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EFFECT OF PLANT OILS ON *USTILAGO MAYDIS* INFECTING MAIZE AND TEOSINTE

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ABSTRACT

Trials were conducted on bio-control of the common smut disease caused by *Ustilago maydis* (DC.) Cda on teosinte (*Zea mexicana*) and maize (*Zea mays*). Soybean, maize, sunflower and vegetable oils were used as foliar spray at 30 cc/L of water under artificial inoculation under greenhouse and field conditions. Oil foliar applications wear done one day before, on the same day and one day after plant inoculation by the smut pathogen, or before and after. The results on teosinte showed the efficacy of maize and soybean oils without significant differences. The overall results showed that the ranked order of treatments revealed the effectiveness of maize oil followed by the fungicides, vegetable oils (in one experiment), and soybean oil in a descending order in greenhouse. Repeated greenhouse test indicated significant differences between both treatments of soybean and maize oils, and the check (not-treated). Foliage oil spray after plant inoculation ranked first for oils followed by simultaneous spray and plant inoculation for soybean and sunflower without significant differences. Results on maize were similar to teosinte. Both results showed the efficacy of the used oils to control the smut disease on both crops.
Keywords: *Ustilago maydis* – teosinte – maize – plant oils – oil analysis – fatty acids

INTRODUCTION

Common smut caused by *Ustilago maydis*, (DC.) Cda is a worldwide occurring disease on maize for several decades early in the sixties and later on teosinte (*Zea mexicana*) green fodder cereal plant similar to maize. Fahmy, Zeinab and Oushy (2001) reported that teosinte is a maize-like summer fodder crop of currently minor acreage in Egypt. The crop never gained wide importance, mainly because of difficult seed production beside susceptibility to common smut.

Under Egyptian conditions, Moursy, Maysa *et al.* (1988) found that repeated inoculation indicated either stability or change in isolate virulence. Mansour *et al.* (1994) found that most of the maize cultivars tested for resistance to common smut were susceptible to highly susceptible. Certain inbreds, single crosses, and double crosses showed between 10-15% infection and classified as resistant. Fahmy, Zeinab *et al.* (1994) studied the efficacy of certain systemic fungicides (Raxil, Rizolex, Rixolex-T, Saprol, Tilt-100, Impact, Sumi-eight and Benlate) against common smut on maize. Seed dressing was not effective. Foliage spray application was feasibly effective when applied one week before artificial inoculation or before and after inoculation. Certain fungicides (Sumi-eight and Tilt) suppressed gall enlargement and disease development. Pre-inoculation spray protects the plant from infection. Up-dated work by Fahmy-Zeinab and Oushy (2001) showed that genotypes from teosinte (*Zea mexicana*) and maize (*Zea mays*) were highly susceptible to common smut disease.

Oil analysis and fatty acids composition:

Hafez (1984) reported that fatty acids are the integral constituents of every fat and oil. The degree of complexity of the glycerides basically depends on the number and amount of various fatty acids linked to the glycerol moiety. Also, the physical and chemical characteristics of lipids largely depend on their fatty acid composition.

Northover and Schneider (1993) reported that glyceridic oils are usually trisubstituted and most usually with long chain C_{18} acids. If monounsaturated oleic acid ($C_{18:1}$) or diunsaturated linoleic acid ($C_{18:2}$) predominante, the products are liquids at 25^{0}C and are oils.

Oil activity against plant diseases:

Martin and Salmon (1931 and 1933), early showed that several emulsified oils acted therapeutically by inactivating lesions of the powdery mildew of hop (*Sphaerotheca macularis*). Locke (1990) found that, surfactant-emulsified neem oil was more effective as a prophylactic treatment, giving excellent protection of bean plants against bean rust (*Uromyces appendiculatus*). Clayton *et al.* (1943) used multiple spray programs to protect tobacco seedlings from blue mold caused by *Peronospora tabacina* D.B. Adam and found that plant oils with high proportions of linoleic acid ($C_{18:2}$) or other polyunsaturated acids were fungicidal, whereas oils with high proportions of the monounsaturated oleic acid ($C_{18:1}$) were nonfungicidal.

The objectives of this study is to: (1) achieve disease biocontrol methods on teosinte through the use of natural plant oils to avoid chemical pollution, and (2) to increase teosinte seed production through disease crop protection to face crop area increase. The prospect of using plant oils as fungicides is very appealing, because refined oils are now readily available and safe for human consumption. (3) Disease control tests on maize were done in green house only being the alernative host of the pathogen.

MATERIALS AND METHODS

***Ustilago maydis* isolates:**

Teliospores of the pathogen were collected from both infected maize and teosinte field plants during 1998 crop season. Teliospores from mature galls were rinsed in 2% copper sulphate aqueous solution (Christensen and Stakman, 1926) for 48 hours. They were then streaked on potato dextrose agar (PDA) and incubated on 30°C. Single colonies (small and mostly derived each from a single teliospore) were asceptically picked and placed onto PDA slant until used (Moursy, Maysa *et al.*, 1988).

Preparation of inocula and inoculation:

Three cm^3 of either maize isolate and/or a teosinte isolate sporidial suspension randomly prepared at $13x10^5$ sporidia/ml prepared from 4-day old agar cultures were injected into the spindle above the growing apices of the treated plants. Isolates from each host were used to inoculate the same host.

Maize and teosinte seeds:

Maize G2 and teosinte local varieties were kindly supplied by the respective research sections of the Field Crops Research Institute, ARC.

Seed Planting:

a) Teosinte tests:

The first preliminary experiment was conducted under greenhouse conditions in 1999 to evaluate the activity of soybean, maize, sunflower and vegetables oil against common smut disease incidence on teosinte under artificial inoculation. Occidar fungicide was added for comparison. In this experiment, simultaneous plant inoculation and either oil or fungicide foliar spray was the only treatment for evaluation. Oils were used at 30 cm^3/L of water in quantities enough for complete plant coverage. Plant inoculation served as check treatment.

1. **Greenhouse tests:** When plants were 60-day old, they were inoculated by the pathogen sporidia as mentioned above. Seeds were planted under greenhouse conditions in summer seasons during may (25-30°C) in No. 25 clay pots. Eight seeds were planted in each of five replicates and thinned later to five, irrigated and manured as usual.
2. **Field tests:** Seeds were planted during July (1999) in rows; in ten hills/each of four rows and two rows were provided for each control treatment. When plants were 70-days old, they were inoculated each with 3 ml of sporidial suspension in the spindle above the growing apices and receive the control treatments simultaneously in the same time.
3. During 2000 summer season in greenhouse the same above control materials were applied on artificially inoculated local teosinte plants in different application sequences. The experiment was designed to show the best control material efficacy related to application time and plant inoculation.

b) Maize tests (in green house):

Seeds were planted and treated as those of maize. When plants reached 45-day old, they were inoculated by the pathogen sporidia (Fahmy, Zeinab and Oushy, 2001).

Natural plant oils and application treatments:

Oils of maize, soybean, and sunflower were obtained as food commodities from the local market. Natural oil 93% (w/v) of Stoller Chemical LTD-England was also obtained. Its active ingredient is a mixture of vegetable oils (93%). If not other wise stated, oils were applied on treated plants at 3 sequences; before, at, and after pathogen inoculation. Oils were applied at 30 cc/L of water as oil emulsions. Tween 20 as surfactant was added to the oil water mixture at the rate of 0.25 g/L.

Disease readings:

Disease readings were expressed as % infected plants 15-20 day after inoculation.

Oil analysis and fatty acids composition:

Gas-liquid chromatography by James and Martin (1952) was used for separation of micro-quantities of fatty acid esters. The % fatty acid composition in oils was determined.

Statistical design and analysis:

One factor randomized complete block design was used. Readings expressed as% infection were transferred to degrees. Least significant differences test (LSD) and Duncan Multiple Range Test described by Duncan (1955) and Snedecor and Cochrane (1967) were performed.

RESULTS

Teosinte tests:

First control test (Oil screening for efficacy):

Results Table,1 expressed as % infection transferred to degrees showed significant difference between check and both maize oil and Occidar fungicide. Soybean, sunflower, maize, vegetable oils and the fungicide showed no significant differences. However, the overall results showed that the ranked order of treatments revealed the effectiveness of maize oil followed by Occidar (fungicide), vegetables oil, soybean oil arranged in a descending order, indicating the priority of maize oil under this condition followed by the fungicide with significant differences.

Table (1): *Ustilago maydis* inoculation into teosinte variety Local in greenhouse and screening for oils control efficacy using oil foliar spray (30 cc/L of water), simultaneously with plant inoculation; 1999 planting season. Results are expressed as % infection transferred to degree.

Plant oils and treatments*	Degree of infection	Ranked order
Soybean		
*Spray + inoculation**	45.000 AB	4
Maize		
Spray + inoculation	38.775 B	1
Sunflower		
Spray + inoculation	48.775 AB	5
Vegetable oil		
Spray + inoculation	45.000 AB	3
Occidar (fungicide)		
Spray + inoculation	38.950 B	2
Inoculation only (check)	*56.900 A*	*6*

LSD at 0.05 = 12.16

*** Simultaneous plant inoculation and oil foliar spray.**

Second control test (Oil screening for efficacy):

The second test was conducted under field conditions in Sids Experimental Station Farm (Middle Egypt, to the south) in the summer season of 1999. The materials were examined by the same treatment as above (greenhouse test). In this case, significant differences were detected between both soybean and maize oils and the check treatments. However, the overall results showed the effectiveness of soybean oil followed by maize oil, Occidar (fungicide), sunflower oil, and vegetables oil arranged in a descending order indicating the priority of soybean oil followed by maize oil without significant difference.

Third control test (Screening for oil application sequences and/or time:

The results (Table 3) showed the same activity for the same oils in greenhouse (Tables 1 and 2), i.e. maize oil ranked first and followed by soybean oil and sunflower without significant differences. As time of control material application in relation to plant inoculation is concerned, foliage spray after plant inoculation ranked first for oils and Occidar (fungicide) followed by simultaneous spray and plant inoculation in soybean and sunflower oils without significance. Spray before plant inoculation was the least effective. The overall results in the three above tests are in agreement regarding oils control efficacy.

Table (2): *Ustilago maydis* inoculation into teosinte variety Local in field (Sids Station) and screening for oils control efficacy using oil foliar spray (30 cc/L of water), simultaneously with plant inoculation; 1999 planting season. Results are expressed as % infection transferred to degrees.

Plant oils and treatments*	Degree of infection	Ranked order
Soybean		
Spray + inoculation*	9.000 B	1
Maize		
Spray + inoculation	18.000 B	2
Sunflower		
Spray + inoculation	36.000 AB	4
Vegetable oil		
Spray + inoculation	40.500 AB	5
Occidar (fungicide)		
Spray + inoculation	31.500 AB	3
Inoculation only (check)	54.000 A	6

LSD at 0.05 = 24.23

Simultaneous plant inoculation and oil foliar spray.

Maize tests:

First control test (Season 1999):

Results, Table 4, show the effect of different sequences of oil application on artificial infection by *U. maydis* under greenhouse conditions. Oil foliar spray after *U. maydis* plant inoculation was the top effective in case of soybean and maize oils while effectively ranked first and third, respectively (without significant difference) after the fungicide (Sumi-eight). Sunflower oil was the least effective. Other application sequences were of lower effects. Results agree with those on teosinte and oils were the same effective as fungicides.

Second control test (Season 2000):

The application treatments were repeated under the same conditions in greenhouse with one more oil application treatment; spray before and after *U.maydis* plant inoculation. Results, Table 5, show considerable differences between oil application times without particular trend like that in the above tests on maize and/or teosinte. Effective treatments were either those including simultaneous spray and plant inoculation, and those including spray before and after inoculation. In this test maize oil ranked first followed by soybean and the fungicides. Sunflower was the least effective and ranked the fifth.

Table (3): *Ustilago maydis* inoculation into teosinte variety Local in greenhouse and evaluation of control treatments sequences. Results expressed as % infection transferred to degrees 15 days after treatment; 2000 planting season. Oils applied at 30 cc/L of water.

Plant oils and treatments*	Degree of infection	Ranked order
Soybean		
Spray before inoculation	53.950 BC	11
Spray + inoculation	45.000 CDE	7
Spray after inoculation	32.900 F	2
Maize		
Spray before inoculation	48.750 CD	8
Spray + inoculation	49.650 CD	9
Spray after inoculation	32.725 F	1
Sunflower		
Spray before inoculation	51.675 BC	10
Spray + inoculation	41.100 DEF	6
Spray after inoculation	36.300 EF	3
Occidar (fungicide)		
Spray before inoculation	60.000 AB	12
Spray + inoculation	38.175 EF	4
Spray after inoculation	38.775 ED	5
Inoculation only (check)	63.400 A	13

LSD at 0.05 = 8.931
LSD at 0.01 = 11.970
*** Sequences and/or time of oil application in relation to plant inoculation.**
**** Plant foliar spray with oil emulsion.**

Table (4): *Ustilago maydis* inoculation into G2 maize plants in greenhouse and control treatments application sequences. Results expressed as % infection transferred to degrees 15 days after inoculation; 1999 planting season.

Plant oils and treatments*	Degree of infection		Ranked order
Soybean			
Spray before inoculation	52.800	CD	11
Spray + inoculation	42.100	FG	8
Spray after inoculation	32.725	I	1
Maize			
Spray before inoculation	38.950	GH	5
Spray + inoculation	45.000	EF	9
Spray after inoculation	35.100	HI	3
Sunflower			
Spray before inoculation	42.100	FG	7
Spray + inoculation	54.900	BC	12
Spray after inoculation	39.200	FGH	6
Sumi-eight (fungicide)			
Spray before inoculation	60.000	AB	13
Spray + inoculation	36.050	HI	4
Spray after inoculation	47.900	DE	10
Spray before and after inoculation	34.000	HI	2
Inoculation only (check)	60.850	A	14

LSD at 0.05 = 5.353 **LSD at 0.01 = 7.167**

Spray = Plant foliar spray with oil emulsion in water (30 cc/L).
Inoculation = Plant inoculation with Ustilago maydis.

Table (5): *Ustilago maydis* inoculation into G2 maize plants in greenhouse and evaluation of control treatments application sequences. Results expressed as % infection transferred to degrees 15 days after inoculation; 2000 planting season.

Plant oils and treatments*	Degree of infectionorder		Ranked
Soybean			
Spray before inoculation	49.200	BC	15
Spray + inoculation	38.775	CDEF	9
Spray after inoculation	46.250	CD	13
Spray before and after inoculation	28.300	F	2
Maize			
Spray before inoculation	38.950	CDEF	10
Spray + inoculation	15.700	G	1
Spray after inoculation	35.025	DEF	7
Spray before and after inoculation	32.750	EF	6
Sunflower			
Spray before inoculation	47.475	BC	14
Spray + inoculation	32.550	EF	5
Spray after inoculation	35.100	DEF	8
Spray before and after inoculation	40.050	CDEF	11
Sum-eight (fungicide)			
Spray before inoculation	60.025	A	17
Spray + inoculation	32.075	EF	4
Spray after inoculation	41.272	CDE	12
Spray before and after inoculation	29.750	EF	3
Inoculation only (check)	57.975	AB	16

LSD at 0.05 = 10.16 **LSD at 0.01 = 13.56**

Spray = Plant foliar spray with oil emulsion in water (30 cc/L).
Inoculation = Plant inoculation with Ustilago maydis.

Oil analysis for fatty acids composition

Results, Table 6, show that linoleic acid in both soybean and maize oils (commercial commodities) were 65.31% and 42.83%, respectively and showed the higher percentage. It is known that oils having high content of this fatty acid are characterized by fungicidal activity (Clayton *et al.*, 1943). Other fatty acids occurred in markedly low percentage.

Table 6: Fatty acid composition % of soybean and maize oil (commercial commodities).

Fatty acids and composition		Soybean oil		Maize oil	
		RRT*	% Fatty acid	RRT*	% Fatty acid
Myristic	$C_{14:0}$	0.63	0.50	-	-
Palmatic	$C_{16:0}$	0.74	3.15	0.70	15.55
Palmitoleic	$C_{16:1}$	0.76	0.74	0.73	0.10
Stearic	$C_{18:0}$	0.81	0.80	0.88	2.16
Oleic	$C_{18:1}$	0.89	20.18	0.92	35.60
Linoleic	$C_{18:2}$	1.00	65.31	1.00	42.83
Linolenic	$C_{18:3}$	1.013	9.32	1.09	0.62

*** RRT: Relative retention time.**

Northover and Schreinder (1993) (after Daninco Inc. Mississauga, Ontario, Canada) reported that fatty acid composition of sunflower oil are 80% and 10% for oleic and linoleic acids (unsaturated), respectively (low linoleic acid).

DISCUSSION

The present study was initiated to find out a feasible bio-control method of common smut caused by *U. maydis* (DC.) Cda on maize (*Zea mays*) and teosinte (*Zea mexicana*). Teosinte, is highly susceptible to the smut disease which is the main constrain to seed production and increase of cultivated area during summer to face animal feed. Soybean, maize, sunflower and vegetables oil were suggested as foliar spray on both crops. The use of plant oils against certain plant disease is now widely known. McGee *et al.* (1989) used soybean oil to control storage fungi in grain (*Penicillium* and *Aspergillus* spp.).

Northover and Schneider (1993) studied the activity of plant oils on diseases caused by *Podosphaera leucotricha*, *Venturia inaqualis* and *Albugo occidentalis* and revealed the effectiveness of sunflower, olive, canola, corn, soybean and rapeseed oils. Bekheet *et al.* (1999) found that soybean oil and triton X-100 showed similar high prophylactic and/or therapeutic activity comparable to Dorado to control powdery mildew of sugar beet. The present results showed the efficacy of maize and soybean oils to control the common smut disease caused by *U. maydis* on teosinte and maize as foliar spray at 30 cc/L of water. Oils have control effect on pathogensis of plant pathogen, which is most likely through contact suppression of the pathogen structures development in plant tissues (deep seeted pathogens).

Soybean and maize oil analysis for fatty acids composition showed their high contents of lenoleic acid ($C_{18:2}$); 65.31% and 42.83%, respectively. Lenoleic acid showed a fungicidal effect on common smut disease caused by

U. maydis on teosinte and maize. Present results agree with Clayton *et al.* (1943) who concluded that lenoleic acid occurs in large amounts in most of the fungicidal oils. Cohen *et al.* (1990) found that against *Phytopathora infestans* linoleic acid were fungicidal, whereas oleic acid was non fungicidal. Calpouzos (1966) and Calpouzos *et al.* (1959) found that parffinic oils were fungicidal to *M. musicola* and *Sphaerotheca pannosa* (both of which are ascomycetes). These oils reduced photosynthesis in banana leaves and Calpouzos (1969) suggested that this might have suppressed the pathogenesis of *M. musicola*. This theory agrees with the present theory (present investigation; the authors) as to the effect of soybean and maize oils on common smut disease caused by *U. maydis* on teosintle and maize.

The results are promising and need to be further implemented, especially under field conditions to control the common smut disease.

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تأثير الزيوت النباتية على فطر يوستلاجو ميدس فى الذرة الشامى والذرة الريانة

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أجريت تجارب مقاومة مرض التفحم العادى المسبب عن الفطر يوستلاجو ميدس (.DC) .Cda على الذرة الريانة والذرة الشامى. إستخدمت زيوت فول الصويا والذرة الشامى وعباد الشمس وزيوت الخضروات رشاً على المجموع الخضرى للنبات بمعدل 30 سم3 لكل لتر ماء تحت ظروف العدوى الصناعية فى الصوبة الزجاجية وفى الحقل. أجرى الرش بالزيت قبل العدوى الصناعية بيوم أو فى نفس يوم العدوى أو بعد العدوى بيوم - أظهرت النتائج على الذرة الريانة كفاءة زيت الذرة وزيت فول الصويا ولكن بدون فروق معنوية وكان مجمل النتائج يدل على أولوية زيت الذرة يتبعه المبيد الأوميدار وسومى ايت ثم زيت الخضروات (فى أحد التجارب) يتلوه زيت فول الصويا فى ترتيب تنازلى فى الصوبة. وعند إعادة التجربة ظهرت فروق معنوية بين كل من معاملتى زيت فول الصويا وزيت الذرة بينهما وبين معاملة المقارنة (عدوى فقط) وأظهرت مواعيد رش الزيوت كفاءة الرش بعد العدوى الصناعية بالفطر يتبعها الرش والعدوى فى نفس اليوم بالنسبة لزيت فول الصويا وزيت عباد الشمس ولكن بدون فروق معنوية وأتفقت نتائج الرش على الذرة الشامى مع مثيلاتها على الذرة الريانة, وفى كلتا الحالتين ظهرت كفاءة الزيوت المستعملة فى مقاومة مرض التفحم العادى على المحصولين