STUDIES ON THE CONTROL OF CORN COMMON SMUT DISEASE [*USTILAGO MAYDIS*]

By

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ABSTRACT

Among six geographical isolates of *Ustilago maydis* collected from different locations, the Beni-Sueif isolate was the most pathogenic followed by isolates of Qalubiya, Fayoum, Gharbiya, Giza and Kafr El-Sheikh. Pathogenicity of an isolates seems correlated with germinability of its teliospores. Sporidia caused higher disease than teliospores.

Cultivation of garlic, sugar beet, oat, lupine, faba bean or lentil caused considerable decline in viability of teliospores that remained or buried in soil. Root exudates of these plants exhibited similar effect in vitro. The chickpea, barley and wheat as well as their root exudates caused the lowest suppressive effect on viability of teliospores. Viability of teliospores was decreased to some extent when left for 4-5 months in fallow irrigated or non-irrigated soils.

The reaction of tested maize accessions against artificial infection with common smut was considerably varied. The maize accession TWC-Baraka was the most resistant during 1998 and 1999 seasons followed by the accessions TWC-322 and the SC-129 meanwhile, the local varieties Giza-2 and Boushi, and the hybrids TWC.320, TWC.324, TWC.321, and DC Dahab were the most susceptible.

The plant oils eucalyptus, clove, cinnamon, peppermint, and anise at 750 and 1000 ppm and clove and anise oils only at 500 ppm caused 100% inhibition of the in vitro fungal growth. The promising effect of these oils at different concentrations in controlling common smut disease and increasing corn grain yield under stress of artificial infection was emphasized under greenhouse and field conditions. The best results were obtained when maize plants were injected with oil simultaneously with pathogen inoculation. Spraying plants with plant oils 48 h before or after pathogen inoculation was partially effective.

Additional key word: common smut, U. maydis, isolates, teliospores, viability, root exudates, preceding crops, varietal resistance, disease control.

INTRODUCTION

Ustilago maydis [*U. zeae*] is a pathogen specific to maize and teosinte and is the causal agent of common smut of maize that is distributed worldwide. (Shurtleff, 1980). All above ground plant parts can be infected, but galls that form on the apical meristem of seedlings or those on infected ears cause the greatest losses (Smith and White, 1988). William *et. al.* (1991) reported that the greatest damage of common smut results from infection of the corn ear or the stalk above the ear.

Rudenko (1969) recorded that the common smut disease symptoms appeared earlier and were more sever after inoculation with sporidia than with teliospore. Mills and Kotzel (1981) reported that teliospore germ tubes seem to be more important than sporidia in the epidemiology of the disease under field conditions. Pope and McCarter (1992) indicated that certain pairwise combinations of compatible sporidial lines were more effective than others in inducing galls. Wilcoxson and Covey (1960) noticed decrease in tassel smut when maize followed maize than when it followed other cereal crop. Predko (1972) stated that the fewest smut infected maize plants occurred after winter wheat, pea and sugar beet. Kozhevn (1975) found that monoculture of maize increased significantly infection with common smut whereas, barley and millet reduced the disease incidence after 2 years of continuous cultivation of maize. William *et. al.* (1991) reported that common smut in corn fields adjacent to wheat fields, has been particularly damaging.

Growing maize smut-resistant varieties is the main practical means for disease control. El- Shafey *et. al.* (1988) found that all Egyption local varieties were highly susceptible to the common smut pathogen *U. maydis*. Pataky, (1991) reported that, selection based on natural infection in breeding programs have been somewhat unreliable because of the fortuitous occurrence of environmental conditions conducive to infection and an association between host growth stage during infection periods and the plant tissues on which galls form. More recently Moursy *et. al.* (2001) found that maize oil was more effective against *Ustilago maydis* followed by soybean and sunflower oils in controlling the disease. Foliage oil spray after plant inoculation

ranked first for control the disease followed by simultaneous spray and plant inoculation, in general. Popov (1972) found that infected plants usually yielded 40% less than healthy plants. Kostandi and Geisler (1989) stated that loss in maize grain yield associated with smut galls on ear, stem and tassel were 57.1%, 24.3% and 9.3% respectively. El-Assiuty *et. al.* (1990) found that the ear infection reduced grain yield per plant by from 84 to 93%. Infection with one gall above the ear drastically affected the yield than infection with one or more galls below the ear.

The present investigation aimed to studying variability between different isolates of *U. maydis*. Effect of preceding crops and their root exudates, effect of some plant oils on controlling common smut disease and grain yield as well as reaction of different maize accessions against common smut disease was also investigated.

MATERIALS AND METHODS

Collection of smut gall samples:

At the end of the local growing season and before harvesting corn fresh smut galls [*Usilago maydis*] were collected from six locations belonging to the Egyptian provinces Giza, Beni-Sueif, Fayoum, Qalubiya, Gharbiya, and Kafr El-Sheikh. Smut galls were allowed to dry out, if wet, and gently sieved. The obtained powder of teliospores was used in the following studies.

Germinability of U. maydis teliospores and pathogenicity test:

A loopful of smut powder containing teliospores of a given *U. maydis* isolate were mixed with few drops of distilled water on sterilized glass slides, four slides each, and left for 24 h in moistened chambers at 30°C then percentage of germination was determined.

As for pathoginicity test, maize plants 30 days old grown in pots No.25 (5 plants/pot) were injected near their leaf whorls with spore suspensions containing sporidia or teliospores of *Ustilago maydis* at rate of 2ml/plant. Each treatment was conducted in four replicated pots.

The inoculum containing teliospores was prepared by mixing teliospores powder of a known isolate with distilled water [5 g/l] and thoroughly agitated before use. While sporidial suspension was prepared as follow: surface sterilized teliospores [by soaking for 48 h in 1.5% copper sulphate] (Elseoud, 1999) were streaked on sterile

water agar plates and incubated at 30°C. After 48 hours, individual germinated teliospores were transferred to PDA plates and incubated at 30°C for 7-10 days. The resultant sporidial fungal growth was flooded with sterilized distilled water [10 ml/plate], scraped by a sterile scalpel to release the fungal sporidia. Sporidial suspensions were filtered through cheesecloth then diluted with sterile distilled water to give final concentration of $5.5x10^5$ sporidia ml^{-1} immediately before use.

For pathogenicity test, maize plants 30 days old grown in pots No.25 (5 plants/pot) were injected near their leaf whorls with the spore suspension containing sporidia or teliospores of *Ustilago maydis* at the rate of 2ml/plant. Each treatment was conducted in four replicated pots.

Control maize plants were injected with sterilized distilled water. Plants were covered with transparent plastic bags for 24 hrs to maintain relative humidity for inducing spore germination and infection and observed daily to follow up disease development and recognize gall formation. After 15 days disease incidence [% infection] and disease severity was determined according to the gall diameter scale suggested by Khalil (1973). The teliospores of *U. maydis* Beni-Sueif-isolate was used in the following studies because it's high germination.

Effect of root exudates on teliospore germination:

Seeds of maize and other winter-crop plants [Garlic, Onion, Chick-pea, Faba bean, Oat, Clover (G.1 Sewa), Clover (Misqawy), Sugar beet, Lupine, Wheat, Barley, Lentil] were germinated and allowed to grew aseptically on glass beads-sterilized distilled water mixture for 15 days. Then glass beads were removed and the resultant crude root exudates were examined for their effect against distilled water on teliospore germination as above mentioned.

Effect of some plant oils on the linear growth of U. maydis In vitro:

The crude oils of eucalyptus, clove, cinnamon, peppermint, and anise caused complete inhibition of *U. maydis* growth *in vitro* during the preliminary lab test. Each of these plant oils was added to melted Richard's agar medium at 250, 500, 750, and 1000 ppm emmediately before pouring into the plates. Plates without plant oils were served as control. Plates were inoculated as above with sporidial fungal growth. Reduction in diameter of the fungal colony was recorded 10 and 20 days after incubation at 30 °C.

Greenhouse experiments:

Unless other wise stated, 30 days old plants of maize Giza-2 variety grown in potted soil [5 plants/pot No.25] at 30-35 °C and sporidial [$5.5x10^5$ sporidia/ml] and teliospores suspensions of *U. maydis* [Beni-Sueif isolate] were used throughout the following studies.

Effect of the maize-preceding crops on teliospores longevity:

Known amounts [about 1g] of teliospores [with known initial germination] of a known isolate of *U. maydis* were placed in nylon bags. Four bags for each isolate were buried individually at 10-cm depth in potted soils [pots No.30]. Pots were planted with Chinese garlic, G6-onion, G88-chickpea, G2-faba bean, commercial oat, G1-Sewa-clover, miskawy-clover, Rass poly- sugar beet, G1-lupine, G133-wheat, G126-barley or G9-lentil. Eight pots were left without sowing (fallow) and used as control but half of them was normally irrigated meanwhile the other was non. Four to five months after sowing, plants were removed and the nylon bags containing teliospores were get out and germinability of the enclosed teliospores was examined. Teliospores were incorporated in drops of free distilled water on sterilized glass slides placed on U-shaped glass tubes in a moisture chambers made up of a sterile Petri dishes lined with blotting paper saturated with distilled water. After 24 h from incubation at 30 C % germinated spores was calculated.

Reaction of some maize accessions against common smut disease:

Several maize accessions (see Table 5) were screened for 2 successive seasons under greenhouse conditions for their reaction against common smut infection. Maize plants were injected nearly below the growing leaf whorl by sporidial spore suspension or water (in control treatment) at rate of 1.5 ml/plant. Incidence and severity of the disease was recorded 15 days later as above mentioned.

Effect of some plant oils on common smut disease incidence:

Peppermint, eucalyptus and cinnamon oils were used at conc. of 750 ppm and 1500 ppm [in tap water with few drops of Tween 20 as spreading agent]. Maize plants were injected at same time with both sporidial suspension (as mentioned above) and plant oil or sprayed with the plant oil 48 h before or after spore injection.. Disease reading was recorded 15 days after oil's application as mentioned above.

Field experiment:

Effect of infection with U. maydis on corn grain yield:

Corn grain yield as affected with both natural and artificial infection with common smut was investigated in field trials carried out at locations of Sakha and Gemmeiza. Maize plants were injected nearly below the growing leaf whorl with sporidial suspension (2-3 ml/plant) 45, 55 days after planting and in the ear after silk emergence (5-10cm long) (Pope & McCarter, 1992). This timing of inoculation induces formation of smut galls under the ear, above the ear and in ear, respectively. In inoculation trials, 4 replicates [rows], 10 plants per each were used for each time. Disease readings under stress of both artificial and natural infection were carried out 100 days after planting. At the harvest time, grain yield in gm/plant was estimated and reduction in the grain yield was calculated. For comparison, grain yield of healthy plants was also determined.

Effect of some plant oils on disease incidence and grain yield:

Tow field trials at Sakha and Gemmeiza Agricultural Experimental Stations were carried out to investigate effect of some plant oils on the infection with common smut and grain yield of maize plants under field conditions. Maize plants, 45 days old, were injected nearly below the growing leaf whorl with sporidial suspension at rate of 2ml/ plant. The plant oils i.e. peppermint, eucalyptus and cinnamon at 750 or 1500 ppm were used for treating maize plants either simultaneously or 48 hrs before or after inoculation with the sporidial suspension. Disease readings (disease incidence and severity) were taken 15 days after oil's applications. Four rows, ten plants per each were used in each treatment. At the end of the growing season, yield per plant was estimated. For comparison, grain yield of healthy plants was also determined.

Statistical analysis:

All data were subjected to statistical analysis and means were compared by Duncan`s multiple range test (Armitage, 1971). As for our presend tabulated result, unless otherwise stated, the means in the same column with the same letters don't differ significantly according to Duncan's Multiple Range test at level 5%.

RESULTS

Pathogenicity test and germinability of teliospores:

Results shown Table (1) indicate that inoculation with teliospores or sporidiospores of the different isolates resulted in considerable significant variations in disease incidence (30.0 to 90.0%) and disease severity (19.0 to 56.0%). Sporidia of any isolate caused higher disease incidence and severity than teliospores.

The isolate obtained from Kafr El-Sheikh seems to be the least pathogenic particularly when its teliospores were used while Beni-Sueif, Fayoum, and Qalubiya isolates obtained from were significantly equal and more severe than the isolates from Giza and Gharbiya. The same data showed also that the highest limits of teliospore germination was produced by Beni-Sueif isolate (41.6%) followed by Qalubiya (32.2%), Fayoum (30.0%), Gharbiya (25.2%), Giza (25.1%) and Kafr El-Sheikh (19.4%).

Table (1): Efficiency of *U. maydis* -teliospores and -sporidiospores (collected from different locations) to infect the susceptible Giza 2 maize variety, greenhouse, 1998.

Type of inoculum and fungal isolate	% Germination	Disease incidence %	Disease severity %
Teliospores			
Giza (Giza)	25.1	40 e	21 gh
Beni-Sueif (Sids)	41.6	60 bc	39 cd
Fayoum (Tameia)	30.0	50 cde	31 e
Qalubiya (Benha)	32.2	55 cd	35 de
Gharbiya (Gemmiza)	25.2	45 de	25 fg
Kafr El-Sheikh (Sakha)	19.4	30 f	19 h
Sporidiospores			
Giza (Giza)		60 dc	42 cd
Beni-Sueif (Sids)		90 a	56 a
Fayoum (Tameia)		75 ab	49 ab
Qalubiya (Benha)		80 a	53 a
Gharbiya (Gemmiza)		60 bc	43 bc
Kafr El-Sheikh (Sakha)		55 cd	30 ef

Effect of root exudates of some winter crops and maize on viability of teliospores of U. maydis:

Data in Table (2) illustrated that spore germination was negatively and significantly affected by root exudates of tested plants. The root exudates of garlic, onion, sugar beet, and faba bean plants were highly effective in reducing spore germination [90.9- 97.5%] without significant differences in between. In point, the root exudates of lentil, oat, and lupine plants were moderately effective [69.1-85.3%]. Root exudates of clover "G.1 Sewa", chickpea, and wheat plants produced little but significant suppressive effect [22.8-33.1%]. On the opposite side, root exudates of barley, clover "Misqawy", and maize showed no significant suppressive effect on teliospore germination when compared with germination in free water. The root exudates of barley plants reduced germination by 8.3%, while those of clover "Misqawy" and maize plants increased it by 6.6% and 7.8%, respectively.

Table (2): Effect of root exudates of certain winter crops and maize on germination of teliospores of *U. maydis*

Root exudates of	% Spore germination	% Decrease or increase
Garlic	01.0 g	- 97.5
Onion	01.4 g	- 96.6
Chick-pea	31.0 bc	- 24.0
Faba bean	03.7 fg	- 90.9
Oat	09.1 de	- 77.7
Clover (G.1 Sewa)	27.3 c	- 33.1
Clover (Misqawy)	43.5 a	+ 06.6
Sugar beet	01.7 g	- 95.8
Lupine	12.6 d	- 69.1
Wheat	31.5 bc	- 22.8
Barley	37.4 ab	- 08.3
Lentil	06.0 ef	- 85.3
Maize	44.0 a	+ 07.8
Control [Free water]	40.8 a	

Effect of some plant oils on the linear growth of U. maydis In vitro:

The obtained results shown in Table (3) indicated clearly that the fungal growth was completely inhibited at concentrations of 750 and 1000 ppm of any of the tested plant oils. At 500 ppm clove and anise oils only caused 100% inhibition of fungal growth after 20 days from incubation. However, at 250 ppm clove oil shows the highest satisfactory efficiency for inhibiting fungal growth (88.5%). It is worth mentioning that no growth was occurred when the non-grown fungal discs taken from the plates of the higher concentrations were transferred to oil-free medium, indicating the fungicidal effect of these oils for the smut fungus.

Greenhouse experiments:

Effect of the maize preceding crops on teliospores longevity:

Viability of *U. mayds* teliospores that buried in soils cultivated with any tested crop plants or in fallow irrigated or non-irrigated potted soils was significantly declined (Table 4). Burying teliospores in soils cultivated with garlic, sugar beet, oat, lupine, faba bean or lentil reduced their germination by 72.7, 66.5, 63.3, 59.0, 57.6 and 55.1%, respectively. The lowest reduction in germination of teliospores was

observed teliospores buried in fallow irrigated soil (34.6%), soils cultivated with chickpea (33.3%), wheat (32.3%), fallow non-irrigated soil (25.0%), and soil cultivated with clover "Misqawy" (20.0%) without significant differences in between.

Table (3): Effect of some plant oils on the linear growth of *U. maydi*

Plant oil	Conc. (ppm)	Days after incubation			
		10 days		20 days	
		Growth (mm)	% Reduce	Growth (mm)	% Reduce
Eucalyptus	250	21.0	16.1	27.0	37.3
	500	15.0	54.8	18.5	65.4
	750	0.0	100.0	0.0	100.0
	1000	0.0	100.0	0.0	100.0
Clove	250	10.0	87.1	11.5	88.5
	500	0.0	100.0	0.0	100.0
	750	0.0	100.0	0.0	100.0
	1000	0.0	100.0	0.0	100.0
Cinnamon	250	20.0	22.6	29.0	30.7
	500	11.0	19.4	15.0	23.1
	750	0.0	100.0	0.0	100.0
	1000	0.0	100.0	0.0	100.0
Peppermint	250	17.5	38.7	24.5	45.5
	500	14.0	61.3	19.0	63.7
	750	0.0	100.0	0.0	100.0
	1000	0.0	100.0	0.0	100.0
Anise	250	9.0	93.6	9.5	95.1
	500	0.0	100.0	0.0	100.0
	750	0.0	100.0	0.0	100.0
	1000	0.0	100.0	0.0	100.0
Control	0	23.5	0.0	38.3	0.0

Effect of some plant oils on common smut disease incidence under greenhouse conditions:

The obtained results (Tables 5) indicated that the all tested oil treatments caused significant reduction in disease incidence (0-40%) and disease severity (0-33%) compared with 70% and 48%, respectively in the non-treated control. Simultaneous injection with sporidial suspension and any of the plant oils peppermint, eucalyptus at 750 and 1500 ppm and cinnamon at 1500 ppm were the best treatment for suppressing common smut infection (0-5%) and disease severity

(0-2%). Less significant reduction in disease incidence and severity was observed also when plants were sprayed with plant oils weather 48 hrs before or after the fungal inoculation. In general, the higher oil concentration (1500 ppm) was more effective in this respect than the lower one (750 ppm). In single treatment i.e. spraying maize plants with cinnamon oil at 750 ppm after 48 hrs from their inoculation with sporidial suspension has no significant effect on disease incidence (45%).

Table (4): Effect of certain winter crops preceding maize on longevity of teliospores of *U. maydis* expressed as% of germination.

The preceding crop	% Germination	% reduction in germination
Garlic	15.3 g	72.7
Onion	27.1 de	51.7
Chick-pea	37.4 bc	33.3
Faba bean	23.8 def	57.6
Oat	20.6 efg	63.3
Clover (G.1 Sewa)	29.9 cd	46.7
Clover (Misqawy)	44.9 b	20.0
Sugar beet	18.8 fg	66.5
Lupine	23.0 def	59.0
Wheat	38.0 bc	32.3
Barley	40.0 b	28.7
Lentil	25.2 def	55.1
Fallow irrigated soil	36.7 bc	34.6
Fallow non-irrigated soil	42.1 b	25.0
Control [Initial germination]	56.1 a	00.0

Table (5): Effect of plant oils applied 48 hrs before, after or at same time of inoculation with sporidia of the common smut pathogen on percentages of infection and disease severity under greenhouse.

Plant oil And conc. [ppm]	Time of oil application					
	% infection			% Disease severity		
	48 hrs before	48 hrs after	At same time	48 hrs before	48 hrs after	At same time
Peppermint						
750	30 bcde	35 bcd	5 fg	17 de	20 cd	2 gh
1500	15 ef	20 de	0 g	8 f	10 ef	0 h
Eucalyptus						
750	35 bcd	40 bc	5 fg	21 cd	24 bcd	2 gh
1500	15 ef	20 cde	0 g	8 f	10 ef	0 h
Cinnamon						
750	40 bc	45 ab	15 ef	30 bc	33 b	6 fg
1500	20 cde	25 bcde	5 fg	9 f	11 ef	2 gh

Control	70 a	70 a	70 a	48 a	48 a	48 a

Reaction of maize accessions against common smut disease:

The obtained results (Table 6) indicated that the disease incidence was relatively higher in 1998 than 1999 season. The reaction of tested maize accessions against artificial infection with common smut was considerably varied. Along tow successive seasons, the incidence and severity of the disease were ranged between 10-70% and 4-49%, respectively. The maize accession TWC-Baraka was the most resistant to infection with *U. maydis* as it exihibited the lowest disease readings during both 1998 and 1999 seasons followed by the accessions TWC-322 and the SC-129. Six accessins viz. Giza-2, Boushi, TWC.320, TWC.324, TWC.321, and DC Dahab were the most susceptible accessions as they showed the highest disease incidence (50-70%) and disease severity (38-49%). The rest accessions reacted moderately and distributed between these two extremes.

Table (6): Reaction of some single (SC), double (DC), three way (TWC.) crosses and open pollinated (O.P) maize varieties against artificial inoculation with common smut pathogen (*U. maydis*) under greenhouse conditions during 1998 & 1999 seasons.

Accession	Infection %		Disease severity %	
	1998	1999	1998	1999
SC10	40 cde	35 cde	36 bcde	32 abc
SC123	25 ef	15 fg	15 hi	12 ef
SC124	45 bcd	40 bcd	38 bcde	35 ab
SC129	20 f	20 ef	12 i	11 fd
DC Amoun	35 cde	35 cde	32 ef	30 abc
DC Dahab	50 abc	45 abc	41 abcd	38 a
DC Taba	30 def	25 def	25 fg	22 bcd
TWC. 310	30 def	20 ef	20 gh	16 de
TWC. 320	65 ab	60 ab	45 ab	44 a
TWC. 321	50 abc	45 abc	42 ab	40 a
TWC. 322	20 f	20 ef	13 i	11 def
TWC. 323	40 cde	35 cde	33 def	30 abc
TWC. 324	60 ab	50 abc	44 abc	40 a
TWC. 352	50 abc	45 abc	45 ab	36 ab
TWC. 3052	30 def	25 def	22 g	20 cd
TWC. Baraka	10 g	10 g	4 j	7 f
TWC.Nefertiti	40 cde	35 cde	34 cde	30 abc
TWC.Nagah	45 bcd	40 bcd	37 bcde	35 ab
O.P. Giza 2	70 a	65 a	49 a	45 a
O.P. Boushi	50 abc	50 abc	44 abc	41 a

Grand means	40.25	35.75	31.55	28.75

Field Experiments:

Effect of the artificial infection with U. maydis the causal of corn common smut disease on corn grain yield:

The data in Table (7-A) proved that the smut galls were formed more frequently below ear [63.2-65.1%] than above the ear [51.4-56.2%] or in the ear itself [47.5-52.8%] in the artificial inoculation trials conducted at locations of Sakha and Gemmeiza. However, percentage of smut galls that formed at all plant portions on the naturally infected plants was ranged between 0.7-1.0% (Table 7-B). The galls in the ear itself were significantly larger in size than those formed above or below ears. The greatest reduction in grain yield/plant was associated with smut galls formed in the ear itself (87.2-92.2%) followed by smut galls formed on plant stem above ear (36.7-38.9%). Little but significant loss in grain yield was detected also when smut galls were formed on plant stem below the ears (23.2-19.5%). Closely similar trend was also reported under stress of natural infection (Table 6-B).

Table (7-A): Percentage of common smut disease incidence, gall diameter, grain yield and% reduction in grain yield as affected by the artificial infection with common smut at location of Sakha Gemmeiza [Gemm.].

Location of gall	Disease incidence%		Diameter of smut gall [cm]		Grain yield [gm/plant]		% Reduction in yield	
	Sakha	Gemm.	Sakha	Gemm.	Sakha	Gemm.	Sakha	Gemm.
On ear	47.5 b	52.8 b	10.3 a	12.3 a	31.5 d	17.1 d	87.2	92.2
Above ear	51.4 b	56.2 b	5.1 b	3.9 c	155.3 c	133.4 c	36.7	38.9
Below ear	62.3 a	65.1 a	5.1 b	7.6 b	188.4 b	175.7 b	23.2	19.5
Healthy	0.0 c	0.0 c	0.0 c	0.0 d	245.3 a	218.2 a		

Table (7-B): Percentage of common smut disease incidence, gall diameter, grain yield and % reduction in grain yield as affected by the natural infection with common smut at location of Sakha Gemmeiza [Gemm.].

Location of gall	Disease incidence %		Diameter of smut gall [cm]		Grain yield [gm/plant]		% Reduction in yield	
	Sakha	Gemm.	Sakha	Gemm.	Sakha	Gemm.	Sakha	Gemm.

On ear	0.8 a	0.7 a	8.0 a	12.0 a	33.1 d	17.9 d	86.7	91.9
Above ear	0.7 a	0.9 a	3.1 c	4.9 b	158.9 c	131.2 c	36.1	40.7
Below ear	0.7 a	1.0 a	6.2 b	5.0 b	187.5 b	171.2 b	24.6	22.6
Healthy	0.0 b	0.0 b	0.0 d	0.0 c	248.5 a	221.2 a		

Effect of some applications of plant oils on common smut disease incidence and corn grain yield:

All tested applications of plant oils were significantly effective in reducing common smut disease incidence on the artificially infected maize plants and increasing corn grain yield under field conditions at locations of Sakha (Table 8-A) and Gemmeiza (Table 8-B).

Table (8-A): Effect of plant oils applied 48 hrs before, after or at same time of inoculation with sporidia of the common smut pathogen on % infection, % disease severity and grain yield (gm)/plant at location of Sakha.

Time of treatment & Plant oil	ppm	Disease criterion		
		% Infection	% Severity	Grain yield
Spraying 48 hrs. before inoculation				
Peppermint	750	26.7 de	15.0 e	292.0 de
	1500	11.8 g	05.3 g	320.3 c
Eucalyptus	750	30.2 cd	17.5 de	286.2 ef
	1500	13.1 g	06.8 fg	318.0 c
Cinnamon	750	37.4 bc	26.0 c	271.0 g
	1500	16.9 fg	07.9 f	301.0 d
Spraying 48 hrs. after inoculation				
Peppermint	750	28.1 d	17.2 de	287.0 ef
	1500	12.3 g	7.0 fg	315.0 c
Eucalyptus	750	32.7 bcd	18.5 d	278.0 fg
	1500	15.3 fg	8.0 f	312.2 c
Cinnamon	750	41.6 b	31.8 b	243.0 gh
	1500	20.1 ef	9.0 f	295.0 de
At the same time				
Peppermint	750	0.0 h	0.0 i	344.7 ab
	1500	0.0 h	0.0 i	345.1 a
Eucalyptus	750	2.8 h	1.1 i	332.9 b
	1500	0.0 h	0.0 i	343.5 ab
Cinnamon	750	10.3 g	3.5 h	315.5 c
	1500	0.0 h	0.0 i	344.0 ab
Control		61.2 a	44.1 a	161.2 i

Control [Healthy]		0.0	0.0	343.9 ab

Applying plant oils [peppermint and eucalyptus oils at 750 and 1500 ppm and Cinnamon oil at 1500 ppm] at the same time of inoculation with spore suspension of the common smut pathogen resulted in the best disease control and the highest significant increase in grain yield/plant. The common smut disease incidence was reduced to 0.0-2.8% at Sakha and 0.0-3.6% at Gemmeiza. compared with 61.2% and 44.1% in control untreated plants at both locations, respectively. The results about disease severity showed similar trend. It is worthy to state that the corn grain yields/plant in these treatments was significantly equal and comparable with that produced from the healthy maize plants that grown under the natural field conditions.

Table (8-B): Effect of plant oils applied 48 hrs before, after or at same time of inoculation with sporidia of the common smut pathogen on% infection,% disease severity and grain yield (gm)/plant at location of Gemmeiza.

Time of treatment & Plant oil	ppm	Disease criterion		
		% Infection	% Severity	Grain yield
Spraying 48 hrs. before inoculation				
Peppermint	750	28.7 de	16.2 c	273.2 fgh
	1500	13.0 g	7.0 de	299.0 bc
Eucalyptus	750	31.0 cd	18.6 c	261.1 hi
	1500	14.6 fg	7.9 de	293.0 bcd
Cinnamon	750	39.7 bc	28.2 b	250.0 ij
	1500	18.4 fg	8.5 de	286.2 de
Spraying 48 hrs. after inoculation				
Peppermint	750	30.2 cd	17.7 c	261.0 hi
	1500	14.0 fg	7.5 de	290.0 cde
Eucalyptus	750	33.1 bcd	20.1 c	248.0 j
	1500	16.9 fg	8.3 de	279.5 efg
Cinnamon	750	42.5 b	32.6 b	235.9 k
	1500	21.1 ef	9.5 d	269.9 gh
At the same time				
Peppermint	750	2.5 hi	1.5 f	303.5 b
	1500	0.0 i	0.0 f	317.6 a
Eucalyptus	750	3.6 h	1.8 f	300.0 bc
	1500	0.0 i	0.0 f	318.1 a
Cinnamon	750	12.3 g	5.3 e	283.6 def

	1500	2.5 hi	1.5 f	302.0 bc
Control		66.7 a	47.3 a	143.8 l
Control [Healthy]		0.0	0.0	316.5 a

DISCUSSION

The six isolates of *U. maydis,* which collected from different Egyptian locations, were significantly varied in their pathogenicity on the local maize variety Giza 2. Inoculation with sporidia was more effective than teliospores for inducing common smut infection. Beni-Sueif isolate was the most virulent meanwhile isolate of Kafr El-Sheikh was the least virulent. The virulence of a known isolate was proportional with germinability of its teliospores. The high virulent isolate has the highest germination of teliospores. Mills and Kotzel, (1981) reported that teliospore germ tubes seem to be more important than sporidia in the epidemiology of the disease under field conditions. Kim and Lee (1996) found also that, teliospore inoculation produced smut symptoms but far less severely than the sporidial inoculum. The variations in virulence between isolates might attributed to genetic, physiologic and metabolic factors. Pope and McCarter (1992) indicated that certain pairwise combinations of compatible sporidial lines were more effective than others in inducing galls; Martínez *et. al.* (1997) recorded that the cultures of 10 *U. maydis* strains contained different levels of IAA. Guevara-Lara *et. al.* (2000) suggested that production of IAA by *U. maydis* might be involved in tumour formation. They found that the high levels of pathogenicity in general, were associated to high levels of IAA production by the strains. Mutual inhibition might exist between some strains in the same sporidial suspension. Clausen *et. al.* (1997) observed interstrain inhibitions between different *Ustilago maydis* strains. Sherwood, *et. al.* (1998) reported that the *Ustilago hordei*, the cause of barley covered smut, produce a factor that inhibited its own mating. The mating inhibition factor (MIF) specifically inhibited mating of *U. hordei* and other *Ustilago* spp., but not teliospore germination or sporidial growth. They added that since smut fungi must first mate to become pathogenic, mating inhibition has the potential to be an effective method of disease control for these pathogens. Whatever, the present results are holds fairly good with Moursy *et. al.* (1988) who found that isolates of *U. maydis* differed in pathogenicity to certain maize inbreds and Giza 2 variety. Isolates showed selective effect and certain isolates were aggressive than others. Rudenko, (1969); Tseng, (1988); Kim and Lee (1996) reported also

that the common smut disease symptoms appeared earlier and were more sever after inoculation with sporidia than with teliospore. On contrast, Fahmy (1983) found that the infection was more prevalent and maximized when inoculation was made with teliospores than with sporidia.

The present results indicated that, the root exudates of the tested crops reduced teliospores germination to different extents [22.8-97.5%]. In this regard, root exudates of garlic, onion, sugar beet, and faba bean were the most effective while clover "G.1 Sewa", chickpea, and wheat were the least effective. However, root exudates of barley, clover "Misqawy", and maize had no significant effect on teliospore germination when compared with germination in free water. In nature, the inhibitory effect of root exudates may occurred when spores were in contact to the roots. This inhibition effect might be due to different toxic metabolites that may existed at different concentration. The present results, however, are in agreement with William *et. al.* (1991); Harender *et al.* 1994); Gomes *et al.* (1997) and Santiago *et. al.* (2000). Burial of teliospores in fallow irrigated and non-irrigated soils reduced their germination by 34.6% and 25.0%, respectively. This may be due to rapidly decomposition of spores that germinated in wet soils and this will resulted in reducing its survival. Similar results and explanation were reported by Ragab *et. al.* (1984). The obtained results suggested, however, that cultivation of any of the aforementioned winter crops particularly garlic, sugar beet, oat, lupine, faba bean as preceding crop in maize-crop rotation might be more effective to reducing viability of *U. maydis* teliospores and infection with common smut than fallow-soil.

Incidence and severity of the common smut disease were relatively higher in 1998 than 1999 and considerably varied between the screened 20 maize accessions. The maize accession TWC-Baraka was the most resistant maize accession followed by the TWC-322 and the SC-129. We could recommend using these accessions under natural infestation where inoculum of teliospores is rarely being endemic in maize fields. On the other side, Giza-2 and Boushi, TWC.320, TWC-324, TWC-321, and the DC-Dahab were the most susceptible. Similar results were recorded by Asran (1993); Bader *et. al.* (1997); Kostandi *et. al.* (1997); and Soliman and Kostandi (1998). In fact, field resistance is required because presence of many pathogenic races or biotypes of *Ustilago maydis* (Shurtleff, 1980). Pataky, (1991) reported that resistance to common smut probably has been incorporated serendipitously in dent corn because infected breeding lines usually are

eliminated from breeding programs. The nature and durability of resistance, however, remains unknown. In some cases, resistance may be a polygenic trait that involves few genes that most likely condition functional, physiological and morphological characters such as tightness and thickness of husks are the logic means of resistance (Christensen, 1963 and Smith and White, 1988). Resistance of some hybrids to common smut may be attributed to harboring more antagonistic microorganisms on their leaves (Ragab, (1994). Kostandi *et. al.* (1997) tested the reaction of 10 different maize hybrids to artificial infection with smut disease under field conditions in Egypt and found that, the highly susceptible hybrids were the highest in total N content (N_t), while the reverse was true for the moderately or highly resistant ones. Variations in smut severity among hybrids were attributed to the lack in the efficiency of N translocation and its utilization by the plant.

Under stress of artificial inoculations with sporidia of *U. maydis* under field conditions, smut galls were formed more frequently below ear [63.2-65.1%] than above ear [51.4-56.2%] or in the ear itself [47.5-52.8%]. Percentage of galls that were formed at any of these sites on the naturally infected plants don't exceed 1.0%. The smut galls in the ear have larger diameter than those formed above or below ears. The largest galls in ears caused the greatest reduction in grain yield/plant (86.7-92.2%). However, the smallest galls that formed above and below ear reduced it by 36.1-40.7% and 19.5-24.6%, respectively. In fact, when the damage occurs to plant portion above ear, translocation of photosynthetic products and other nourishment to the newly formed ear would be affected directly and negatively. These results coincided with those obtained by Kostandi and Geisler (1989) and Tolba (1996).

Moursy *et.al* (2001) controled common smut infection significantly by spraying maize plants with oils of soybean, maize and sunflower. During this work the eucalyptus, cinnamon, and peppermint oils could inhibit the in vitro fungal growth completely at conc. more than 500 ppm while clove and anise oils only do this at conc. more than 250 ppm. The fungicidal effect of these oils was emphasized, as no fungal growth was occurs by transferring fungal discs from the plates showing no growth to Richards's oil-free medium. These plant oils probably affected fungal enzymes responsible for metabolism, permeability and/or fungal penetration into cell or cell membranes (Ismail *et al.*, 1989 and Sharaf-El-Din, 2000). Whatever, the promising effect of these oil applications against *U. maydis* was supported during

our greenhouse and field trials. Remarkable reduction in disease incidence and severity was occurred in greenhouse trials by injecting maize plants simultaneously with sporidial suspension and oils of peppermint, eucalyptus at 750 ppm or cinnamon at 1500 ppm. Spraying maize plants with any of these oil's application either 48 hrs before or after pathogen inoculation reduced disease incidence significantly but to less extent than simultaneous injection method. Applying the same treatments under the field conditions resulted in similar reduction in disease occurrence in addition to considerable increase in grain yield [302.0-345.1 g/plant] compared with control [143.8-161.2 g/plant]. These results indicate that these oils could be applied to maize plants as protective or curative substances. In general, eucalyptus, peppermint, and cinnamon oils, as reported by several investigators, to be efficient in controlling some other maize diseases rather than maize smut. Eucalyptus oil is known to be the most important component in the leaves which has the activity against plant pathogens (Singh and Dwivedi, 1987; Paran *et al.,* 1996; Montes and Carvajal 1998; and Fahmy and Mahmoud (2001).

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