Effects of some aqueous plant extracts and sulphur compounds on the control of maize late wilt disease caused by *Cephalosporium maydis*

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ABSTRACT

The antifungal activities of aqueous extracts of barbados aloe fleshy leaves, onion bulbs, garlic cloves, jimsonweed and peppermint leaves, as well as sulphur substances of Zn SO_4 and Cu SO_4 against *Cephalosporium maydis* causing late wilt disease of maize were investigated. Results showed that, onion and garlic aqueous extracts were the most effective against all tested properties of the causal pathogen, i.e. against fungal dry weight *in vitro*, for controlling of disease incidence and losses in yield under field conditions. Results indicated also that all tested extracts and sulphur substances were effective against the pathogenic fungus under laboratory conditions with different inhibitory effects.

The aqueous extracts of barbados aloe, onion, garlic and jimsonweed were the most effective against dry weight of the fungus, with lower IC_{50} values of 12.43, 15.02, 15.35 and 16.25%, respectively, followed by the two sulphur substances and then peppermint leaves extract. Field experiments showed that the most effective reduction for late wilt disease incidence was obtained by Cu SO_4 and garlic cloves extract, followed by Zn SO_4 and onion bulb aqueous extract. On the other hand, significant increases in crop production due to peppermint, barbados aloe leaves extracts and Zn SO_4, in addition to onion and garlic aqueous extracts were obtained, while the other tested control agents had slight or no effect in comparison with control in this respect.

Keywords: *C. maydis*, maize, Antifungal activity, plant extracts.

INTRODUCTION

Maize (*Zea mays* L.) is known to be one of the most important grain crops in Egypt. It is the third crop after wheat and rice crops

for human consumption, as well as for animal feeding. The cultivated area of maize in Egypt was gradually increased to reach 1.927.000 feddans yielding 25.3 ardab/feddan in 2005 season (National campaign report of maize yield improvement, 2005).

Late wilt of maize, caused by *Cephalosporium maydis*, is economically the most important fungal disease of maize in Egypt (Samra *et al.* 1971). *Acremonium maydis* is the synonym preferred scientific name of the late wilt pathogenic fungus on maize, but *Cephalosporium maydis* is the common scientific name in Egypt. *C. maydis* is a soilborne vascular wilt pathogen that penetrates root tissue and colonizes the xylem (Sabet *et al.* 1970). Less commonly, this pathogen also can be transmitted through the seeds (El-Shafey *et al.* 1988), and may occasionally cause seed rot or pre-emergence damping-off under heavy inoculum potential (Samra *et al.* 1966). 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 became infected, and grain yield losses may approach 37% of wilting plants, and about 15% of the total yield in Egypt (Samra *et al.* 1971).

A considerable progress has been achieved to control the late wilt disease by breeding for resistant varieties (Labib *et al.* 1975). On the other hand, the chemical control by systemic fungicides has, so far, been unsuccessful (El-Assuity, 1976). Therefore, the need for increasing maize production and controlling such disease by using cheaper and environmentally safer alternative technique instead to the traditional synthetic fungicides, are the world wide aims. However, plant products received a special attention concerning a strategy for developing environmentally safe methods against plant diseases. Recently, there is a world wide interest in identifying plants having compounds possessing antifungal properties with fungicidal formulations (Al-Abed *et al.* 1993).

The main objective of this study is to confirm the efficacy of certain aqueous plant extracts and some sulphur substances for controlling maize late wilt disease under field conditions. It is also aimed to evaluate the antifungal activities of all selected aqueous

plant extracts and some sulphur compounds against growth of the late wilt pathogenic fungus on maize (*Cephalosporium maydis*) under laboratory conditions.

MATERIALS AND METHODS

1. Preparation of tested control agents:

1. 1. Preparation of aqueous plant extracts:

In this study, five plant species belonging to four families were used for controlling late wilt disease on maize caused by *Cephalosporium maydis* (Table 1). Fresh materials of the selected plants were washed with sterile water, cut into small pieces, air dried and weighted according to the concentrations needed. Weighted samples with some distilled water were homogenized using a blender. To the needed volume, the homogenized solutions were diluted by distilled water and then filtered using filter paper.

1. 2. Preparation of sulphur compounds:

Two sulphur compounds i.e. Zn SO_4 and Cu SO_4 were also used to investigate their antifungal activities against C. *maydis* in this study. The used sulphur chemicals were weighted, dissolved in distilled sterilized water and filtered using filter paper.

Table (1): Plants used for controlling late wilt disease on maize

No	English name	Scientific name	Family name	Used parts
1	Onion	*Allium cepa* L.	Liliaceae	bulbs
2	Garlic	*Allium sativum* L.	Liliaceae	cloves
3	Peppermint	*Mentha piperita* L.	Lamiaceae	leaves
4	Barbados aloe	*Aloe vera* (L.) Burm. f.	Aloeaceae	leaves
5	Jimsonweed	*Datura stramonium* L.	Solanaceae	leaves

2. Laboratory (*in vitro*) studies:

2. 1. Isolation and identification of the causal pathogen:

Samples of the third to fifth internodes of the diseased plants were washed by running water, divided into small pieces, surface sterilized using 0.5% sodium hypochlorite solution for 3 minutes, washed several times in sterilized distilled water and then blotted between two sterilized filter papers. Surface sterilized internal plant

tissues were transferred into Petri plates containing Potato Dextrose Agar medium (PDA), mixed with 2 g yeast extract for liter. Plates were incubated at 28 °C ± 2 C° for 3-7 days and fungal growth was daily observed. Using a hyphal tip technique, mycelium was purified, transferred to PDA slants and kept in a refrigerator at 4 °C as stock cultures for maintaining the cultural growth.

According to Barnett (1960) and Singh (1982), the isolated fungus of *Cephalosporium maydis* was identified at Agric. Botany Dept., Faculty of Agriculture, Kafr El-Sheikh and at Dept. of Maize, Sugar and Foliages crop diseases, Plant Pathology Research Institute, Giza, Egypt, in which preparation of pathogen inoculums for diseased nursery was also done.

2. 2. Effect of the tested aqueous plant extracts and sulphur compounds on dry weight of *C. maydis*:

This experiment was done using three concentrations i.e. 5, 15 and 25 % of all tested agents, in addition to untreated control. Aliquots of 20 ml liquid PD medium mixed with 2 g L^{-1} yeast extract were placed in 100 ml Erlenmeyer flasks, and then inoculated with discs, 5 mm in diameter, of the fungus and then incubated at 28 °C ± 2 C°. Three replicates were used for each concentration. After two weeks, the cultures were filtered using weighted filter paper. The mycelial mats were collected, washed by distilled sterilized water and dried in an oven at 60 °C until a constant weight, then the dry weight was determined. Compared with the data obtained from control, percentage of inhibition (I %) was calculated for each concentration according to the formula suggested by Topps and Wain (1957) as follows:

$$I\% = \frac{A - B}{A} \times 100$$

Where: I % = Percent of inhibition
A = Mean fungal dry weight of the control.
B = Mean fungal dry weight of the given treatment.

Linear regression equations of the certain treatment concentrations against percentage of fungal dry weight inhibition were fitted, so that slope of the fitting data and IC_{50} values could be interplotted (Finney, 1971).

3- Field experiments:

Field experiments were carried out in Sakha Agricultural Research Station during 2004 and 2005 growing seasons. Two concentrations, 15 and 25 % of all tested agents, in addition to untreated control, were used. Soil was infested by adding the preparing pathogenic inoculums in plots of 9.6 m^2 in area. Each plot contains two rows with 6 m. long and 80 cm. apart. Three replicates (plots) were used in these experiments.

3. 1. Treatment and sowing of maize grains:

Grains of highly susceptible maize cultivar "Balady" were soaked in all tested agents for overnight before sowing. Grains soaked in sterilized distilled water were acted as control. Sixty grains were used for each row. After one week from soil infestation, soaked grains were planted in the disease nursery. So, plants were thinned to one plant per hill and 25 cm between hills.

3. 2. Virulence of certain pathogen:

The virulence of *C. maydis* on maize was carried out under field conditions using the soil infestation technique (Sabet *et al.* 1966). Disease incidences 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 incidence data were reused to calculate percentages of disease reduction of each concentration as follow:

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

Losses of yield were calculated by Calpouzos *et al.* (1976) as:

$$\text{Loss \%} = \frac{yh - yd}{yh} \times 100$$

Where: yh = yield of healthy plants, yd = yield of infected plants.

A complete randomize design was used in this study. Least significant differences (LSD) and Duncan's multiple range tests were applied for comparing means (Duncan, 1955).

RESULTS AND DISCUSSION

For their importance in food preparation and in traditional public medicine, aqueous extracts of five plant species of *Allium cepa*, *A. sativum*, *Mentha piperita*, *Aloe vera* and *Datura stramonium* were selected. Also, two sulphur solutions of Zn SO_4 and Cu SO_4, used in preparing some synthetic fungicides, were also used in this study. Antifungal activities of these treatments against late wilt pathogen (*C. maydis*) of maize under laboratory and field conditions were investigated.

1. *In vitro* studies:

A laboratory study was performed to examine sensitivity of *C. maydis* to the tested aqueous plant extracts and sulphur compounds. To evaluate their antifungal activities, IC_{50} of each treatment, using three concentrations, in addition to control, were obtained.

1. 1. Effect of different concentrations of the tested plant extracts and sulphur compounds on dry weight of *C. maydis*:

Table (2) showed increasing of the inhibitory effects by increasing concentrations of the tested five plant extracts and two sulphur compounds on dry weight of *Cephalosporium maydis* under laboratory conditions. At concentrations of 15 and 25 %, highly significantly inhibition of fungal dry weight of all tested control agents was observed. The lowest amount of dry weight was 6.70 mg obtained when the fungus was grown on 25 % onion bulb extract. It was ranked, therefore, as the first one with inhibition percentage of 93.69 %, followed by 86.93 % obtained by barbados aloe extract. Garlic cloves were also effective extract in providing reduction in fungal dry weight of 75.49 % at concentration of 15 %.

The results indicate that, there are two types of all tested control agents in this study. The first type is the five aqueous plant extracts, while the second refers to the two sulphur compounds. To compare response of *C. maydis* to these types at different concentrations, general means for all inhibitory percentages of fungal dry weight obtained by each type for each concentration were grouped, separately recalculated and plotted in Fig. (1). It shows highly response of the fungal dry weight via increasing of the plant extract concentrations comparing to the sulphur substances. The general

inhibitory effects calculated for the first type were strongly increased from 18.04 to 53.64 and then to 67.70% for concentrations of 5, 15 and 25%, respectively. While, the inhibitory effects caused by sulphur substances were increased from 15.60 to 49.44% at concentrations of 5% and 15%, respectively, while, less fungal sensitivity at concentrations more than 15% was observed. It means that, increasing of sulphur substances more than 15% is not economic comparing to the aqueous plant extracts. It means that, increasing concentrations of all tested plant extracts was cheaper and safer technique in comparing to sulphur compounds *in vitro*.

Table (2): Effect of different concentrations of five aqueous plant extracts and two sulphur compounds on dry weight of *Cephalosporium maydis*.

Treatments	Conc %	Mean mg	Rank	Inhibition		IC_{50}	Slope
				mg	%		
Barbados aloe	5	79.42 f-i	14	-26.78 *	25.22	12.43	3.52
	15	29.70 ab	4	-76.50 **	72.03		
	25	10.70 a	2	-95.50 **	86.93		
Onion	5	91.95 ghi	20	-14.25 ns	13.41	15.02	3.65
	15	66.03 d-g	12	-39.41 **	37.82		
	25	6.70 a	1	-99.50 **	93.69		
Garlic	5	83.25 f-i	17	-22.95 ns	21.61	15.35	2.63
	15	26.03 ab	3	-80.17 **	75.49		
	25	42.70 bcd	6	-63.50 **	59.79		
Jimsonweed	5	82.98 f-i	16	-23.22 ns	21.86	16.25	2.69
	15	44.70 b-e	7	-61.50 **	57.91		
	25	35.70 abc	5	-77.50 **	70.50		
Peppermint	5	97.62 hi	21	-8.58 ns	8.08	36.56	1.33
	15	79.70 f-i	15	-26.50 *	24.95		
	25	71.70 e-h	13	-34.50 **	32.49		
Zn SO_4	5	90.86 ghi	19	-15.34 ns	14.45	20.23	2.33
	15	58.70 c-f	10	-47.50 **	44.73		
	25	45.70 b-e	8	-60.50 **	56.97		
Cu SO_4	5	88.41 ghi	18	-17.79 ns	16.75	23.53	1.79
	15	48.70 b-e	9	-57.50 **	54.14		
	25	62.70 d-g	11	-43.50 **	40.96		
Control	0.0	106.20 i	22	-	0.0		
L.S.D. 5%				25.075			

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

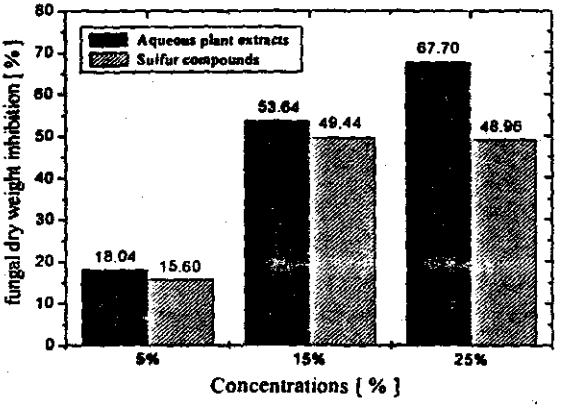

Fig. (1): A comparison between the mean inhibitory effects of fungal dry weight recalculated for five plant extracts and two sulphur compounds at different tested concentrations.

1. 2. Evaluation of antifungal activities:

To evaluate performance of the antifungal activities, IC_{50} of each treatment was calculated. Linear regressions of fungal dry weight inhibition versus the tested concentrations were plotted (not shown) to obtain IC_{50}. The obtained IC_{50} and slopes values for each treatment are also represented in Table (2). It is obvious that, the IC_{50} of *Aloe vera*, *Allium cepa*, *A. sativum* and *Datura stramonium* aqueous extracts were the lowest values obtained of 12.43, 15.02, 15.35 and 16.25%, respectively compared to the others. Therefore, these plants are considered the most toxic extracts towards mycelial growth of *C. maydis*. These results are in agreement with Abd El-Ghani and Heweidy (1997), who found that the growth of *Cephalosporium maydis*, *Fusarium* sp., *Macrophomina phaseolina*, *Helminthosporium turicum* and *Ustilago maydis* were greatly reduced in the medium containing garlic cloves juice than in the control. Against *Rhizoctonia solani* and *Sclerotium* sp., garlic extract was also effective (Carcia and Lawas, 1990). These results were also in accordance with the results of Atta-Alla *et al.* (2003), who stated that the extracts of onion and garlic individually inhibited growth of *Sclerotinia sclerotiorum* (Lib.) de Bary. The fungal growth was completely suppressed at concentration of 15%

under different incubation periods for onion or garlic extracts. Dubey and Dwivedi (1991), reported also that onion and garlic extracts had fungitoxic effect against *Macrophomina phaseolina in vitro*, and against the pathogenic fungus caused downy mildew disease of maize (El-Moghazy, 2003). Singh *et al.* (1979) found that growth of *Fusarium oxysporium* f. sp. *ciceri* and *Sclerotinia sclerotiorum* in liquid media incorporated with 5000 to 7000 ppm of garlic cloves juice was greatly reduced. The effectiveness of *Aloe vera* against the fungal dry weight was also obtained versus growth of some yeast (Zommara and Rashed, 2005). For *Datura*, Malhotra and Rai (1990) stated that the water extract of *Datura alba* decreased soybean mycoflora.

Dry weight of *C. maydis* was also inhibited via Zn SO_4 and Cu SO_4 compounds with IC_{50} values of 20.23 and 23.53% respectively. While, the highest IC_{50} value was 36.56% obtained by peppermint extract. It means that the last three treatments were less effective against growth of late wilting disease pathogen in comparison with the four previous agents. These results are in agreement with several reports. Fahmy and Mohamed (1984), effectively used some sulphur compounds against some plant pathogens. Yegen *et al.* (1992) indicated that aqueous extract and essential oils of *Mentha spicata* was effective versus mycelial growth of *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phytophthora capsici*. However, role of higher plants as a source of fungitoxic agents and their importance in controlling different plant pathogens are many (Al-Abed *et al.* 1993 and Baharat *et al.* 1997).

2. Field studies:

For controlling late wilting disease and increasing maize production, two field experiments were carried out. Effectiveness of the selected control agents on the rate of disease incidence and on maize yielding was investigated. Regarding performance of the tested control agents, a basic question is therefore: Will the antifungal activities realized *in vitro* could be applied under field conditions? So, the *in vitro* results were used. With exception of *Mentha piperita* extract, the *in vitro* data indicated that IC_{50} values are ranged from 12.43% to 23.53%. Moreover, the inhibitory effects of all control agents against fungal dry weigh at 5%

concentrations were not significant. Therefore, 5% was used out, while concentrations of 15 and 25%, in addition to control, were applied for field experiments.

2. 1. Effect of the tested aqueous plant extracts and sulphur compounds on level of late wilt disease incidence on maize:

Data presented in Table (3) show decreasing in the disease incidence with increasing in the treatment concentrations during both seasons. At concentration of 25%, Zn SO_4, Cu SO_4 and garlic extract were the most effective. They ranked as the first three, which reduced late wilt disease incidence by 64.75, 63.89 and 63.28% during 2004 season and by 60.63, 62.43 and 56.13% during 2005 season, respectively. These are in agreement with Abd El-Ghani and Heweidy (1997), who found that 25% was the best concentration of garlic juice reduced late wilt disease incidence.

Table (3): Effect of the tested plant extracts and sulphur compounds on rating of maize late wilt disease incidence and disease reduction during two seasons.

Treatments	Con %	First season (2004)			Second season (2005)		
		Rank	Disease incidence %	Disease reduction %	Rank	Disease incidence %	Disease reduction %
Barbados aloe	15	15	12.33 f	-	14	12.70 d	-
	25	12	10.93 ef	6.02	11	10.77 cd	2.97
Onion	15	6	8.00 bc	30.61	6	8.57 a-d	22.79
	25	4	7.60 b	34.39	5	8.25 a-d	25.68
Garlic	15	8	8.77 bcd	24.59	4	7.77 abc	30.00
	25	3	4.20 a	63.28	3	4.87 ab	56.13
Jimsonweed	15	14	12.20 f	-	13	12.63 d	-
	25	7	8.50 bc	26.91	7	8.63 a-d	22.25
peppermint	15	10	10.47 def	9.97	10	10.17 cd	8.38
	25	9	9.70 cde	16.60	8	8.90 bcd	19.82
Zn SO_4	15	11	10.70 ef	8.00	9	9.73 cd	12.34
	25	1	4.10 a	64.75	2	4.37 ab	60.63
Cu SO_4	15	5	8.00 bc	31.21	6	8.57 a-d	22.79
	25	2	4.20 a	63.89	1	4.17 a	62.43
Control	0.0	13	11.63 ef	-	12	11.10 cd	-
L.S.D. 5%			1.76			4.01	

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

Onion bulb extract was also reduced late wilt disease at concentrations of 25% by 34.39 and 25.68 %, and by 30.61 and 22.79% at concentrations of 15% during 2004 and 2005 seasons, respectively. Sulphur compounds were reported by Coley-Smith and King (1969) to be the major constitutes of garlic and onion exudates as inhibitors. On the other hand, no disease reduction was obsearved when maize grains were soaked in 15% of either *Aloe vera* or *Datura stramonium* extracts during both seasons. Our results are in contrast to the known effect of peppermint extract against some fungal disease incidence. Yegen *et al.* (1992) stated that the aqueous extract of *Mentha spicata* exhibited fungitoxicity towards *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phytophthora capsici*. Peppermint extract reduced seed infection by *Drechslera oryzae* and treated seeds had higher viability (Alice and Rao, 1986).

2. 2. Effect of the tested aqueous plant extracts and sulphur compounds on the yield of healthy and infected maize plants:

Table (4) shows also that 25 % of Zn SO_4 and peppermint were the best concentration to gain high maize yield during two studied seasons in comparison with control. The lowest loss percentages of the yield were 10.37 and 12.90% for Zn SO_4 as well as 13.36 and 11.98% for peppermint during 2004 and 2005, respectively. Loss of maize production was also reduced by onion, barbados aloe and garlic extracts. Conversely, not significant loss of yield due to jimsonweed was obtained. Whereas, higher loss percentage of maize production was obtained as a result of Cu SO_4 compared with control during both growing seasons.

The present study showed that the inhibitory effects of all tested control agents on the fungal dry weight, disease incidence and losses in maize production were increased by increasing the treatment concentrations. Some plant extracts show antifungal activities against a wide range of pathogenic fungi (Papadopoulou *et al.* 1999). To evaluate will the antifungal activities realized *in vitro* could be applied under field conditions, general averages of all data obtained from different concentrations *in vitro* and in field experiments were combined and then plotted together in Fig. (2).

Table (4): Effect of the tested control agents on the yield of healthy and late wilted maize plants during two seasons.

Treatments	Con %	Yield (g / 1000 grains)					
		First season (2004)			Second season (2005)		
		Healthy	Infected	Loss %	Healthy	Infected	Loss %
Barbados aloe	15	388.7 d	264.2 d	32.03	392.1 d	262.9 b	32.95
	25	387.7 d	308.8 j	20.35	394.6 e	304.1bcd	22.93
Onion	15	371.4 b	290.8 h	21.70	350.7 a	296.8 f	15.37
	25	425.8 f	213.0 a	49.98	420.2 g	208.9 a	50.29
Garlic	15	459.1 h	260.5 c	43.26	451.5 j	270.3 bc	40.13
	25	387.1 d	302.8 i	21.78	399.8 f	302.1bcd	24.44
Jimsonweed	15	425.0 f	266.2 e	37.36	425.0 h	259.1 b	39.04
	25	344.9 a	215.9 b	37.40	468.6 l	210.7 a	55.04
Peppermint	15	371.0 b	269.9 f	27.25	360.4 b	266.5 b	26.05
	25	410.2 e	355.4 l	13.36	400.1 f	352.1 e	11.98
$Zn SO_4$	15	461.9 i	283.5 g	38.62	442.3 l	282.5 bc	36.13
	25	374.0 c	335.2 k	10.37	389.2 c	339.0 de	12.90
$Cu SO_4$	15	451.6 g	263.4 d	41.67	456.1 k	264.7 b	41.96
	25	507.0 k	265.1 de	47.71	497.8 n	263.6 b	47.05
Control	0.0	471.6 j	285.2 g	39.53	478.5 m	277.9 bc	41.92
L.S.D. 5%		2.55	1.81		1.61	39.58	

Averages in a column followed by a different letter are significantly different at 0.05 level after a Duncan's multiple range test.

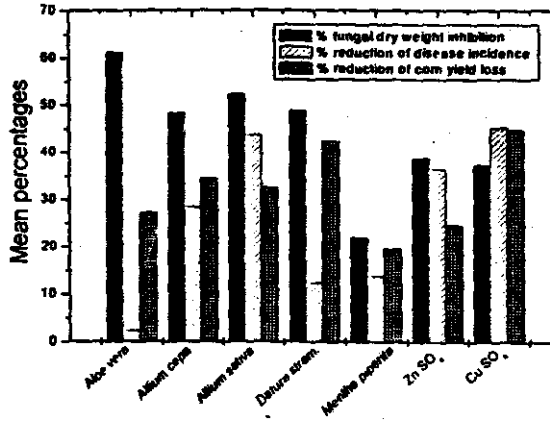

Fig. (2): General means percentages of the *in vitro* and field experimental data combined for all tested control agents.

It indicates that the most effective four plant extracts for reducing dry weight of *Cephalosporium maydis* were *Aloe vera, Allium sativum, Datura stramonium and Allium cepa*, where they inhibited the fungal dry weight by 61.14, 52.30, 48.72 and 48.31% respectively. While the most effective four control agents reducing disease incidence were Cu SO_4, *Allium sativum*, Zn SO_4 and *Allium cepa*, where they reduced percentage of late wilt disease incidence by 45.08, 43.51, 36.43 and 28.37%, respectively. *Mentha piperita* and Zn SO_4 followed by *Aloe vera*, *Allium sativa* and *A. cepa* were the superior five treatments reducing loss of yield by 19.67, 24.50, 27.07, 32.41 and 34.33 %, respectively.

From the previous results, *Allium sativum* cloves and *A. cepa* bulbs were the most effective aqueous extracts acted against all studied properties. Significant enhancement of crop production due to reduction of both disease incidence and fungal dry weight were together obtained using both extracts. Wilson *et al.* (1997) reported that, among 345 plant extracts, species of *Allium* were the most effective showing antifungal activity. *Allium* spp. may have an antifungal compounds play an important role in controlling several diseases (Kurucheve et al. 1997). The inhibitory effect of garlic or onion extracts might be attributed to the presence of some antifungal ingredients. For corn yielding, Zn SO_4 was one of the best treatments reduced loss percentage. Due to their probable toxic effect against both pathogen and the host, Cu SO_4 extract caused lack plants and fewer yields.

Although, moderate reduction of fungal dry weight and disease incidence was obtained, highly increase in crop production by *Mentha piperita* was obtained. It means that the fungal sensitivity and symptomes appearance were not affected, while the plant properties were changed to the better by using mint extract. So, indirect effect of *Mentha piperita* extract against late wilt disease was obtained, leading to increase of the maize production. Also, mint is considered as an activator to peroxidase enzyme which associated with induced resistance in plants against pathogens (Ye *et al.* 1990). It was in agreement with the findings of El-Shoraky (1998), who reported lower influence of mint extract against dry weight of *Fusarium poae*, *F. oxysporium*, *F. moniliforme*, *F. solani* and *Macrophomina phaseolina.*

It might be also suggested that soaking of maize grains in the tested control agents was effective technique to induce production of some components acted as inhibitors against growth or / and fungal penetration. So, some plant defense substances involving the systemic induction of resistance against *Cephalosporium maydis* was activated. Systemic acquired resistance (SAR) is a suggested phenomenon by Hatcher (1995) to describe the systemic induction of resistance against a broad spectrum of phytopathogens.

It can be also concluded that the toxicity of different extracts may varied or enhanced if we used another solvents instead of water. These solvents may have the capacity to extract another compounds existed in plants which increase induced resistance (Hussien *et al.* 2005). So, using of plant extracts actually offer a cheaper and environmentally safer alternative instead to convential synthetic fungicides for controlling late wilt disease on maize, and on the other hand, to obtain economic yield. Consequently, such fungicidal, non-residual treatments minimized chemical hazard and environmental pollutions should be in consideration.

REFERENCES

Abd El-Ghani, Haifaa S. and Heweidy, M. A. (1997). Effect of juice of garlic cloves on some fungi with special reference to late wilt disease of corn caused by *Cephalosporium maydis*. 8 th Congress of the Egyptian Phytopathol. Soc., Cairo. 147-159.

Al-Abed, A. S., Qassem, J. R. and Abu-Blan, H. A. (1993). Anti-fungal effects of some common wild, plant species on certain plant pathogenic fungi. Dirasat (Pure and Applied Science), 20: 149-158.

Alice, D. and Rao, A. V. (1986). Management of seed borne *Drechslera oryzae* of rice with plant extracts. International Rice Research Newsletter, 11(3): 19 p.

Atta-Alla, S. I., El-Sheikh, M. A., Rahhal, M. M. and Alghandour, S. A. (2003). Certain bioagents, botanical extracts and intercropping for controlling the white stem rot of chickpea in El-Behera governorate. J. Agric. Res., Tanta Univ., 29(4): 577-596.

Baharat, R. A. I., Jariwala, S. and Manjari, K. (1997). Efficiency of fungicides and antibiotics on seed mycoflora and seed

germination of some species. Indian Phytopathology, 50(2): 261-265.

Barnett, H. J. (1960). Illustrated genera of imperfect fungi. Burgess. Minneapolis, USA, 226 pp.

Boulos, L. (1983). Medicinal plants of North Africa. Reference Publications, Inc., USA, 286 p.

Calpouzos, L., Roellfs, A. P., Mdson, M. E., Maetin, R. B., Welsh, J. R. and Wilcoxson, R. D. (1976). A new model to measure yield losses caused by stem rust in spring wheat. Agric. Exp. Sta. Univ. Minnesota, Tech. Bull. 307: 1-3.

Carcia, R. P. and Lawas, M. V. P. (1990). Note: Potential plant extracts for the control of *Azolla* fungal pathogens. Philippine Agric., 73(3-4): 343-348.

Coley-Smith, J. R. and King, J. E. (1969). The production by species of *Allium* of alkyl sulphide and their effect on germination of Sclerotia of *Sclerotium cepivorum* Berk. Ann. Appl. Biol., 64, 2, 289.

Dubey, R. C. and Dwivedi, R. S. (1991). Fungitoxic properties of some plant extracts against vegetative growth and sclerotial viability of *Macrophomina phaseolina*. Indian Phytopathol., 44(3): 411-413.

Duncan, D. B. (1955). Multiple range and multiple F. test. Biometrics, II: 1-42.

El-Assiuty, E. M. (1976). Studies on the control of stalk rots of maize, chemical control of the late-wilt disease, M. Sc., Fac. Agric., Cairo Univ.

El-Moghazy, S. M. (2003). Studies on the integrated control of downy mildew disease on maize and sorghum. Ph. D. Thesis, Fac. Of Agric., Tanta Univ., 128 pp.

El-Shafey, H. A., El-Shorbagy, F. A., Ikbal I. Khalil, and El-Assiuty, E. M. (1988). Additional sources of resistance to the late-wilt disease of maize caused by *Cephalosporium maydis*. Agricultural Research Review, 66(2): 221-230.

El-Shoraky, Fathia S. A. (1998). Using extracts and oils of some plants in controlling plant diseases. Ph. D. Thesis, Fac. Agric., Tanta Univ.

Fahmy, F. G. and Mohamed, M. S. (1984). Effect of onion root exudates, sulphide compounds and filterates of certain

microorganisms on infectivity of tobacco mosaic and po: viruses. Egypt. J. Phytopathol., 16(1-2): 65-69.

Finney, D. J. (1971). Probit analysis. Cambridge Univ. Press 318 p

Hatcher, P. E. (1995). Three-way interactions between pla pathogenic fungi, herbivorus insects and their host plant Biological Reviews, 70: 639 – 694.

Hussien, Mamdouha M., Mostafa, Nabila A. and El-Shamy, M. M (2005). Effect of gama rays and some other compounds on the control of wheat loose smut disease. J. Agric. Res., Tanta Univ., 31(3): 347-360.

Kurucheve, V., Gerard, E. A. and Jayaraj, J. (1997). Screening of higher plants for fungitoxicity against *Rhizoctonia solani in vitro*. Indian Phytopath. 50(2): 235-241.

Labib, H. A., Abd El-Rahim, S. A. and Abd El-Fattah, A. (1975). DCI a new maize hybrid seed resistant to late-wilt disease caused by *C. maydis*. Agric. Res. Rev., Min. Agric., 53: 1-4.

Malhotra, D. and Rai, P. K. (1990). Observations on the effect of leaf extracts on germination and fungi associated with seeds of *Glycine max* L. Indian Journal of Applied and Pure Biology, 5(1): 53-56.

National campaign report of maize yield improvement, (2005). Economic Affairs Sector of Agricultural Statistics, Ministry of Agriculture and Land Reclamation.

Papadopoulou, K., Melton, R. E., Leggett, M., Daniels, M. J. and Osbourn, A. E. (1999). Compromised disease resistance in saponin-deficient plants. PNAS, October 26, 96(22):12923-12928.

Sabet, K. A., Samra, A. S. and Khalil, I. K. (1966). Variation in *Cephalosporium maydis*, the cause of the late wilt disease. In: Samra, A. S. and Sabet, K. A. [Eds.] Investigations on stalk-rot disease of maize in U. A. R. pp. 46-93. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.

Sabet, K. A., Samra, A. S. and Mansour, I. M. (1966). Late-wilt of maize and a study of the causal organism. In: Samra, A. S. and Sabet, K. A. [Eds.] Investigations on stalk-rot disease of maize in U. A. R. pp. 8-45. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.

Sabet, K. A., Zaher, A. M., Samra, A. S. and Mansour, I. M. (1970). Pathogenic behavior of *Cephalosporium maydis* and *C. acremonium*. Ann. Appl. Biol., 66: 257-263.

Samra, A. S., Sabet, K. A. and Abd El-Rahim, M. F. (1966). Seed transmission of stalk-rot fungi and effect of seed treatment. In: Samra, A. S. and Sabet, K. A. [Eds.] Investigations on stalk-rot disease of maize in U. A. R. pp. 94-116. Ministry of Agriculture, Government Printing Offices, Cairo, Egypt.

Samra, A. S., Sabet, K. A., Kamel. M. and Abd el-Rahim, M. F. (1971). Further studies on the effect of field conditions and cultural practices on infection with stalk-rot complex of maize. Arab Republic of Egypt, Min. of Agriculture, Plant Protection Dept., Bull. No. 2.

Singh, R. S. (1982). Plant pathogens " The Fungi ". Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta, 443 pp.

Singh, U. P., Pathak, K. K., Khare, M. N. and Singh, R. B. (1979). Effect of leaf extract of garlic on *Fusarium oxysporium* f. sp. *ciceri*, *Sclerotinia sclerotiorum* and on gram seeds. Mycologia, 71: 556-563.

Topps, J. H. and Wain, R. L. (1957). Investigation on fungicides. III. The fungitoxicity of 3- and 5-allyl salcylanilide and pavachlovanilines. Ann. Appl. Biol. 45: 506-511.

Wilson, C. L., Solar, J. M., El Ghaouth, A. and Wisniewski, M. E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. Plant Dis. 81(2): 204-210.

Ye, X. S., Pan, S. Q. and Kuc, J. (1990). Activity, isozymy pattern and cellular localization of peroxidase as related to systemic resistance of tobacco to blue mould (*Peronospora tabacina*) and to tobacco mosaic virus. Phytopathology, 80: 1295-1299.

Yegen, O., Berger, B. and Heitefuss, R. (1992). Studies on the fungitoxic effects of extracts of six selected plants from Turkey on phytopathogenic fungi. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 99(4): 349-359. (c. f. Review of Plant Pathology, 1993, 72(4): 2483).

Zommara, M. A. and Rashed, M. A. (2005). Inhibition of mould growth on the surface of ras cheese by plant water extracts or P^H control. J. Agric. Res. Tanta Univ., 31 (3): 457- 470.

الملخص العربى

تأثير بعض المستخلصات المائية النباتية و المركبات الكبريتية فى مقاومة مرض الذبول المتأخر فى الذرة الذى يسببه فطر *Cephalosporium maydis*

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درس تأثير خمس مستخلصات مائية نباتية وهى الأوراق العصيرية لنبات الصبار و أبصال البصل وفصوص الثوم وأوراق كل من الداتوره و النعناع، بالاضافة الى كبريتات الزنك و كبريتات النحاس على الفطر المسبب لمرض الذبول المتأخر فى الذرة. ولقد أثبتت النتائج أن المستخلصات المائية النباتية لكل من البصل و الثوم كانت أكثر المعاملات تأثيراً على كل الخصائص المختبرة للمسبب المرضى، مثل الوزن الجاف للفطر فى المعمل، و فى السيطرة على شدة المرض و الفقد فى المحصول فى الحقل. وقد دلت النتائج على أن كل المستخلصات النباتية و كذلك المركبات الكبريتية كانت ذات فعالية متفاوتة التأثير التثبيطى على الفطر المسبب المرضى فى المعمل. فقد كانت المستخلصات المائية لنباتات الصبار و البصل و الثوم و الداتوره أكثرها تثبيطا للوزن الجاف للفطر، حيث حققت أقل قيم للتركيز المثبط لـ ٥٠ % من نمو الفطر فكانت التركيزات ١٢,٤٣ ، ١٥,٠٢ ، ١٥,٣٥ ، ١٦,٢٥ % على التوالى، متبوعة بمركبى الكبريتات ثم مستخلص أوراق النعناع. ولقد أوضحت النتائج المتحصل عليها من التجارب الحقلية أن كبريتات النحاس و مستخلص فصوص الثوم و كذلك كبريتات الزنك و مستخلص البصل كانت أكثر المعاملات تأثيرا فى تقليل شدة الأصابة بالمرض. على الجانب الاخر، تم تحقيق زيادات معنوية فى انتاج محصول الذرة كنتيجة للمعاملة بمستخلص أوراق النعناع و الصبار و كبريتات الزنك، بالاضافة الى البصل و الثوم، بينما كان لباقى المعاملات تأثيرا ضعيفا والبعض ليس له تأثير وذلك مقارنة بالنباتات الغير معاملة (الكنترول).