BIOLOGICAL CONTROL OF MAIZE LATE WILT DISEASE CAUSED BY *CEPHALOSPORIUM MAYDIS*

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ABSTRACT

Antagonistic activity of different microorganisms against *Cephalosporium maydis*, a soil-borne pathogen that causes late wilt disease of maize, was investigated. *In vitro* antagonistic screening indicated that the bacterial isolate coded B5 which was identified as *Bacillus subtilis* proved to be the most effective antagonist with high RPA-value which reached 2.25, followed by 1.49 for *Pseudomonas fluorescens* P6. Linear growth of the pathogen was suppressed strongly *in vitro* due to inoculation with the fungal isolate coded T8 which identified as *Trichoderma harzianum*. Under infested field conditions, results indicate that percentages of pre-emergence damping-off reduced to 29.17 and 32.22 % due to seed inoculation by T8 and B5, respectively, compared with control (47.22 %). Efficiency of the selected antagonists was also extended to reduce incidence of late wilt disease of the survival plants in the disease nursery. Results illustrate that T8, P7 and B5 were the most effective antagonists which reduced late wilt disease of 58.93, 58.72 and 57.26 %, respectively in comparison with 58.38 % for the commercial bio-product P9 during both infestation seasons. Plant growth parameters and productivity of the treated plants were also clear enhanced. Accordingly, grain yield was increased to 52.38 and 38.10 % due to seed inoculation by the bacterial and fungal isolates (B5 and T8), respectively, in comparison to control.

Keywords: *C. maydis*, biological control, maize, antagonism, growth-promotion.

INTRODUCTION

Late wilt of maize, caused by *Cephalosporium maydis* Samra, Sabet & Hingorani, is the most economically important fungal disease of maize (*Zea mays* L.) in Egypt (El-Shafey and

Claflin, 1999). *Acremonium maydis* is the synonym preferred scientific name of the late wilt pathogenic fungus on maize, but *C. maydis* is the common scientific name in Egypt (El-Moghazy and Shalaby, 2006). *C. maydis* is a soilborne vascular wilt pathogen that penetrates root tissue and colonizes the xylem (Sabet *et al.*, 1970). More rarely, this pathogen also can be transmitted through the seeds as seedborne (El-Shafey *et al.*, 1976), and may occasionally cause seed rot or pre-emergence damping-off under heavy inoculum potential (Samra *et al.*, 1966). Disease symptoms first become apparent during teaseling as a rapid wilting of the lower leaves, and progress to produce characteristic hollow to brown or black stained pith (El-Shafey and Claflin, 1999). Moreover, *C. maydis* is the primary causal fungus of the, so-called, stalk-rot complex. Wilt and drying up symptoms only appear when plants approach maturity. The magnitude of losses largely depends upon the susceptibility of the grown cultivars and the degree of soil infestation. In infested fields, up to 80 % of the susceptible plants may become infected, and grain yield losses may reach 37 % of wilted plants, and about 15 % of the total yield in Egypt (Samra *et al.*, 1971).

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to chemical treatment methods (Osman *et al.*, 1986). These microorganisms, that display antagonistic effect against target pathogens, are capable of survival and propagation in target locations. In agriculture, the use of bacteria and fungi antagonistic towards plant pathogenic fungi has been suggested as an alternative to chemical pesticides for control of plant diseases (Walsh *et al.*, 2001 and Whipps, 2001). Therefore, wide range of different microorganisms was used to suppress late wilt pathogen of maize. *Trichoderma harzianum* (El-Assiuty *et al.*, 1986) and *Bacillus subtilis* (El-Shafey *et al.*, 1985 and Ishac *et al.*, 1985) were successfully used for biological control of the Cephalosporium-wilt disease of maize. Rhizosphere actinomycetes and yeast fungi were also used (El-Mahalawy *et al.*, 2004 and Hassanein *et al.*, 2002) for suppressing *C maydis* successfully. Biological control of soil borne plant pathogens can also be achieved by seed treatment with antagonists. *Saccharomyces cerevisiae* was successfully applied as

seed treatment by Shalaby and El-Nady (2008) to suppress sugar beet damping-off caused by *Fusarium oxysporum*. Parke *et al.* (1991) and Xi *et al.* (1996) noted that *Pseudomonas cepacia, P. fluorescens* and *B. subtilis* were effective as seed treatment against different soil borne pathogenic fungi.

Several possible plant-microbe interactions were developed which were benefit to plants through different mechanisms, such as the production of plant growth regulators, siderophores, phosphate, nutrient uptake and availability (Höflich and Kühn, 1996 and Bowen and Rovira, 1999). Therefore, the present study was designed to investigate potentiality of seed treatment with different bio-agents on incidence of late wilt disease in soil infested by *C. maydis*, as well as their beneficial effect for promoting growth and yield production of maize plants.

MATERIALS AND METHODS

Pathogen:

A virulent isolate of *Cephalosporium maydis* previously isolated from naturally infected maize (*Zea mays*, the susceptible "Baladi" variety) in Kafr El-Sheikh governorate was used through this study. For *in vitro* tests, samples showed typical late wilt symptoms were collected, divided into small pieces, surface sterilized using 0.25% sodium hypochlorite solution for 4 minutes, washed several times in sterilized distilled water and then blotted between two sterilized filter papers. These pieces were grown on potato dextrose agar medium (PDA) supplemented with yeast extract. Cultures were incubated at 28 °C ± 2 C° for 3-7 days and then purified by the hyphal tip technique according to Booth (1977). Pure cultures were examined microscopically and maintained on PDA slants supplemented with 0.1 % yeast extract at 4 °C for further experiments according to the method described by Khalifa (1991). According to Barnett (1960) and Singh (1982), the pathogenic isolate was identified as *Cephalosporium maydis*. To test the pathogenicity of *C. maydis*, soil infestation technique was used and the inoculums were prepared as described by El-Shafey *et al.* (1988) at Dept. of Maize, Sugar and Foliages crop diseases, Plant Pathology Research Institute, Giza, Egypt.

Antagonists:

Antagonists were isolated from rhizosphere soil samples of healthy maize plants using the soil dilution plate method (Johnson and Curl, 1972). Healthy plants were selected, pulled up and carried to laboratory. The adhering soil was removed from the roots, collected, air dried and mixed. Ten grams mixed with 90 ml sterilized distilled water in conical flasks (250 ml). After thoroughly shaking for 10 min., dilution series was prepared. The later dilutions were used to inoculate plates containing PDA and nutrient agar media. 100 µl of soil suspension were spread on plate surfaces using sterilized Drigalsky glass triangle. Plates were incubated at 28-30 °C for 3 days. The isolates were screened for their *in vitro* antagonism to *C. maydis*, the causal agent of late wilt disease of maize. Of these, one fungal (T8) and two bacterial isolates (B2 & B5) were found to be strongly antagonistic to the pathogen.

Fungal isolate was identified using the key of imperfect fungi according to Barnett and Barry (1972) and by Rifai (1969). Antagonistic activity of the fungal isolate against *C. maydis* was tested *in vitro* using linear growth technique. A disk bearing mycelium of the pathogen was placed in the center of plate containing PDA medium supplemented by yeast extract and at periphery, similar disk of potential antagonist was placed. Antagonistic effect was carried out as the scale described by Bell *et al.* (1982) consists of five classes. On the other hand, the bacterial isolates were identified according to Parry *et al.* (1983) and Bergy's manual of systemic bacteriology (1984). Additionally, three antagonistic strains of Pseudomonads (*Pseudomonas fluorescens* and two strains of *Pseudomonas cepacia*) were kindly obtained from the stock culture of Agric. Res. Center, Giza, Egypt. For simplicity, the antagonistic Pseudomonads strains were coded as P6, P7 and P11, respectively and that re-cultivated in king's B medium until application. As well as, commercial Pseudomonad bio-product called P-Suraksha (a PGPR with an ISO 9001, New Delhi) which coded as P9 was also selected for comparison in this study.

To determine efficiency of all bacterial antagonists, relative power of antibiosis (RPA) was estimated for each antagonist. PDA

medium was poured into 9 cm Petri dishes with 15 ml/dish. After solidification, plates were inoculated in the center with discs (5 mm in diameter each) obtained from three days old cultures of *C. maydis*. Plates were simultaneously inoculated at the periphery by standered amounts of the bacterial growth. Plates free from bacterial antagonists were acted as control. Experiments were designed in three replicates. Plates were incubated at 20 °C until full growth of control treatment. Diameter of inhibition zone surrounding each antagonist was recorded and RPA of each bioagent was estimated according to the formula described by Ibrahim *et al.* (1987):

$$RPA = Z / C$$

Where: Z = Diameter of inhibition zone.

C = Diameter of spotted antagonistic isolate.

The effective antagonists were re-cultured in nutrient broth for bacteria and potato dextrose broth for fungus isolate T8 up to 10 days in shaking incubator. Bacterial cultures were adjusted to 10^8 cell ml^{-1} and the commercial Pseudomonad was concentrated 10 % solution.

Evaluation of antagonism under field conditions:

Antagonists, exhibiting broad spectra of antagonism were used to control the late wilt disease under infested field conditions. Two field experiments were conducted during 2007 and 2008 growing seasons at the disease nursery of the experimental farm of Sakha Agricultural Research Station. Soil infestation was carried out one week before planting. The previously prepared pathogenic inoculums were added and well mixed thoroughly with the surface soil layer of the disease nursery in plots of 9.6 m^2 in area as described by El-Shafey *et al.* (1988) and watered before planting. Each plot contains two rows with 6 m. long and 80 cm. apart. After one week from soil infestation, grains of susceptible maize cultivar "Balady" were immersed in suspensions of the selected fungal and bacterial antagonists for overnight before sowing. Grains soaked in sterilized distilled water were acted as control. Sixty grains were planted for each row in the disease nursery. Three replicates (plots) were used in these experiments. After three weeks from planting, germination rates were recorded and pre-emergence damping off was resulted as follows:

$$\% \text{ pre-emergence damping-off} = \frac{\text{No. of non emerged seeds}}{\text{No. of sown seeds}} \times 100$$

After that, plants were thinned to one plant per hill and 25 cm between hills. Degrees of disease incidences of the survival plants were recorded, as percentage of infected plants, 35 days after silking (El-Shafey, *et al.*, 1988) as follow:

$$\text{Disease incidence (DI \%)} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

Disease incidcnce data were reused to calculate percentages of disease reduction of each treatment based on this formula:

$$\text{Reduction \%} = \frac{\text{DI \% of control} - \text{DI \% of treatment}}{\text{DI \% of control}} \times 100$$

On the other hand, some growth and yield parameters such as perimeter and dry matter of the stalks, weight of 1000 grains and weight of the cob were determined. Efficacy of each treatment on the grain yield productivity in comparison with control plants were calculated as described by El-Assiuty *et al.* (1986) as:

$$\text{Efficacy \%} = \frac{\text{Yield of treatment} - \text{Yield of control}}{\text{Yield of control}} \times 100$$

Statistical analysis:

Data were statistically tested for analysis of variance (ANOVA) using Irristat version 3/93. A complete randomize design was applied in this study. Least significant differences (LSD) and Duncan's multiple range tests were used for comparing means (Duncan, 1955).

RESULTS AND DISCUSSION

1. *In vitro* studies:

A laboratory study was performed to examine the antagonistic activities of some microorganisms against the pathogenic fungus *Cephalosporium maydis* the causal of late-wilt of maize plants.

Screening and identification of antagonistic microorganisms:

Isolation of microorganisms originated from rhizosphere-soil samples of healthy maize plants resulted in isolation of various microbial isolates comprising bacteria and fungi. The *in vitro*

screening of these isolates exhibited antagonistic action on plates against the causal pathogen. Only one fungal isolate and two bacterial isolates were conducted to suppress growth of *C. maydis* as biocontrol agents. Fungal isolate, coded T8 was identified as *Trichoderma harzianum* according to Rifai (1969). To identify both bacterial isolates, their morphological characteristics and biochemical activities were recorded in **Table (1).**

Table (1) Morphological characteristics and biochemical activities of the antagonistic tested isolates coded B2 and B5.

Test	Isolates B2 and B5
Shape of cell	Rods
Sporulation, spore shape	+
Motility	Motile
Gram reaction	+
Anaerobic growth	-
Growth in 7% NaCl	+
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin hydrolysis	+
Catalase reaction	+

\+ Positive - Negative

Results indicate that both isolates B2 and B5 were identified as *Bacillus subtilis*. Unfortunately, B2 was damaged during the *in vitro* tests, therefore it was excluded. To investigate their antagonistic activities against *C. maydis*, relative power of antibiosis (RPA) of all selected bacterial antagonists were estimated under laboratory conditions. Data which are presented in **Table (2)** indicate that *B. subtilis* (isolate B5) proved to be the most effective antagonist with RPA-value which reached 2.25, followed by 1.49 for the strain *P. fluorescens* (P6). RPA-values obtained by the commercial bioproduct (P9) and *P. cepacia* (P7) were 1.42 and 1.41, respectively. Due to lower RPA-value (1.21), *P. cepacia* (P11) was also excluded from the *in vivo* trials.

The antagonistic activities of *Bacillus spp.* and *Pseudomonas spp.* were recognized by many investigators. Ferreira *et al.* (1991) reported that several *Bacillus spp.* produce at least 66 different

antibiotic compounds against bacteria and fungi. Many biocontrol agents of *Pseudomonas spp.* produce extracellular secondary metabolities such as 2,4- diacetylphloroglucinol, pyoluteorin, phenazine pyrolnitrin and pyoverdine that inhibit the growth of some soil-borne fungal pathogens (Mavrodi *et al.* 2001 and Kraus and Loper, 1992).

Table (2) Relative Power of Antibiosis (RPA) of different bacterial antagonists *in vitro* against *C. maydis*.

Bacterial antagonists	RPA
Bacillus subtilis (B5)	2.25 b
Pseudomonas fluorescens (P6)	1.49 a
Pseudomonas cepacia (P7)	1.41 a
Pseudomonas cepacia (P11)	1.21 a
Bioproduct P-Suraksha (P9)	1.42 a
LSD 5%	0.397

Regarding to the antagonistic fungal isolate T8, it was identified as *Trichoderma harzianum*. Antagonistic efficiency of the fungal isolate was estimated according to the scale of Bell *et al.* (1982). Data show that the antagonist was scaled as the second level, that the antagonist over grow and covered at least two third of the medium surface, while the pathogen was limited at the third of the medium surface. El-Kazzaz *et al.* (2002) found that the antibiotic gliotoxin was found to be produced by *T. harzianum*. This antifungal substance (gliotoxin) probably affects the respiratory sites of the host fungus and causes reduction in the mycelial growth (Hadler *et al.*, 1973). Such antibiotic proved to be an inhibitor effect against a wide spectrum of the pathogenic fungi. Dennis and Webster (1971) found also that *Trichoderma spp.* were able to produce diffusible inhibitory substances such as acetaldehyde or other acidic volatiles.

II. Field trials

Effectiveness of the selected biocontrol agents (B5, P6, P7 and T8) in addition to the commercial bio-product (P-Suraksha, coded as P9) were tested, on the late wilt disease expressions. Growth and yield parameters of maize plants were also included. These experiments were carried out during two successive seasons

of 2007 and 2008 in soil infested by C. maydis (disease nursery) under field conditions.

Biological control of late wilt disease:

Influence of the tested antagonists on the disease expressions under field conditions are represented by pre-emergence damping-off and disease incidence in this study. Inoculation of maize grains with the antagonists increased emergence of maize seedlings grown in soil infested with *C. maydis*. This was reflected on the reduction of pre-emergence damping-off as plotted in **Fig. (1).**

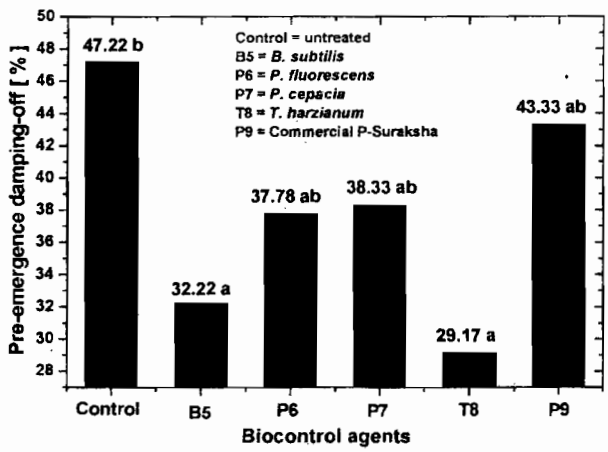

Fig. (1). Pre-emergence damping-off caused by using different biocontrol agents inoculated maize grains.

T8 and B5 isolates were the most effective biocontrol agents, which decreased percentages of pre-emergence damping-off 29.17 and 32.22 %, respectively. Pre-emergence damping-off was also reduced to 37.78, 38.33 and 43.33 % by using P6, P7 and P9, respectively in comparison with 49.22 % for control. Therefore, the efficiency of the antifungal biocontrol agents to protect maize grains against damping-off has been clearly proved throughout the present study. Similar findings against *C. maydis* were stated by El-Mehalawy *et al.* (2004) using actinomycetes and yeast fungi.

Due to seed inoculation of the selected antagonists, incidence of the late wilt disease of maize plants were significantly ($p<0.05$) reduced in comparison to control during both seasons (**Table 3**). Data show that, T8, P7 and B5 were the most effective agents reduced late wilt disease of 58.93, 58.72 and 57.26 % during 2007 season, respectively. Marked reductions were also achieved by the commercial bio-product P9 (58.38 %) and B5 (56.49 %) during 2008 season. The antagonistic effect of the *P. cepacia* (P7) at the second season gave the least disease reduction (0.59 %), indicating its lowest antagonistic activity.

Table (3). Effect of the tested biocontrol agents on rating of maize late wilt disease incidence and disease reduction during 2007 and 2008 seasons.

Treatments	First season (2007)		Second season (2008)	
	Disease incidence %	Disease reduction %	Disease incidence %	Disease reduction %
Control	4.80 c	0.00	9.01 c	0.00
B. subtilis (B5)	2.05 a	57.26	3.92 a	56.49
P. fluorescens (P6)	2.68 b	44.13	4.10 ab	54.50
P. cepacia (P7)	1.98 a	58.72	8.96 c	0.59
T. harzianum (T8)	1.97 a	58.93	4.31 b	52.20
Bioproduct (P9)	2.45 ab	48.93	3.75 a	58.38
L.S.D. 5%	0.52		0.37	

The obtained results indicate that the antagonistic agents have great potential against *C. maydis*. Data obtained are in agreement with those obtained by El-Shafey *et al.* (1985) and Ishac *et al.* (1985) who used *B. subtilis* for controlling late wilt disease of maize. The antagonistic activity of *T. harzianum* against *C. acremonium* the causal of late wilt disease of sorghum was also conducted by El-Assiuty *et al.* (1986). El-Assiuty *et al.* (1991) reported the same potential of *P. fluorescens* against *C. maydis*. For suppression of the soil borne plant pathogens, production of hydrolytic enzymes such protease, lipase, pectinase, amylase by some plant growth rhizobacteria (PGPR) inhibited the growth of *Fusarium culmorum* and *F. oxysporum* (Egamberdiyeva, 2007). Nielson and Sorensen (1999) demonstrated that *P. fluorescens* was antagonistic to *R. solani* and *Pythium ultimum*, and produced lytic enzymes.

The present data revealed that *B. subtilis* (B5) and *T. harzianum* (T8) were the most effective antagonists followed by the other antagonistic agents. Both antagonists which were found to be strongly effective to *C. maydis in vitro* were effective producers of antifungal metabolites. Since the cell wall of *C. maydis* consists largely of chitin and ß-glucan (Bartnicki-Garcia and Lippman, 1982), *T. harzianum* excretes chitinase and ß-1, 3-glucanase responsible for destroying the hyphae of the fungal pathogen (Elad *et al.*, 1982) and penetration of the hyphae becomes more easy (Chet and Baker, 1981). On the other hand, the well known antagonistic effect of *B. subtilis* against most pathogenic fungi is due to produce at least of 66 different antibiotic compounds (Ferreira *et al.*, 1991). Therefore, the antagonistic isolates of *B. subtilis* and *T. harzianum* in the present study are capable to grow totally at the expense of *C. maydis* hyphae, indicating their potential for pathogen suppression where the antagonism takes place outside the limits of rhizosphere.

Plant growth and productivity:

Significant increase in the perimeter and dry matter of maize stalk was also obtained by application of the tested microorganisms previously acted as biocontrol agents during two seasons **(Table 4)**.

Table (4). Effect of the tested biocontrol agents on stalk perimeter of the survival maize plants during 2007 and 2008 seasons.

Treatments	Stalk properties			
	First season (2007)		Second season (2008)	
	Perimeter (cm)	Dry matter (%)	Perimeter (cm)	dry matter (%)
Control	7.50 a	35.56 a	5.75 a	21.54 a
B. subtilis (B5)	8.75 ab	44.85 c	6.75 ab	23.07 bc
P. fluorescens (P6)	8.13 a	55.13 f	7.38 b	28.40 d
P. cepacia (P7)	8.00 a	53.57 e	7.63 b	23.99 c
T. harzianum (T8)	9.38 b	49.68 d	7.25 b	46.82 e
Bioproduct (P9)	8.75 ab	41.14 b	6.88 b	22.64 ab
L.S.D. 5%	1.18	1.41	1.04	1.12

Data show also that the tested bioagents inoculants varied in their effects on both growth parameters in comparison with control plants. It reflects the role played by these agents to stimulate cell division and elongation as well as formation of dried tissues in the plants. It was explained by production of plant growth regulators due to the associated microorganisms. Plant growth regulators are known to stimulate both rapid (increase in cell elongation) and long term responses in plants (Cleland, 1971). Maize plant growth measurements were increased significantly using rhizosphere inoculants (El-Mehalawy *et al.*, 2004). Regarding to productivity of maize grains, weight of cob and the1000 grains and their efficiency were recorded in **Table (5)** under experimental conditions. It shows significantly increase of grain yield in inoculated plants with the antagonists compared with non-inoculated control plants.

Table (5). Effect of the tested antagonists on the grain and cob yield of the survival maize plants during 2007 and 2008.

Treatments	1000 grain yield				cob yield	
	2007		2008			
	Weight (Kg)	Efficacy (%)	Weight (Kg)	Efficacy (%)	Weight (Kg)	Efficacy (%)
Control	0.32 ab	0.00	0.21 a	0.00	0.17 a	0.00
B. subtilis (B5)	0.44 c	37.50	0.32 b	52.38	0.29 b	70.59
P. fluorescens (P6)	0.34 ab	6.25	0.27 ab	28.57	0.27 b	58.82
P. cepacia (P7)	0.30 a	- 6.25	0.28 ab	33.33	0.27 b	58.82
T. harzianum (T8)	0.44 c	37.50	0.29 ab	38.10	0.30 b	76.47
Bioproduct (P9)	0.39 bc	21.88	0.28 ab	33.33	0.28 b	64.71
L.S.D. 5%	0.079		0.077		0.088	

A remarkable increase in grain productivity reached to 37.50 % was obtained for both bioagents *B. subtilis* B5 and *T. harzianum* T8 in comparison with control during the first season. These values became 52.38 and 38.10 %, respectively during 2008 season. It was reflected on the mean weight of each cob. The enhancement of the cob weight reached 70.59 % and 76.47 % due to seed inoculation with *B. subtilis* and *T. harzianum*, respectively compared with control. On the other hand, effects of the other bioagents varied on the yield productivity. So, efficiency of the tested microorganisms as biocontrol agents was also extended to stimulate growth and

productivity of maize plants as plant growth-promoters. Such promoters are playing a significant role in the biofertilization of crops (Cakmakci *et al.*, 2005). The stimulatory effect of these inoculants was extended to increase plant growth, speed up seed germination, improve seedling emergence, response to external stress factors, protect plants from diseases and root growth pattern (Lugtenberg *et al.*, 2002).

The stimulatory effect of different species of Bacillus, Pseudomonas and Mycobacterium as plant growth-promoting rhizobacteria (PGPR) for maize was investigated by Egamberdiyeva, (2007). He found increasing plant growth and grain maize productivity due to the stimulatory effect of these microbes. Therefore, the most acceptable mechanisms of these associative bioagents are production of phytohormones (Bothe *et al.*, 1992 and Kloepper *et al.*, 1980) as well as antagonism against soil borne plant pathogens (Höflich *et al.*, 1994). It might be also suggested that inoculation of maize grains in the tested control agents was effective technique to induce systemic acquired resistance (SAR) against *C. maydis*. SAR is a suggested phenomenon by Hatcher (1995) to describe the systemic induction of resistance against a broad spectrum of phytopathogens. This study indicates that *B. subtilis* isolate B5 and *T. harzianum* isolate T8 proved to be the most effective antagonism. This is in agreement with the findings of Kloepper *et al.* (2004) and El-Assiuty *et al.* (1986), respectively. Therefore, the successfully biocontrol actions given by both microbes could be candidates for the commercial productions of such bioagents to control late wilt disease on maize.

From the results, it can be concluded that *C. maydis* was suppressed strongly *in vitro* by the tested antagonists. Also, pre-emergence damping-off and disease incidence were reduced strongly by the application of the tested antagonists at the time of the planting as seed treatment. Moreover, growth measurements and productivity of maize grains were increased by inoculation with these antagonistic microorganisms. But it is still not clear, what is the future of the microflora established rhizosphere which inoculated with biocontrol agents? So, further study is necessary to find a suitable formulation to optimize antagonism conditions to

obtain more effective biological control with guarantee safe conditions of the rhizosphere microorganisms.

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الملخـــص العربـــــى

المكافحة البيولوجية لمرض الذبول المتأخر فى الذرة الشامية المتسبب عن *Cephalosporium maydis*

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درس النشـاط التضـادى لعدد من الكائنـات الحيـة الدقيقـة فـى مواجهـة فطر *Cephalosporium maydis* ، المسبب المرضى المحمول بالتـربة والذى يسبب مرض الذبـول المتأخر فى الذرة الشـامية . وقد أثبـت الحصر التضـادى معمليـا أن العزلـة البكتـيرية التـى تحمل الكود B5 ، والتـى تم تعريفهـا علـى أنهـا *Bacillus subtilis* ، كانـت أكثـر العوامـل الحـيوية المضادة تحقـيقا لأعلى قدرة نسـبية علـى التضـاد RPA بلغت ٢,٢٥ يليها ١,٤٩ % بواسطة البكتـيريا P6 *Pseudomonas fluorescens* . كذلك فـان النمو الخطـى للمسبب المرضـى المذكور قد ثبـط بقـوة فـى المعمل بواسـطة العزلـة الفطريـة صـاحبة الكـود T8 وعرفـت علـى أنهـا *Trichoderma harzianum* . وتحـت ظـروف حقـل العدوى ، أثبتـت النتائج أنخفاض واضح فى نسبة الذبول قبل الأنبثـاق وصل الـى ٢٩,١٧ و ٣٢,٢٢ % للحبوب الملقحة بكل من T8 و B5 على التوالى ، مقارنـة بـ ٤٧,٢٢ % للكنتـرول. ولقـد أمتـدت الكفـاءة التضـادية للعوامـل الحيويـة المختـارة الـى خفـض نسـبة حـدوث مـرض الذبـول المتـأخر فـى النباتـات المتبقيـة فى حقـل العدوى . وأوضـحت النتـائج أن العزلات T8 و P7 وB5 كانـت الأفضـل فـى خفـض نسـبة حـدوث المـرض بـ٥٨,٩٣ ، ٥٨,٧٢ و٥٧,٢٦ % على التـوالى ، مقارنة بـ ٥٨,٣٨ % للمنتج الحيوى التجارى صـاحب الكـود P9 فـى كـلا الموسمين . وكـذلك تحسـنت بوضـوح قياسـات النمو وأنتاجية النباتات المعاملـة. ووفقـا لذلك ، فقد أزداد محصـول الحبوب معنويا الى ٥٢,٣٨ و ٣٨,١٠ % بسبب تلقيح الحبوب بـالعزلات البكتـيرية والفطرية (B5 و T8) على التوالى مقارنة بالكنترول .