

BIOLOGICAL CONTROL OF THE LATE WILT DISEASE OF MAIZE USING *Streptomyces griseus*

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ABSTRACT

This work was carried out during 2004 of maize growing season in two locations first at Dakahlia and the second at Gharbeia governorate.

Biological control trails against the late wilt disease caused by *Cephalosporium maydis* were conducted using actinomycetes (*Streptomyces griseus*).

Strains No. 7, 9, 15 and 40 were more and highest active against *C. maydis*. The concentration of *Streptomyces griseus* suspension a, b and c were used against the pathogen in laboratory or greenhouse conditions gave a good action antifungal with *C. maydis* and succeeded in inhibition the fungal growth. The percentage of infection decreased with the increasing of antibiotic dose.

Seed treatment with *Streptomyces griseus* suspension was effective in controlling the late wilt disease of maize caused by *Cephalosporium maydis*.

Fourty streptomycetes strains were isolated from collected soil samples. All these isolates were purified and screened for their antifungal activity against some soil pathogenic fungi especially *C. maydis*. Ten aggressive strains from *Streptomyces griseus* were selected for further studies. The antibiotics were extracted and purified. Antifungal (Antibiotics) from liquid media are very important in control of the mycotic diseases.

The need for new safe and more effective antifungal especially with the increase of infection with plant diseases.

INTRODUCTION

Late wilt disease caused by the soil born fungus; *Cephalosporium maydis* has been degraded as the most serious disease of maize in Egypt. This disease first described by Samra *et al.* (1962). The use of actinomycetes in controlling this disease was previously reported [Broadbent *et al.* (1971); Brown, (1987); Chi *et al.* (1965); Cook, (1981); Cooper *et al.* (1950); Fahmy, (1986); Gubta *et al.* (1979); Knouss, (1976); Reddie *et al.* (1971); Deacon, (1991); Dik *et al.* (1998). Laboratory and greenhouse experiments were conducted to study the antagonistic effect of *Streptomyces sp.* against the causal organism of the late wilt disease; *Cephalosporium maydis* Turhan, (1981 a and b) and Turhan *et al.* (1986) in two locations, first at the experimental Laboratory of Tag AL-Ezz Research Station at Dakahlia Governorate and the second at the experimental greenhouse of Gemmiza Research Station Gharbeia Governorate.

The biocontrol agent *Streptomyces griseus* was used as coating of maize seeds, it produces substances that affects mycelial growth of *Cephalosporium maydis* in laboratory and in rhizosphere of maize plant, Sing *et al.* (1980) and Harman (1991). These substances suggested the microorganisms especially *C. maydis* Rangaswami *et al.* (1962 and 1963); Rothrock *et al.* (1984); Sabaou *et al.* (1987); Tahvonon *et al.* (1990);

Tahvonen, (1988); Tahvonen *et al.* (1995); Augustine *et al.* (2005) and Gupta *et al.* (1997).

The need for corn (*Zea mays*) as a nutrient for human and animals increasing so that the total area in Egypt about (1.9) million feddan produced 5.871 million tons (2004). In Dakahlia the area (49.405) feddan produced 1.348517 (Ardab) (27.30 ardab/feddan) while the area in Gharbeia governorate was 79.031 ardab/feddan (2004), respectively, according to the statistical data of Corn Crop Council, Ministry of Agriculture, 2004.

Seed treatment of corn (*Zea mays*) was tested for the control of late wilt disease caused by *C. maydis* (Wood *et al.* 1955). Ten aggressive strains of *Streptomyces griseus* with strong antifungal activity were effective in controlling the pathogen at rhizosphere in soil artificially infected with *Cephalosporium maydis*.

MATERIALS AND METHODS

The present investigation was carried out during 2004 growing season in two locations first at Dakahlia and the second at Gharbeia governorate.

Isolation of microorganisms :

Isolation of the late-wilt pathogen :

Diseased maize plants showing characteristics symptoms of the wilt disease, were collected from different locations. The stalk was split length-wise and small parts of the interior were aseptically transferred into plates of potato dextrose agar (PDA) supplemented with 0.1% yeast extract. Isolation plates were incubated at 30 °C for 7 days.

The pathogenicity, cultural and microscopical characteristics of the isolated fungus were examined and identification was confirmed in comparison to the culture collection of the Maize and Sugar Crops disease Res. Section, Plant Pathology Institute, Agric. Res. Center.

Pathogenicity test :

To test the pathogenicity of *C. maydis*, the soil infestation technique was used. The inoculum of the fungus was raised on sorghum seeds in 0.5 kg capacity milk bottles. After sufficient growth had been obtained, the contents of the bottles were thoroughly mixed. Fifty grams of the inoculum were added and mixed with 8-10 cm. top layer of autoclaved Nile silt in each 25 cm. diameter/pot. The seeds of susceptible variety Balady were planted at the rate of 8 seeds/pot. and thinned later to 5 plants. The percentage of infected plants were recorded 80 days after sowing.

Mechanical analysis of the soil samples :

Samples were analyzed in Gemmeiza Agricultural Research Station (Laboratory of Soil & Water). Soil samples representing different types under diversified cropping systems were taken from the top 20 cm layer, packed in polyethylene bags and transported to the laboratory for mechanical (physical analysis). The sampling sites as well as the standing crops of the different samples are shown in Table (1).

Table (1) : Mechanical analysis of the soil samples.

Samples	Standing crops	Location	Sand %	Silt %	Clay%	Soil texture
1. D	Onion	Aga (Aldears)	6.0	65.0	20.0	Loamy clay
2. D	Banana	Meet Ghamr	3.0	20.0	70.0	Clay loam
3. D	Rice	AL-Senbellaween	2.0	17.0	72.0	Clay loam
4. D	Rice	Sherbeen	4.0	20.0	68.0	Clay loam
5. D	Sugar beet	Belqas	65.0	2.0	20.0	Sandy clay
6. D	Maize	Talkha	1.0	70.0	18.0	Loamy clay
7 Gh.	Cotton	AL-Mahalla	3.0	20.0	75.0	Clay loam
8 Gh.	Maize	Zefta	4.0	17.0	70.0	Clay loam
9 Gh.	Cotton	Tanta	2.0	20.0	66.0	Clay loam
10 Gh.	Maize	AL-Santa	0.0	80.0	14.0	Loamy clay

1. D : Sample No. 1 from Dakahlia Governorate.

7. Gh : Sample No. 7 from Gharbela Governorate.

C- Isolation of actinomycetes (streptomycetes) from soil sample:

Isolation was performed by soil dilution plate technique using starch nitrate agar medium. Ten gm of dried soil was taken in 90 ml sterilized water, agitated vigorously and allowed to stand, to make soil suspension. Serial dilution were made and the different dilution were applied into plates and 20 ml of melted starch nitrate agar medium at around 45 °C was added.

After gently rotating, the plates were incubated at 28 °C for 5-7 days. Selected colonies (rough, chalky) of actinomycetes were transferred from mixed culture of the plates into respective agar 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 maintenance till further examination.

Starch-nitrate agar medium Waksman, (1959).

Starch	20.0 gm
Potassium nitrate	2.0 gm
Dipotassium hydrogen phosphate	1.0 gm
Magnesium sulphate	0.5 gm
Sodium chloride	0.5 gm
Calcium carbonate	3.0 gm
Ferrous sulphate	0.01 ml
Trace salt solution	1.0 ml
Distilled water	1000.0 ml
Agar Agar	20.0 gm

The easy access and inappropriate use of antimicrobials led to selection and spread of resistant microorganisms strains. It is imperative to search for new and more effective antimicrobials. *Streptomyces sp.* were isolated from the soil samples from Dakahlia Governorate using Starch-Nitrate-Agar medium.

The antifungal activity against *Cephalosporium maydis* the causal organism of the late wilt disease of maize in Egypt was tested using the disc diffusion method. For the extraction of active metabolites, culture broth of

antagonistic *Streptomyces* sp. were extracted with organic solvents followed by testing the antibiotic activity.

Identification of actinomycetes :

Identification of the four actinomycetes to species levels was based on characteristics specific to each species as presented in Bergey's Manual of Systematic Bacteriology based on morphological, physiological and biochemical characteristics. The four strains were identified as *Streptomyces griseus* (Pridham, 1958).

Inoculum preparation :

Streptomyces griseus was prepared by placing 50 g of moist wheat bran into 550 ml conical flasks autoclaved at 121 °C for 20 min. as described by Roiger & Jeffers (1991). This mixture was then inoculated aseptically with spore suspension (25 ml) of each actinomycetes in 10% glycerol and incubated at 28 °C in the dark, colonized and control wheat bran suspended in 25 ml of sterile distilled water and 0.2 ml of this suspension was spread in the plats.

Laboratory experiments :

Inhibitory effect of *Streptomyces griseus* metabolites on the growth of *C. maydis* :

The filtrates of the liquid media of *Streptomyces griseus* were obtained by growing the strains in 250 ml flasks were incubated at 28 °C for 7 days. The liquid culture were collected and filtered through wattman filter paper No.1. The filtrates were poured into flasks containing sterilized water agar. Equal amounts of 20 ml were poured into 9 cm diam. petri dishes and inoculated with fungal disk 5 mm. diam. and 4 days old of *Cephalosporium maydis*.

Culture filtrate free medium served as control. All the inoculated plates were incubated at 28 °C and the averages of colonies diameters of radial growth of the fungal pathogen was recorded after 6 days.

Greenhouse experiments :

Biological control trials against the late wilt disease caused by the pathogen *C. maydis* were conducted using actinomycetes antagonists *Streptomyces griseus*.

Seed coating with the antagonist were prepared, also 40 cm plastic pots were filled with (4 kg/pot) autoclaved sandy loam soil about and artificially infested with mycelial suspension of *C. maydis* (20 ml./pot). The mycelia were minced in a sterilized blender for 20 sec. at low speed. Maize seeds var. SC10, H310 and Balady were planted in the artificial soil. Seeds were surface sterilized and coated with spores of *Streptomyces griseus* and the control were carried out by sowing uncoated sterilized in the antagonist suspension. Irrigation took place immediately after planting and repeated every 4 days.

The planted pots were kept under the greenhouse condition and after 75 days the infested plants percentage compared with the control were recorded.

RESULTS AND DISCUSSION

Isolation and Identification of the most active isolates of actinomycetes producing antifungal compounds :

Fourty cultures of actinomycetes were isolated by the methods described before in material and methods according to Waksman, (1959). The isolated cultures were screened for their activity in producing antifungal compounds Sultan (2002).

Ten isolates from the fourty actinomycetes showed very high activity against the tested fungus (*Cephalosporium maydis*) the causal organism of the late wilt of maize in Egypt.

In Table (2) the ten isolates 1, 5, 7, 9, 10, 15, 20, 21, 30 and 40 which identified as *Streptomyces griseus* were considered. Strains No. 7, 9, 15 and 40 were the highest active against *C. maydis*. The effect of three antibiotic concentrations of the selected strains on the growth inhibition % of *Cephalosporium maydis* was recorded in Table (3).

Table (2) : Actinomycetes isolated from the different soil samples from Gharbeia and Dakahlia Governorates.

No. of soil samples	Location	Soil texture	Cultivated plant	Total count of <i>Streptomyces</i> sp.
1	Aga	Loamy clay	Zea maize	27.0
2	Meet Ghamr	Clay loam	Zea maize	20.0
3	Senbellaween	Clay loam	Rice	8.0
4	Sherbeen	Clay loam	Rice	10.0
5	Belqas	Sandy clay	Sugar beet	30.0
6	Talkha	Loamy clay	Onion	40.0
7	EL-Mahalla EL-Kobra	Clay loam	Cotton	15.0
8	Zefta	Clay loam	Banana	5.0
9	Tanta	Clay loam	Rice	4.0
10	EL-Santa	Loamy clay	Zea miaze	60.0

*** The effect of different concentration of the selected strains of *Streptomyces griseus* on the late wilt disease of three maize varieties under green house condition.**

Table (4) shows the percentages of infection with late wilt disease and disease severity index proved. The increasing of concentration from 20 to 60 ml. of *Streptomyces griseus* suspension in pot gave decreasing in the percentage of infection from 12.0 to 7.0 % in hybrid 310, 4.0 to 2.0 in SC10 and from 20.0 to 8.0 in Balady variety comparing with the control (18.0, 7.0 and 30.0 %).

Table (3): The effect of different antibiotic concentrations of the selected strains of *Streptomyces sp.* at 30 °C after 7 days of inocubation on the growth and inhibition rate % of *Cephalosporium maydis* under laboratory conditions.

Isolate No. of the tested strains	Growth inhibition % of <i>C. maydis</i>			
	* Conc. a	** Conc. b	*** Conc. c	Control
1	10.0	30.0	45.0	0.0
5	15.0	40.0	50.0	0.0
7	80.0	85.0	90.0	0.0
9	70.0	90.0	95.0	0.0
10	5.0	10.0	10.0	0.0
15	75.0	85.0	95.0	0.0
20	18.0	20.0	20.0	0.0
21	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0
40	100.0	100.0	100.0	0.0

* Conc. a : 20 ml of P.D.A medium + 20 ml *Streptomyces sp.* filtrate (sterile).

** Conc. b : 20 ml of P.D.A medium + 40 ml *Streptomyces sp.* filtrate (sterile).

*** Conc. c : 20 ml of P.D.A medium + 60 ml *Streptomyces sp.* filtrate (sterile).

Control : 20 ml of P.D.A medium + 20 ml dist. water.

Table (4) : The effect of different metabolites concentrations of the selected strains of *S. griseus* at 30 °C after 7 days incubation on the Late wilt disease of three maize varieties under greenhouse condition using the soil infestation method.

Treatments	Late wilt disease caused by <i>Cephalosporium maydis</i>					
	Hybrid 310		S.C 10		Balady	
	* Perc. %	** D.S.I	Perc. %	D.S.I	Perc. %	D.S.I
Conc. a	12.0	2.0	4.0	1.0	20.0	4.0
Conc. b	8.0	1.0	3.0	1.0	12.0	2.0
Conc. c	7.0	1.0	2.0	1.0	8.0	1.0
Control	18.0	3.0	7.0	1.0	30.0	3.0
L.S.D at 0.05	2.2	1.7	1.2	0.0	2.4	1.1

Conc. a : 20 ml of *Streptomyces sp.* suspension / pot (soil infestation).

Conc. b : 40 ml of *Streptomyces sp.* suspension / pot (soil infestation).

Conc. c : 60 ml of *Streptomyces sp.* suspension / pot (soil infestation).

Control : without the bioagent (*S. griseus*).

* Perc. % : percentage of late wilt disease.

** D.S.I : disease severity index.

The concentration of *Streptomyces griseus* suspension a, b and c which were used against the pathogen in laboratory or greenhouse conditions gave a good antifungal action with *C. maydis* and succeeded in inhibition the fungal growth. The percentage of infection decreased with the increasing of metabolites dose. Table (4) comparing the strains of *Streptomyces sp.* included in International Streptomyces Project (I.S.P) William, (1994) entry as aerial and substrate mycelium color, reverse pigment and the source of carbon utilized. These keys lead to the identification of experimental organism as *Streptomyces griseus*.

Table (5) : Effect of different antibiotic concentration of the selected strains of *Streptomyces griseus* on the late wilt disease of three maize varieties under greenhouse condition (using seed coating method with the bio agent).

(Treatments) concentration of bioagent	Late wilt disease caused by <i>Cephalosporium maydis</i>					
	Hybrid 310		S.C 10		Balady	
	Perc. %	D.S.I	Perc.%	D.S.I	Perc.%	D.S.I
Conc. a	15.0	3.0	7.0	1.0	18.0	4.0
Conc. b	13.0	2.0	9.0	2.0	15.0	3.0
Conc. c	10.0	2.0	3.0	1.0	18.0	4.0
Control	25.0	4.0	10.0	2.0	30.0	5.0
L.S.D at 5 %	4.3	1.6	2.2	1.3	4.6	1.7

Conc. a : 20 ml of *Streptomyces sp.* suspension / liter dist. water.

Conc. b : 40 ml of *Streptomyces sp.* suspension / liter dist. water.

Conc. c : 60 ml of *Streptomyces sp.* suspension / liter dist. water.

Control : dist. water without bioagent.

Identification and characteristics of the isolate :

The color of colony, aerial mycelium, substrate mycelium, soluble pigments and growth were recorded in Table (6).

Strains were different in the color and growth of the colony from strain to another. Strains No. 9 and 15 were the best in the growth on the same media. While, strains No. 1, 10, 20 and 21 gave week growth. Also, strains No. 40 was very week growth.

Table (6) : Morphological and characterization of isolates of *Streptomyces sp.* on the specific media.

Isolate No. of the tested strains	The color of			
	Aerial mycelium	Substrate mycelium	Soluble pigment	Growth
1	Whitsh yellow	Yellow	Yellow	* W.G
5	Whitsh yellow	Yellow	Yellow	** M.G
7	White	White	White	M.G
9	White	White	White	***V.G.G
10	White	White	White	W.G
15	White	White	White	V.G.G
20	Yellow	Yellow	Yellow	W.G
21	White	Yellow	Yellow	W.G
30	White	Yellow	Yellow	****V.W.G
40	White	White	White	****V.W.G

* W.G : Weak growth.

** M.G : Moderate growth.

*** V.G.G : Very good growth.

**** V.W.G : Very week growth.

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المقاومة البيولوجية للذبول المتأخر في الذرة الشامية باستخدام الإستربتومايسز

جريسيسوس

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مركز البحوث الزراعية - معهد بحوث أمراض النبات - قسم بحوث أمراض الذرة والمحاصيل
السكرية - الجيزة - مصر

أقيمت تجربتان الأولى معملية في معامل قسم بحوث الأمراض بمحطة البحوث الزراعية بتاج العز دقهلية والثانية في الصوبة البحثية لمحطة البحوث الزراعية بالجيزة غربية في موسم واحد (٢٠٠٤م) وذلك لدراسة مقاومة مرض الذبول المتأخر في الذرة الشامية تحت ظروف نوعين من التربة الأولى (طينية سلتية) والثانية (سلتية طينية) بنوع واحد من الأكتينومايسز وهو (ستربتومايسز جريسيسوس) والذي تم إستخدامه على هيئة معلق حيوي تعامل به البذرة كما تلقح به تربة القصارى بتركيزات مختلفة قبل الزراعة مباشرة لصنفين من هجين الذرة الشامية البيضاء هما (هـ ف ١٠ ، هـ ث ٣١٠) مقارنة بالصنف البلدي الكنترول وقد تم دراسة تطور المرض بالفحص كل شهر من تاريخ الزراعة وتقدير نسبة وشدة المرض وذلك بغرض تقدير كفاءة هذا المقاوم الحيوي في مقاومة أو تقليل المرض الذي لوحظ أنه بدأ يفتزو معظم الهجن الفردية والثلاثية بنسب معقولة لا يستهان بها وأيضاً لزيادة الإكراه نحو إستخدام المقاومات البيولوجية في مقاومة بعض أمراض الذرة الخطيرة قليلاً من نفقات المقاومة الكيماوية التي تلوث البيئة وقد زاد الإهتمام بإستخدام هذا الميكروب لقدرته على إنتاج المضادات الحيوية ذات القدرة العالية تثبيط نمو المسبب المرضى والهدف من هذا البحث هو تقدير إمكانية إستخدام كائنات حية لا تلوث البيئة ومقاومة المسببات المرضية حتى ولو لم تكن على مستوى المبيدات الكيماوية المخلقة التي ما زالت للأسف الشديد تستخدم في مصر حتى وقتنا هذا .