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CONTROL OF LEAF BLIGHT DISEASE OF MAIZE USING TWO BIOAGENTS AND SOME NATURAL PLANT OILS

BY

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ABSTRACT

Four of each of (*Bacillus* sp and *Streptomyces* sp.), isolated from maize phyllosphere and some natural plant oils (Jojoba, Garlic and Castor) as well as El-Kanz 2000 (a natural compound) were evaluated for their antagonistic efficiency against *Helminthosporium turcicum* causing leaf blight disease of maize under laboratory conditions. As plant oils El-Kanz 2000 (1%) is being the best effective treatment with inhibition zone in diameter (30 mm), followed in descending by Garlic oil (2%), Jojoba oil (15%), and Castor oil (40%) with inhibition zones in diameters (24,19,18 mm, respectively). On the other hand, *Bacillus* sp. (No.4) gave the highest inhibition zone followed by *streptomyces* sp. (No3) .

Application of all control materials mentioned above were used simultaneously with *H. turcicum* showed the highest effect on leaf blight disease incidence followed by both application (72h. before and 72h. after *H. turcicum* spores spraying) under greenhouse conditions. Mixture of *Bacillus* sp. and *Streptomyces* sp (1: 1 v/v) was the most efficient treatment followed by El-Kanz 2000 after the chemical fungicide.

INTRODUCTION

Leaf blight caused by *Helminthosporium turcicum* is an important foliar disease of maize *(Zea mays)* in temperate areas of the world including Egypt. The disease is sporadic in occurrence, depending on the environmental conditions (Bentalila *et al.,* 1991). In Egypt, it is mostly found in the northern and north western regions of the Delta in late summer (off-season) maize planting, where favorable weather conditions are prevailing at that time of the year (El-Shafey, 1978; El-Assiuty, 1987 and Gouda, 1996). When infection became severe, high yield losses can occur when susceptible varieties or hybrids are grown in these areas. In 1970 the disease infection reached destructive proportions where yield was decreased by two-third or more in many areas (Hooker, 1975; Perkins and Hooker, 1981). Evaluation of maize inbreds, hybrids and varieties for resistance to *H. turcicum* were made by (El-Assiuty *et al.,* 1987; Diab *et al.,* 1993; Khalifa and Zein El-Abedeen 2000).

Jojoba oil, extracted from Jojoba seeds was used to control efficiently potato tuber rots (Hanna, 2001 and Tawfik *et al.*, 2001).

Antagonistic bacteria have been intensively studied as bio-control agents effective against air-borne pathogens. Among 20 genera of bacteria, *Bacillus* sp. and *Streptomyces* sp. are widely used for their characteristics as bio-control agents (Cook and Baker, 1983; Phae *et al.*, 1992; Lazzaretti *et al.*, 1995; Constantinescu *et al.*, 2002).

The objectives of this investigation was to study: **a-** The response of 12 genotypes to leaf blight disease caused *H. turcicum* under both artificial and natural infection. **b-** The efficiency of natural oils (Jojoba, garlic and castor) and two bio-agents (*Bacillus* sp. and *Streptomyces* sp.) in controlling the disease in an attempt to bio-control leaf blight disease to avoid the harmful effects of chemical fungicides.

MATERIALS AND METHODS

Isolation of the causal organism:

Samples of maize plants showing leaf blight disease symptoms were collected from different localities, Alexandria (Nobariya), Minoufia (Minouf), Kafr El-Sheikh (Sakha), Dakahliya (El-Mansoura), Sharkeya (El-Zagazig), Qualubiya (Qaliop) and Giza (Giza). Small pieces infected leaves with fresh lesions were cut, thoroughly washed with running water and left to dry, then were dipped in sodium hypochlorite solution (5%) for 2min., passed through three changes of sterile water and dried between two sterilized filter papers before they were plated onto sterilized potato Dextrose agar (PDA) medium Booth, (1971).Plates were incubated at 27°c for 7 days and examined daily for the occurrence of fungal growth. The growing fungi were examined microscopically and purified using single spore technique .The obtained isolates were maintained and refrigerated for further studies on the same medium.

Identification of fungal isolates:

The obtained fungal isolates were identified by morphological characteristics and microscopic examination according to (Robert and Jenkins, 1949). and confirmed by comparing these isolates with the culture collection of maize, sugar and forage crops Res-Dis-Dept., Plant Pathology. Res. Inst., Agric. Res. Center, (ARC). Giza, Egypt.

Preparation of spore suspension:

For preparation of fresh spore suspension of the phytopathogen, *H. turcicum* was grown on PDA medium in 9 cm. Petri plate at 27 °C for 15 days. Twenty ml of the sterilized distilled water added to each plate for spores release by using a small sterile brush or scalped to obtain a uniform spore suspension. The concentration of the spore Suspension was adjusted to 4000 spore / ml.

Pathogenicity test:

A Pot experiment was carried out to evaluate *H. turcicum* isolate for their virulence on two different maize cultivars, Giza 2 (susceptible) and three way cross (TWC) 320 (resistant), (Gouda, 1996). Pots were filled with silt soil (30 kg/pot),

seven seeds were sown in each pot, and then thinned to five plants. Plants were fertilized according to the standard method and were irrigated regularly using tap water. Spore suspension of each isolate of the pathogen that prepared previously was sprayed onto the leaves of potted plants (35-days old) or (at the 5-6 leaf stage of plant growth) using an atomizer (20 ml/plant). The conidial suspension was adjusted with a hemocytometer to a concentration of approximately 4000 spore / ml, 0.5ml of Tween 20 was added to 500 ml of the spore suspension. Control pots were sprayed with sterile distilled water. Three replicates were used for each isolate. All pots were kept in moist chambers for 24 h. After which they were exposed to greenhouse conditions at 25°C (temperature that favors development of the disease). Percentages of infected plants were recorded after about 2 weeks of inoculation. The fungus were easily isolated on PDA medium from the fresh lesions that appeared after about 2 weeks of the inoculation with the leaf crush, according to El-Shafey (1970). The obtained fungal isolates were identified as previously mentioned.

Evaluation of 12 hybrids to *H. turcicum*:

In a greenhouse experiment, 12 maize hybrids were evaluated for susceptibility to leaf blight. Eight seeds were planted in 20 cm diameter pots. Two weeks after emergence, plants were thinned to five plants per pot. Pots were arranged in a randomized complete block design with four replicates. Cultural practices, irrigation, fertilization...*etc.* was carried out as mentioned above. Artificial inoculation was carried out following the method adopted by (El-Assiuty *et al*, 1987). Plants were sprayed with spore suspension of the most aggressive isolate (35 days after sowing) Readings were taken after two weeks as percentages of infected plants.

Antagonistic effect of some natural plant oils against *H. turcicum*:

This experiment was conducted to evaluate four natural plant oils for their antagonistic activity against *H. turcicum*, the incitant of leaf blight on maize under laboratory conditions. Well cut diffusion technique according to Brock (1973) was used. Petri plates (9cm in diameter containing PDA supplemented with 5% peptone and seeded with *H. turcicum* (4000 spore/ ml) were also used. our wells were made equidistantly around the center of the plat using sterile cork borer, 0.2 ml of each oil was transferred to the well using sterile micropipette. Which prepared at different concentrations (0.5, 1, 2, 5, 10, 15, 20, 30, 40 and 50 %) Tween 20 was added at the rate of ½ ml/L. The chemical fungicide, Eminent (0.6 ml/L) was used for comparison. Prepared plates were incubated at 27°C for 7 days. Readings were taken as width of inhibition zone diameters (mm).

Antagonistic effect of *Bacillus* sp. and *Streptomyces* sp. against *H. turcicum*:

Four isolates of each *Bacillus* sp. and *Strepotomyces* sp. were examined for their antagonistic activity against *H. turcicum*. Spore suspensions for all treatments were prepared by transferring a loop of each *Bacillus* sp. 2 days-old and *Streptomyces* sp. 10 days-old to100 ml of (Nutrient broth and starch nitrate broth, respectively). The antagonistic reaction between each treatment and *H. turcicum* was conducted using agar diffusion technique Brock, (1973) as mentioned previously. Prepared plates were incubated for at 28 C for 48 h. for *Bacillus* sp and 10 days for *Streptomyces* sp. Readings were recorded as aforementioned.

***In vivo* antagonistic effect of *Bacillus* sp. and *Streptomyces* sp. as well as natural plant oils against *H. turcicum*:**

Spore suspensions of *Bacillus* sp. (No.4) and *Streptomyces* sp (No.3) singly and in combination (1:1 v/v) were used. Natural plant oils (Castor, Jojoba and Garlic) in addition to El Kanz2000 at the rates of (40, 15, 2, 1 ml/L, respectively) as solutions in water were also used. All these control materials were applied at three intervals (72h. before, simultaneously and 72h. after *H. turcicum* spore spraying) after 35 days of sowing. Plants were sprayed with 20 ml of each treatment /plant. Plants sprayed with *H. turcicum* spores only were used as control. Four pots were used for each treatment. Data were taken as % infection transferred to degrees and LSD was calculated.

RESULTS AND DISCUSSION

Pathogenic variation of *H. turcicum* isolates:

Results presented in Table (1) indicate that all the tested isolates varied in their virulence. Giza 2 cultivar was more susceptible to *H. turcicum* with infection rate of (50.2% - 96.3%) whereas, TWC 320 was less susceptible (1.5%-12.0%) infection. As can be seen, isolate (No.6), was the most aggressive one causing the highest infection percentage on both maize cultivars (96.3% and 12.0%).Therefore isolate (No. 6), was selected for further studies.

Evaluation of 12 hybrids to *H. turcicum*:

Twelve hybrids were screened for disease reaction under greenhouse conditions. Ratings were recorded as percentage of leaf area infected, about two weeks after *H. turcicum* spore sprayed (35 days-old). The results tabulated in Table 2. Maize hybrids were classified to six categories according to the 0.5 (very light infection) to 5.0 (plants nearly killed) rating scale as follows:

(Hybrid T.W.C 321)	was highly resistant.
(Hybrid S.C 10)	was moderately resistant.
(Hybrid S.C 123)	was resistant.
(Hybrid S.C.15, 129, TWC 322,323,324)	were moderately susceptible.
(Hybrid S.C 122,124,155)	were susceptible.
(Hybrid TWC 310)	was highly susceptible.
So, Hybrid TWC 310	was used in further studies.

Table (1): Susceptibility of two maize cultivars to infection by different isolates of *H. turcicum*.

Isolates	Governorates	Location	%Infection	
			Susceptible Giza2	Resistant TWC320
1	Dakahliya	El mansoura	82.5	2.0
2	Kafr El-sheikh	Sakha	83.8	2.2
3	Shaekeya	Zagazig	73.8	1.8
4	Qualubiya	Qualube	72.5	1.5
5	Giza	Giza	50.2	2.1
6	Alexandria	Nubariya	96.3	12.0
7	Minoufia	Menouf	84.5	3.2

Table (2): Reaction of 12 mize hybrids to *H.turcicum* disease in greenhouse during 2000/2001.

No.	Hybrids	% Infection			
		2000	2001	Mean	Host Reaction
1	S.C 10	25.5	24.0	24.8	Mr
2	S.C 15	35.3	29.3	32.3	Ms
3	S.C 122	70.0	69.0	69.5	S
4	S.C 123	8.4	9.6	9.0	R
5	S.C 124	51.3	50.6	51.0	S
6	S.C 129	27.2	25.3	26.3	Ms
7	S.C 155	69.3	66.0	67.7	S
8	T.W.C 310	90.0	85.3	87.7	Hs
9	T.W.C 321	3.0	2.7	2.85	Hr
10	T.W.C 322	36.7	42.0	39.3	Ms
11	T.W.C 323	30.6	29.3	30.0	Ms
12	T.W.C 324	43.4	43.9	43.6	Ms
Mean		41.2	39.8	40.3	
LSD 0.05 for Season(S) 0.59		Cultivars (C) 1.33		S×C 1.87	

R=Resistant S=Susceptible Hr= Highly resistant
Hs= Highly susceptible Mr= Moderately resistant Mr=Moderately resistant

Effect of natural plant oils on mycellial growth of *H. turcicum*:

Results in Table (3) show that, all concentrations of natural plant oils under test significantly reduced the mycellial growth of *H. turcicum*, but with different inhibition zones. El- Kanz2000 (1%) is being the best effective treatment with inhibition zone in diameter (45 mm) followed in descending order by Garlic oil (2%), Jojoba oil (1.5%), and Castor oil (4.0%) with inhibition zones in diameters (24,19,18 mm, respectively) compared to control (Tween20) which has no any effect on mycellial growth (0.0 mm).Superiority of El-Kanz2000 was previously recorded by (Zein El-Abedeen and El-Menchawy,2001 and Ata, 2006).

Antagonistic effect of *Bacillus* sp. and *Streptomyces* sp. against *H. turcicum*:

Antagonistic effect of four *Bacillus* sp. and four *streptomyces* sp. against *H. turcicum* was examined according to Brock (1973). Results in Table (4) show that all *Bacillus* sp and *streptomyces* sp. activly inhibited the mycellial growth with varied inhibition zones. With *Bacillus* sp. (No.4) and *Streptomyces* sp. (No.3) showing the highest antagonistic reaction of (32 and 21mm respectively), the highest effect of *Bacillus* sp. may attributed to their advantages over that of *streptomyces* sp. (formation of indospores that are tolerant to heat and desiccation these results are in agreement with (Ibrahim, and Zein El-Abedeen, 2000 and Osman, 2004).

Table (3): Effect of different concentrations of natural plant oils on vegetative growth of *H. turcicm.*

Natural plant oils	Inhibiton zone in diameters (mm) % Concentrations									
	0.5	1.0	2.0	5.0	10.0	15.0	20.0	30.0	40.0	50.0
Kanz2000	35.0	45.0	45.0	-	-	-	-	-	-	-
Castor oil	8.0	10.0	12.0	13.0	14.0	16.0	17.0	18.0	[illegible]	20.0
Jojoba oil	9.0	10.0	13.0	15.0	17.0	19.5	19.0	-	-	-
Garlic oil	17.0	20.0	24.0	24.0	-	-	-	-	-	-
Tween 20 (Control)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table (4): Effect of *Bacillus* sp. and *Streptomyces* sp. on vegetative growth of *H. turcicm.*

No	Bioagent	Main diameter of inhibition zone(mm)
1	*Bacillus* sp	28
2		29
3		30
4		32
1	*Streptomyces sp.*	18
2		20
3		21
4		19

***In vivo* antagonistic effect of *Bacillus* sp. and *Streptomyces* sp. as well as natural plant oils against *H. turcicum*:**

Results presented in Table (5) show that, all treatments at all three dates of application (simultaneously, 72h. before and after *H. turcicum* treatment) affected positively leaf blight disease incidences, on TWC 310 plants but, with different degrees. The most effective and suitable time was when control materials were applied simultaneously with *H. turcicum* treatment followed by application 72h. before and 72h. after *H. turcicum*. spores spraying, respectively. Significant variations were recorded between all control treatments and check (plants sprayed with *H. turcicum*. spores only). Results proved the superiority of the mixture, (*Streptomyces* sp. + *Bacillus* sp.) at the rate of (1:1v/v) as an efficient bio-control of leaf blight in maize when applied simultaneously with *H.turcicum* of infection percentages (6.7 and 6.0 during the two successive seasons, respectively). With in average 6.3 %. This is parallel line with results reported by Ibrahim, (1990), Ibrahim and Zein El-Abedeen (2000) and Osman (2004). Among natural plant oils tested, El- Kanz2000 was the most efficient one after the chemical fungicide (Eminent). These results were confirmed by (Ata, 2005 and Ata, *et al.*, 2006) on sugar beet rust disease.

Table (5): Effect of application times and control materials on *H. turcicum* disease under greenhouse conditions during seasons 2002,2003.

Treatments	% Infection / Application times											
	2002				2003				Average of 2 years			
	X	Y	Z	Mean	X	Y	Z	Mean	X	Y	Z	Mean
St	21.1	18.1	22.4	20.5	20.6	17.6	21.8	20.0	20.9	17.8	22.1	20.3
B	20.7	17.5	22.1	20.1	20.2	17.0	21.3	19.5	20.4	17.3	21.7	19.8
St + B	8.4	6.7	9.6	8.2	7.7	6.0	9.0	7.6	8.1	6.3	9.3	7.9
Kanz2000	8.9	7.3	10.3	8.8	8.1	6.7	9.6	8.1	8.5	7.0	9.9	8.5
Castor oil	25.1	23.3	26.3	24.9	24.6	23.0	25.5	24.4	24.9	23.2	25.9	24.6
Jojoba oil	20.1	17.2	21.3	19.6	19.6	16.8	20.7	19.0	19.8	17.0	21.0	19.3
Garlic oil	14.1	13.5	16.1	14.5	13.6	13.4	15.6	14.2	13.9	13.4	15.9	14.4
Twee20	90.4	89.7	89.0	89.7	87.0	86.4	84.4	85.9	88.7	88.0	86.7	87.8
Eminent	3.5	2.6	4.2	3.4	3.1	2.0	3.4	2.8	3.3	2.3	3.8	3.1
Control	90.3	90.0	90.0	90.1	85.3	85.0	85.0	85.1	87.8	87.5	87.5	87.6
Mean	30.3	28.6	31.1	30.0	29.0	27.4	29.6	28.7	29.6	28.0	30.4	29.3
LSD 0.05 for												
Season (S) 0.24 Application times (A) 0.29 Treatments (T) 0.53												
S× A 0.40 S×T 0.75 A×T 0.91												

X,72h. before spores spray. Y, Simultaneously with spores spray.
Z,72h. after spores spray. St, *Streptomyces* sp. B, *Bacillus* sp

REFERENCES

Ata, A.A. (2005): Studies on sugar beet rust disease in Egypt. M. Sc. Thesis, Plant Path. Dept., Fac. Of Agric., Ain Shams Univ. Cairo, pp124.

Ata, A.A.; El- Samman, G. M.; Moursy, Maysa, A.; Masour, M. I. and Mostafa, H. M. (2006): Control of beet rust by natural plant oils and systemic fungicides. 10 conf. Agric. Dev. Res., Fac. Agric., Ain Shams Univ., Cairo, Egypt, 2006. Anuals Agric. Sci., SP. Issue, 1,193 -205, 2006.

Bentolila, S.; Guitton C.; Bouvet N.; Sailland A.; Nykaza S. and Freyssinet G. (1991): Identification of an RELP marker tightly linked to the Ht1 gene in maize . Theor- Appl. Genet.,82:393-398.

Booth, C. (1971): Methods in microbiology. Vol. 4, 795pp., Acad. Press, London and New York .

Brock, T.D. (1973): Basic Microbiology with Application. Prentic-Hall Inc., Englewood Cliffs, New Jersey, USA.

Constantinescus, F.; Sesan, T. E.; Eland, Y.; Kohl, J. and Shtienberg, D. (2002): Soil-born fungi and host plant influence on the efficacy of *Bacillus subtilis* bio-control agents. Proc. 7th work. group meet , Influence of Abiotic and Biotic Factors on Bio-control Agents. Pin Bay, Kusadasi, Turkey 22-25 May 2002.

Cook, R.J.; and Baker, K.F.(1983): The Nature and Practice of Biological Control of Plant Pathogens. St. Paul, Minn., Am. Phytopathol. Soc., 539 pp.

Diab, M. S.; El-Mersawy, E. M. and Koriem, A. M. (1993): Response of some maize genotypes to leaf blight diseases in Egypt. Eg. J. of App. Sci, 8: 136-150.

El-Assiuty, E.M.; Salma, F.A.; El-Fangary, I.M. and El-Shafey, H.A. (1987): Identification of diverse sources of resistance to *Helminthosporium turcicum* Pass. Agric. Res. Rev. 65: 243-253.

El-Shafey, H.A. (1970): A study of *Helminthosporium* leaf-spot diseases of maize and sorghums in U.A.R. with special reference to leaf blight caused by *H. turcicum*. Ph. D.Thesis, Fac. of Agric. Al-Azhar Univ.

El-Shafey, H.A. (1978): A comparison of three types of resistance to *Helminthosporium turcicum* in maize. Agric. Res. Rev., Min. Agric., 56:8-85.

Gouda, M.I. (1996): Studies on maize leaf blight disease in Egypt. Ms.C. Thesis, Fac. Of Agric., Minofia Univ. 96p.

Hanna, A.I.(2001): Effect of Jojoba plant extracts and antagonistic bacteria on population of rhizosphere organisms and potato tuber rots . Egypt J. Appl. Sci., 16: 436-475.

Hooker, A. L. (1975): *Helminthosporium turcicum* as an a pathogen of corn. Rept. Tottori Mycol. Inst. Japan 1875: 115-125.

Ibrahim, Thanaa F. (1990): Studies on the biological control of the late wilt disease of maize. Ph. D. Thesis, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

Ibrahim, Thanaa F. and Zein El-Abedeen, A. (2000): Biological control of Acremonium wilt of grain sorghum caused by *Acremonium strictum*. Annals Agric. Soc. Moshtohor, 38(1) : 199-208.

Khalifa, K.I. and Zein El-Abeden, A.(2000): Turcicum leaf blight eisestance in some Egyptian maize genotypes under surface and sprinkler irrigation systems. Egypt J. of Appl. Sci 15: 410-421.

Lazzaretti, E.; Menten, J.O.M. and Bettiol, W. (1995): Treatment of wheat seed with *Bacillus subtilis* for the control of *Pyricularia oryzae, Bipolaris sorokiniana* and *Alternaria tenuis*. Phytopatologica, 21(2) : 163-167.

Osman, Ettimad A. H. (2004): Studies on the interaction between soil microflora and the pathogenic organisms causing sorghum diseases. M.Sc. Thesis, Agric., Microbiol. Dept., Fac. Of Agric Cairo Univ. Egypt pp166.

Perkins, J. M. and Hooker, A. L. (1981): Reaction of eighty-four sources of chlorotic lesion resistance in corn to three biotypes of *Helminthsporium turcicum*. Plant Dis. 65:502-504.

Phae, C.G.; Shoda, M.; Kita, N.; Nokano, M. and Ushiyama, K. (1992): Biological control of crown and root rot and bacterial wilt of tomato by *Bacillus subtilis* Ann. Phytopathol. Soc. Japan, 58(3): 329-339.

Robert, A.L. and Jenkins, M. T. (1949): Variability in monoconidial and hyphal-tip isolates of *Helminthosporium turcicum*. Phytopathology, 50: 261-263.

Tawfik, A.E.; Hanna, A.I.; El-Ghareeb, L.A.; Gomah, A.A. and Mahmoud, S.M. (2001): Applied approach for controlling brown rot and bacteria of potatoes. J. Agric. Sci., Mansoura Univ., 26: 3631-3642

Zein El-Abedeen, A and Mervat M. El-Menchawy (2001): Evaluation of some grain sorghum genotypes and detection of oospores of downy mildew fungus. Egypt. J. Appl. Sci.; 16 (1).

مقاومة مرض لفحة الاوراق فى الذرة الشامية باستخدام عاملين حيويين وبعض الزيوت النباتية

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** قسم الميكروبيولوجيا - كلية الزراعة جامعة القاهرة .

فى المعمل درس تأثير ٤ انواع من كل من الباسيلس والاستربتوميسيس والمعزولةمن فللوسفير نباتات الذرة وكذلك زيت الجوجوبا والثوم والخروع والمركب الطبيعى الكنز ٢٠٠٠على نمو الفطر المسبب لمرض لفحة الأوراق في الذرة الشامية فى المعمل - وجد أن الكنز ٢٠٠٠ عند تركيز ١% أعطى أقوى تأثير يلية زيت الثوم عند تركيز ٢% ثم زيت الجوجوبا عند تركيز ١٥%ثم زيت الخروع عند تركيز ٤٠% - كما وجد أن تاثير الباسيلس أقوى من تأثير والاستربتوميسيس فى تثبيط الفطر المسبب للمرض . في الصوبة : عند تطبيق مواد المقاومة رشا على النباتات مع رش الجراثيم الكونيدية للفطر المسبب للمرض أعطت أعلى تأثير يليها عند رش مواد المقاومة قبل رش جراثيم الفطر بـ٧٢ ساعة ثم يليها بعد رش جراثيم الفطر بـ٧٢ساعة- بينما أعطى خليط كل من الباسيلس والاستربتوميسيس أعلى تأثير في مقاومة المرض يليه الكنز ٢٠٠٠ .