.0 ml	60.0
Streptomyces griseus	47.0	55.0
Azotobacter chroococcum	75.0	40.0
* Control	95.0	0.0
L.S.D at 0.05	4.8	2.2

* Control = without pathogen or bioagent.

I. Laboratory experiment :

The results appeared differences between bioagents when used against the pathogenic bacteria. These differences were appeared in growth inhibition.

For example, *Pseudomonas fluorescens* was the first in controlling the pathogen *Erwinia carotovora* var *Zea*. The optimum density which gave the best control was 7 x 10^9 cells/ml. Percentage of bacteria growth was recorded in (Table1). Growth diameter of *E. carotovora* increased from 35 mm while bioagent was *Pseudomonas fluorescens* to 47 mm with *Streptomyces griseus* and 75 mm with *Azotobacter chroococcum* comparing with control 95.0 mm. So the first bioagent *Pseudomonas fluorescens* was the best in decreasing the pathogen growth.

2- Greenhouse experiment :

All treatments with different ratios between bioagent and pathogen were significantly reduced the incidence % of soft-rot disease in maize varieties. SC10 variety gave the lowest percentage of injection with *E. carotovora* or soft-rot disease with all ratios, while TWC 352 (yellow) gave the highest values (25.0 %) at the ratio of (1:3 *Azotobacters* to *Erwinia carotovora* var *Zea*) follow with Balady variety to be 22.0 % with the same ratio.

Table 2 : Effect of biocontrol agents on maize pathogenic bacteria (*Erwinia carotovora* var *Zea*) under greenhouse conditions (2005) using method (1)* at the latest reading.

Biocontrol agents	** Bioagent/ pathogen ratio	Soft rot disease incidence % in maize varieties				
		Balady	SC 30 K8	SC 10	TWC 310	Yellow TWC 352
1- *Pseudomonas fluorescens*	1 : 1	10.0	8.0	6.0	7.0	12.0
	2 : 1	8.0	6.0	4.0	7.0	10.0
	3 : 1	5.0	4.0	3.0	5.0	7.0
	1 : 2	12.0	7.0	9.0	10.0	11.0
	1 : 3	16.0	13.0	12.0	13.0	15.0
Control		5.0	5.0	5.0	6.0	7.0
L.S.D at 0.05		3.0	2.0	2.3	2.4	3.2
2- *Streptomyces griseus*	1 : 1	12.0	9.0	7.0	8.0	13.0
	2 : 1	10.0	7.0	3.0	7.0	12.0
	3 : 1	7.0	5.0	2.0	6.0	8.0
	1 : 2	18.0	8.0	10.0	14.0	13.0
	1 : 3	20.0	16.0	18.0	15.0	17.0
Control		9.0	7.0	5.0	6.0	7.0
L.S.D at 0.05		3.1	2.3	2.7	3.3	2.1
3- *Azotobacter chroococcum*	1 : 1	16.0	10.0	10.0	12.0	17.0
	2 : 1	12.0	8.0	6.0	10.0	13.0
	3 : 1	13.0	9.0	4.0	7.0	10.0
	1 : 2	19.0	16.0	8.0	9.0	20.0
	1 : 3	22.0	20.0	19.0	20.0	25.0
Control		10.0	7.0	5.0	0.0	7.0
L.S.D at 0.05		2.7	2.1	2.2	3.1	2.1

* Method 1 = coating the seeds with the pathogen and bioagent before sawing.
** Pathogen = *Erwinia carotovora* var *Zea*.

The differences in infection percentage with the bacterial stalk-rot of maize may be due to the ability of producing the antibacterial compounds. So the bioagent *Pseudomonas fluorescens* was the first following with *Streptomyces griseus* from actinomycetes and the latest one was *Azotobacter chroococcum* in its action on the percentage of infection.

Table 3 : Effect of biocontrol agents on maize pathogenic bacteria (*Erwinia carotovora* var *Zea*) under greenhouse conditions (2005) using method (2)* at the latest reading.

Biocontrol agents	** Bioagent/ pathogen ratio	Soft rot disease incidence % in maize varieties				
		Balady	SC 30 K8	SC 10	TWC 310	Yellow TWC 352
1- *Pseudomonas fluorescens*	1 : 1	15.0	13.0	8.0	9.0	16.0
	2 : 1	10.0	9.0	10.0	8.0	12.0
	3 : 1	7.0	7.0	6.0	7.0	8.0
	1 : 2	17.0	16.0	15.0	17.0	19.0
	1 : 3	20.0	18.0	15.0	18.0	22.0
Control		5.0	5.0	6.0	5.0	5.0
L.S.D at 0.05		3.0	2.1	2.2	1.9	2.8
2- *Streptomyces griseus*	1 : 1	11.0	12.0	7.0	8.0	15.0
	2 : 1	9.0	9.0	9.0	11.0	11.0
	3 : 1	9.0	8.0	7.0	6.0	7.0
	1 : 2	12.0	15.0	12.0	15.0	17.0
	1 : 3	15.0	17.0	13.0	15.0	20.0
Control		6.0	6.0	5.0	6.0	6.0
L.S.D at 0.05		3.2	2.0	2.3	2.2	2.3
3- *Azotobacter chroococcum*	1 : 1	19.0	10.0	9.0	10.0	17.0
	2 : 1	15.0	8.0	6.0	9.0	16.0
	3 : 1	9.0	7.0	7.0	8.0	9.0
	1 : 2	16.0	13.0	15.0	19.0	20.0
	1 : 3	20.0	19.0	18.0	21.0	22.0
Control		7.0	6.0	6.0	6.0	5.0
L.S.D at 0.05		2.5	2.1	2.0	3.2	2.3

*** Method 2 = injection with the pathogen and bioagent in stalk of maize plant.**
**** Pathogen = *Erwinia carotovora* var *Zea*.**

3- Field experiments:

Pseudomonas fluorescens considered beneficial as biocontrol agents against the pathogen (*E. carotovora* var *Zea*). These bacteria are able to growth inhibitor compounds.

Pseudomonas fluorescens gave the lowest percentage of soft-rot disease. Variety TWC352 (yellow maize) gave the highest value (16.0 %) and followed by Balady variety (15.0 %), while SC10 gave the lowest 12.0 at the same ratio (1:3) Table (4).

Streptomyces griseus (actinomycetes) was the second but *Azotobacter chroococcum* was the latest value to be which gives the lowest action or highest percentage of infection (15.0, 14.0, 13.0, 14.0 and 15.0 %) with all varieties under study and also with using different ratios used, as shown in Table 4. The data in this Table were also taken as the latest reading during the season 2005 of the first method.

Table 4 : Effect of biocontrol agents on maize pathogenic bacteria (*Erwinia carotovora* var *Zea*) under field conditions (2005) using method (1)* at the latest reading.

Biocontrol agents	** Bioagent/ pathogen ratio	Soft rot disease incidence % in maize varieties				
		Balady	SC 30 K8	SC 10	TWC 310	Yellow TWC 352
1- *Pseudomonas fluorescens*	1 : 1	9.0	8.0	6.0	7.0	10.0
	2 : 1	6.0	7.0	3.0	8.0	7.0
	3 : 1	5.0	4.0	3.0	7.0	6.0
	1 : 2	11.0	8.0	10.0	11.0	11.0
	1 : 3	15.0	13.0	12.0	13.0	16.0
Control		7.0	6.0	5.0	5.0	6.0
L.S.D at 0.05		2.3	2.0	2.1	1.8	2.6
2- *Streptomyces griseus*	1 : 1	10.0	7.0	6.0	7.0	9.0
	2 : 1	7.0	7.0	6.0	7.0	9.0
	3 : 1	6.0	4.0	5.0	6.0	7.0
	1 : 2	10.0	9.0	8.0	10.0	10.0
	1 : 3	14.0	13.0	11.0	12.0	15.0
Control		6.0	6.0	5.0	6.0	8.0
L.S.D at 0.05		2.3	2.1	2.0	1.5	2.5
3- *Azotobacter chroococcum*	1 : 1	11.0	9.0	6.0	8.0	9.0
	2 : 1	7.0	8.0	5.0	6.0	7.0
	3 : 1	6.0	5.0	4.0	5.0	6.0
	1 : 2	10.0	10.0	9.0	10.0	11.0
	1 : 3	15.0	14.0	13.0	14.0	15.0
Control		6.0	7.0	6.0	6.0	6.0
L.S.D at 0.05		2.4	1.9	2.3	3.6	2.2

*** Method 1 = coating the seeds of maize with the pathogen and biocontrol agent.**
**** Pathogen = *Erwinia carotovora* var *Zea*.**

Data in Table 5 appeared a good idea about the second method (2) used in this study. It can seen that injection with the pathogen and every one from different biocontrol agents gave a good results in controlling soft-rot disease at all ratios. Also, the second bioagent *Streptomyces griseus* gave the lowest infection percentage especially in variety SC10, while variety TWC325 was high infected (9.0 %) at 1:3 ratio and SC30k8 was moderately (10.0) while TWC352 was 14.0 % at the same ratio (1:3).

Data in Tables 4 and 5 show that stalk maize varieties infection with the pathogen and bioagent at greenhouse or the field were effective than the seeds coated with the pathogen and bioagents.

The effect of these bioagent in protection of maize plants may involve antagonism as a result of secondary metabolites production or extracellularlitic enzymes (Klewnick, 2000).

Table 5 : Effect of biocontrol agents on maize pathogenic bacteria (*Erwinia carotovora* var *Zea*) under field conditions (2005) using method (2)* at the latest reading.

Biocontrol agents	** Bioagent/ pathogen ratio	Incidence % of soft rot disease in maize varieties				
		Balady	SC 30 K8	SC 10	TWC 310	Yellow TWC 352
1- *Pseudomonas fluorescens*	1 : 1	11.0	8.0	6.0	10.0	12.0
	2 : 1	7.0	6.0	4.0	6.0	8.0
	3 : 1	5.0	4.0	2.0	3.0	5.0
	1 : 2	12.0	10.0	6.0	5.0	13.0
	1 : 3	17.0	14.0	12.0	14.0	18.0
Control		5.0	5.0	5.0	5.0	6.0
L.S.D at 0.05		2.9	2.1	2.1	1.8	2.1
2- *Streptomyces griseus*	1 : 1	8.0	6.0	5.0	10.0	11.0
	2 : 1	6.0	5.0	3.0	6.0	7.0
	3 : 1	5.0	4.0	2.0	4.0	5.0
	1 : 2	7.0	6.0	5.0	8.0	12.0
	1 : 3	12.0	10.0	9.0	10.0	14.0
Control		5.0	6.0	6.0	6.0	6.0
L.S.D at 0.05		3.1	2.3	2.2	2.0	2.0
3- *Azotobacter chroococcum*	1 : 1	12.0	7.0	11.0	10.0	13.0
	2 : 1	7.0	5.0	7.0	8.0	6.0
	3 : 1	5.0	4.0	6.0	6.0	5.0
	1 : 2	10.0	7.0	8.0	9.0	12.0
	1 : 3	17.0	10.0	15.0	16.0	18.0
Control		5.5	5.0	6.0	6.0	5.0
L.S.D at 0.05		2.8	2.2	2.4	3.1	2.1

* Method 2 = Injection of maize stalk with pathogen and bioagent.
** Pathogen = *Erwinia carotovora* var *Zea*.

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المقاومة الحيوية لعفن الساق البكتيرى فى الذرة الشامية

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يعتبر مرض عفن الساق البكتيرى (العفن الطري) الذي يصيب نبات الذرة الشامية فى مصر ويتسبب عن الإصابة بالبكتريا *Erwinia carotovora var. Zea.* من الأمراض الخطيرة التي تصيب الساق وخصوصا بعد الإصابة الحشرية بالثاقبات التي تصنع الأنفاق فيها أثناء مرحلة طور البادرات مما يساعد على دخول البكتريا التي تتمكن من إحداث المرض إذا ما توفرت درجة الحرارة والرطوبة المناسبتين. ولم يقتصر حدوث المرض على الصنف البلدى بل تعداه إلى الهجن الفردية والثلاثية كذلك وقد تم زراعة الأصناف الآتية فى ثلاثة مراكز من مراكز محافظة الدقهلية كما هو موضح بالجدول الآتى :

م	الصنف	المنطقة المنزرع فيها	الحوض	تاريخ الزراعة
١	بلدى أبيض	محطة البحوث الزراعية بتاج العز (مركز تمى الأمديد)	الآثار	٢٠٠٥/٦/١
٢	هـ ف أبيض ٣٠ ك ٨	ديسط مركز طلخا	حوض الجزيرة	٢٠٠٥/٦/١
٣	هـ ف ١٠	ديسط مركز طلخا	حوض الجزيرة	٢٠٠٥/٦/١
٤	هـ ث أبيض ٣١٠	منشأة الأخوة مركز أجا	الخرس البحرى	٢٠٠٥/٦/٣
٥	هـ ث أصفر ٣٥٢	منشأة الأخوة مركز أجا	الخرس البحرى	٢٠٠٥/٦/٣

وقد تمت زراعة القصارى البلاستيك فى الصوبة بالأصناف الخمسة المذكورة فى نفس ميعاد الزراعة وتمت العدوى بالبكتريا الممرضة بطريقتين الأولى عن طريق حقن الساق والنبات في عمر خمسين يوما بمعلق البكتريا الممرضة وكذلك المقاوم الحيوى والثانية بإستخدام تقاوى مغلفة بمعلق البكتريا وبتركيز معين قبل الزراعة مباشرة.

وقد تم إستخدام هذه السلالات التالية في المقاومة الحيوية لميكروب الإيرونجيا كاروتوفورا وهى :

١- بكتريا الزيدوموناس فلوروسنز *Pseudomonas flurescens*

٢- بكتريا ستربتومايسز جريسيوس *Streptomyces griseus*

٣- بكتريا الأزوتوباكتر كروكوكم *Azotobacter chroococcum*

بمعلق هذه الكائنات ومشاكل عشرة أيام بعد حقن الميكروب المعدى فى ساق الذرة الشامية (الأصناف المدونة) وتم أخذ القراءات كل شهر من تاريخ العدوى فى طريقة الحقن وفى حالة معاملة التقاوى تم أخذ القراءات أيضا كل شهر من تاريخ الزراعة حيث تم تقييم قدرة المقاومات الحيوية فى إيقاف أو تقليل حدوث الإصابة ومعرفة أعلى الأصناف قدرة على مقاومة المرض ، وقد إعتبرت المعاملة الكنترول فى كل صنف منزرع هي التي لم يتم عدوى البذور فيها. أو حقن الساق بمعلق البكتريا الممرضة أو المقاوم الحيوى. ولقد تم تقدير نسبة حدوث المرض فى الصوبة والحقل على أساس القراءة الأخيرة حتى تكون ممثلة للنسبة الحقيقية للمرض.

ويفيد هذا البحث فى معرفة إمكانية إستخدام بعض طرق المقاومة الحيوية فى مقاومة هذا المرض الخطير توفيرا للنفقات فى حالة إستخدام المقاومة الكيماوية أو حتى عدم الإعتماد كلية عليها حفاظا على البيئة من التلوث.