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OCCURRENCE OF *Exserohilum turcicum* F.SP.*SORGHI* THE CAUSAL ORGANISM OF SORGHUM LEAF BLIGHT IN UPPER EGYPT

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ABSTRACT

In 2005, severe attack of sorghum leaf blight caused by *Exserohilum turcicum* was observed on grain sorghum G-15(*Sorghum bicolar*) and Sudan grass (*Sorghum sudanense*) adjacent to healthy maize plants in Sids Agricultural Research Station (SARS) of Upper Egypt, A.R.C. Therefore, field experiments were conducted in 2006, 2007 and 2008 to determine host specificity type of *E. turcicum* in SARS on a set of ten sorghum and maize varieties. Two *E. turcicum* isolates obtained from lesions of infected sorghum plants were tested for their virulence in the greenhouse on seedlings of the same set of varieties used in the field. Colony and conidial morphology, radial growth at four temperature and serological studies of these sorghum isolates were compared with two maize isolates. One colony type was clearly distinguishable between sorghum isolates from nature at SARS. Fungal colonies from sorghum were generally characterized as having dark olivaceous, determinate margin with scant appressed aerial hyphae. Meanwhile fungal colonies from maize had a determinate margin and profuse aerial hyphae about the center of a gray to green-white colony. Sids isolates of *E. turcicum* attacked only its own hosts, local grain sorghum and Sudan grass, under natural and artificial infections. Our results reveled that sorghum isolates had a fast radial growth in culture than maize isolates at 27°C. At the same time, there were considerable differences in conidial characters among isolates of *E. turcicum* from the two hosts although they were serologically identical. Conidia of sorghum isolates had a long length range and more septa than of maize isolates (66-145 µm, 6 and 40-92 µm, 4 septate respectively). Based on the occurrence of distinct fungal colony type of *E. turcicum* isolates from sorghum along with host-specificity, fungal isolates obtained from Sids were classified as forma specialis.
Keywords: *Exserohilum turcicum* f. sp. *sorghi*, Egypt, colony type, sorghum, maize.

INTRODUCTION

Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* (Pass) K. J. Leonard & E. G. Suggs, teleomorph *Setosphaeria turcica* (Luttrell) K. J. Leonard and E. G.) occurs in corn, sorghum; Johnson grass and Sudan grass (White, 1999) in most humid regions of the world and seems to be favored by moderate temperature and heavy dew formation. In Egypt, the disease was first described by Melchers (1933) and it is found mostly in the northern parts of the country in the Delta during August, September and October (EL-Shafey, 1978; Diab, 1980; EL-Assiuty *et al*., 1987 and Gouda, 1996). Crop losses in maize and sorghum can surpass 50% when infection becomes severe before flowering (Raymundo & Hooker, 1981; Tefferi *et al*., 1996 and Frederiksen & Odvody, 2000) and yield from plants infected at mid-season may be reduced by as much as 68 % (Ullstrup & Miles, 1957). At least seven physiologic races on monogenic corn lines of

E. turcicum have been reported. Races are named on the basis of the *Ht* genes to which they are virulent; *e.g*., race 0 is avirulent on all *Ht* genotypes with the virulence formula *Ht*1, *Ht*2, *Ht*3, *Ht*N / 0, and race 23 is virulent on *Ht*2 and *Ht*3 genotypes with the virulence formula *Ht*1, *Ht*N / *Ht*2, *Ht*3 (White, 1999). Two physiological groups were reported in India by Bhowmik and Prasada (1970) when isolates of the fungus from corn, sorghum, and Sudan grass were tested on these hosts. Masias and Bergquist (1974) found heterokaryons of *E. turcicum* from nature to be pathogenic to both corn and sorghum and homokaryotic isolates to be either host-specific and / or non-host-specific to corn, sorghum or Johnson grass. In Egypt, EL-shafey *et al*. (1982) and El-naggar, A., (2006) reported that the behavior of the collected *E. turcicum* isolates from different host species grown in different governorates were represent three distinct parasitic isolates (infect maize only, infect sorghum only and capable to infect maize and sorghum). In 2005 we observed heavily infection of some grain sorghum and Sudan grass by northern leaf blight adjacent to healthy corn plants in Sids Agricultural Research Station (SARS), A.R.C. Therefore, the current study was an attempt to determine the host specificity type of *E. turcicum* in SARS of Upper Egypt.

MATERIALS AND METHODS

Field trials:

Field experiments were conducted in 2006, 2007 and 2008 to determine the host specificity type of *E. turcicum* in Sids Agricultural Research Station (SARS) of Upper Egypt, A.R.C. Experiment 2006 involved four varieties (two from sorghum and two from maize), meanwhile the other two experiments included ten varieties (Table 1). All varieties tested for their response to *E. turcicum* under natural infection. The experiments were conducted as a randomized complete block design with three replications. Each replicate consisted of two rows, 6m long spaced 70cm apart. Distance between hills was 20 cm. Agronomic practices were followed according to the common recommendations. Seeds were hand planted on the first week of July during the three seasons.

Disease evaluation:

Disease severity was estimated 35 days after flowering by the scale of 0.5 to 5 (Elliott and Jenkins, 1946; Hughes & Hooker, 1971 and Hooker & Kim, 1973).

Morphological characteristics:

Two sorghum isolates of *E. turcicum*, obtained as described by El-Naggar (2006) from lesions of northern sorghum leaf blight appeared at Sids Agricultural Research Station (SARS), one from grain sorghumG-15 (SD.TS-1) and the other from Sudan grass (SD.TS-2). The isolates were used to study their cultural, physiologic and serologic characters compared with the maize isolates (GM.TC-1 and GM.TC-2) obtained from Gemmeiza Agricultural Research station (GARS).

Colony type; agar plugs (5mm in diameter) of the four previous isolates grown on Potato Dextrose Agar (PDA) for 10 days, were transferred to plates of potato lactose agar (PLA) (Borchardt *et al*., 1998) and incubated at 27°C for 7 days. Characters of fungal colonies were estimated.

Conidial size; the dimensions of 60 conidia of each isolate grown on Lactose Casin Hydrolysate Agar (LCHA) (Fallah Moghaddam and Pataky, 1994) for 7 days at 27°C were measured with the aid of 0.01mm micrometer slide (Poland, Warszawa Company).

Temperature effect:

The previous four isolates were used to estimate the radial growth at 20, 23, 27and 35 °C on PLA after 10 days of incubation using 8mm of diameter agar plugs. The experimental design was complete randomized blocks with five replications (9cm plates).

Greenhouse test:

To determine if Sids isolates capable to attack sorghum varieties only or both sorghum and maize varieties the same 10 varieties used on the field were tested for their response in greenhouse. SD.TS-1 and SD.TS-2 isolates with the suspension of about 10^3 conidia per ml were sprayed onto 40 days old plants (four pots, 20 cm diameter, per variety). The sprayed plants (incubated at 25°C) with the aid of atomizer were covered with a poly ethylene sheet for 48 h. Thereafter, covers were removed, and then plants were misted for 5 days. Disease assessed 2 weeks after inoculation as; infected (+) or not infected variety (-) as described by (Carson and Van Dyke, 1994).

Serological test:

Ouchterlony double diffusion test (Ouchterlony, 1949) with a slight modification was conducted to compare serological patterns of *E. turcicum* sorghum-isolates obtained from Upper Egypt (SARS) with the maize-isolates obtained from Delta (GARS) (Fig. 2). Antigen and antiserum preparation were determined as described by El-naggar (2006). Fifteen ml of 1% agarose gel amended with 0.85% sodium chloride and 0.025% thiomerasole poured onto 4 x 8 cm cleaned glass slide. Upper and bottom wells for antigens as well as a trough for antiserum were made by a bencher after gel solidified (Fig. 2). Eighty micro-liter of antigen isolates, SD.TS-1 (1); SD.TS-2 (2) and SK-TS-3(3), obtained from sorghum were loaded onto the upper wells. At the same time antigen of isolates GM.TC-1 (4); GM.TC-2 (5); from maize and SK-TS-3(3) from sorghum were loaded onto the bottom wells. A middle trough filled with 300ul of sorghum-isolate SK-TS-3(AS (3)) antiserum (which capable to infect both sorghum and maize in previous study; El-naggar, 2006). The slide placed in a humidity chamber at 8^oC and observed for precipitin lines after one week. The slid was pressed, dried, stained and de-stained as described by El-naggar (2006). Finally the slides were dried, captured and precipitin lines were recorded.

Statistical analysis:

Data were analyzed, as a randomized complete blocks design (field trails) or a completely randomized blocks design (temperature effect and conidial size experiments). Treatment means were compared by Duncan's

multiple rang test. Analysis of variance (ANOVA) was carried out by using COSTAT software virgin 3.

The obtained data (conidial length, width and septate number as well as the range of length, width and septa number) were subjected to cluster analysis using option Distance Matrix according to **Bray and Curtis** (**1957**) of Excel software to estimate phenotypic distance among sorghum and maize isolates.

RESULTS

Field trails: All the local varieties of grain sorghum (G-15, G-113and G-123) and Sudan grass exhibited a wide range of disease rate ranged from 2 to 4.2 in all seasons. Meanwhile the short grain sorghum (Dorado, Horas, and Izeas) and maize varieties (SC-122, SC-129 and Giza-2) did not show any symptoms (Table, 1). Although all of the local grain sorghum and Sudan grass varieties infected by *E. turcicum*, there were significant differences in disease rate between them. Generally, fungal isolates of Sids station infected some variety of sorghum species, i.e., local varieties of *Sorghum bicolor* and Sudan grass of *Sorghum sudanense*.

Table 1: Mean of disease rate of *Exserohilum turcicum* on a set of sorghum and maize varieties at Sids Agricultural Research Station during 2006, 2007 and 2008 in the field.

Sorghum and maize va	Genus & spec	2006	2007	2008
G-15 (local)	*Sorghum bicola*	4[ay]	3.5[a]	4.2[a]
G-113 (local)	*Sorghum bicola*	- *	3.4[a]	4.1[b]
G-123 (local)	*Sorghum bicola*	-	3.5[a]	4 [c]
Dorado (short)	*Sorghum bicola*	0 [c]	0 [c]	0 [e]
Horas (short)	*Sorghum bicola*	-	0 [c]	0 [e]
Izeas (short)	*Sorghum bicola*	-	0 [c]	0 [e]
Sudan grass (tall)	*Sorghum sudan*	2.4 [b]	2.1 [b]	2 [d]
SC-122 (maize) [z]	*Zea mays*	0 [c]	0 [c]	0 [e]
SC-129 (maize)	*Zea mays*	0 [c]	0 [c]	0 [e]
Giza-2 (maize)	*Zea mays*	-	0 [c]	0 [e]

***Not cultivated.**

[y] Means within each column followed by the same letter are not significantly different at *p*=0.05% according to the Duncan's Multiple Rang Test. [z] Sc= Single cross

Colony morphology: *E. turcicum* colonies (Fig 1) from sorghum were generally characterized by dark olivaceous, having a determinate margin and scant, appressed aerial hyphae. Whereas, colonies of maize isolates had a determinate margin and profuse aerial mycelium about the center of gray to green-white colony.

Conidia were sparsely produced by all isolates on PLA but were abundant on LCHA. Conidia of the four tested isolates which borne singly at the tips of the conidiophores (Fig 2, A&B) were olive gray, spindle shape with a protruding hilum and three to eight septate. Conidia of sorghum isolates (Table, 2) had a mean size (width and length) of 14x95 and 18x114µm with the range from 13-20 x 66-118 to 13-20 x 66-145µm. At the same time, mean size of maize isolates was 19x75 and 20x81µm but ranged from 13-26 x 40-92 to 13-26 x 53-92µm. Generally, sorghum isolates were significantly longer and

more in length and septate number of conidia than maize isolates respectively. Likewise, cluster analysis of all parameters of 60 conidia (Fig, 4) show that the two sorghum isolates separated onto one cluster (with the distance 0.08) regardless the maize isolates which were separated within another cluster (with the distance 0.04).

Table 2: Mean of conidial characters of two sorghum and two maize isolates of *E. turcicum* grown on LCHA medium after 10 days of incubation at 27°C.

Isolates	Length (µm)		Width (µm [y])		Septate number/ conidia	
	Mean[z]	Range	Mean[z]	Range	Mean[z]	Range
SD.TS-1	95 a	66-118	14 a	13-20	6 a	2-8
SD.TS-2	114 b	66-145	18 a	13-20	6 a	2-7
GM.TC-1	75 c	40-92	19 a	13-26	4 b	2-6
GM.TC-2	81c	53-92	20 b	13-26	4 b	2-7

[y] Width measured only at the center of the conidium.
[z] Mean of 60 conidia of each isolate. Means within each column followed by the same letter are not significantly different at *p*=0.05% according to the Duncan's Multiple Rang Test.

Temperature effect: Significant differences were obtained in radial diameters growth after 10 days of the tested isolates under the different temperatures (Table, 3). Results (Table 4) show that, radial diameters growth of sorghum isolates ranged from 11.5 to 90 mm; however it ranged from 9 to 89 mm for maize isolates.

Table 3: Analysis of variance for radial growth (mm) on potato lactose agar after 10 days of incubation at 20, 23, 27 and 35 °C for four isolates of *E. turcicum* from Sids (sorghum isolates) and Gemmeiza (maize isolates) agriculture research stations.

Source of variance	df [a]	MS [c]	F value	P
Isolates	3	1290.7	17.2	0.0000
Temperatures	3	24188.8	323.1	0.0000
Isolates x Temperatures	9	813.0	10.9	0.0000
Error	64	74.86		
Total	79			

[a] df =degrees of freedom and [c] MS = mean square. 0.000=significant at P ≤0.05.

Table 4: Radial growth (mm) on potato lactose agar at 20, 23, 27 and 35 °C for four isolates of *E. turcicum* from two locations; Sids and Gemmeiza agriculture research stations.

Temperature	Sorghum isolates		Maize isolates	
	SD.TS-1	SD.TS-2	GM.TC-1	GM.TC-2
20°C	90	90	89	55
23°C	90	90	85	80
27°C	90	84	58	70
35°C	11.5	15	10	9
Mean	70.4	69.7	60.5	53.5

LSD 5% for interaction (isolates x temperatures) = 9.7.

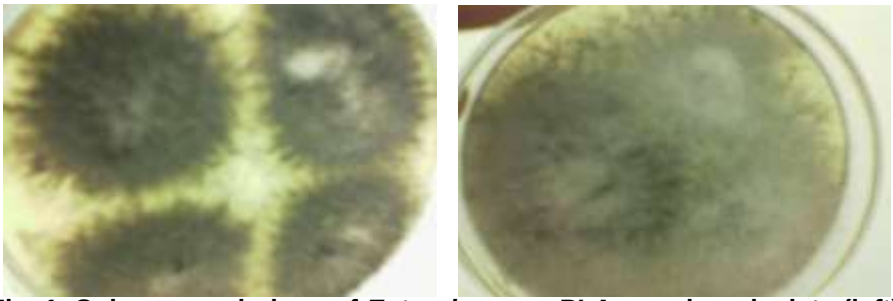

Fig. 1. Colony morphology of *E. turcicum* on PLA; sorghum isolate (left) and maize isolate (right)

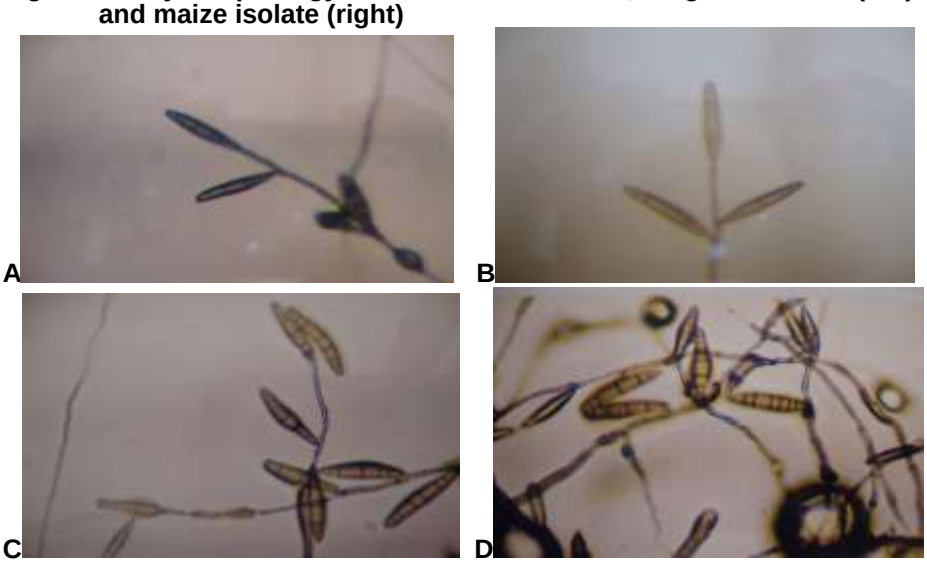

Fig. 2. Conidial morphology of *E. turcicum* isolates from; Sorghum (A and C) and maize (B and D) by 20X and 25 X respectively.

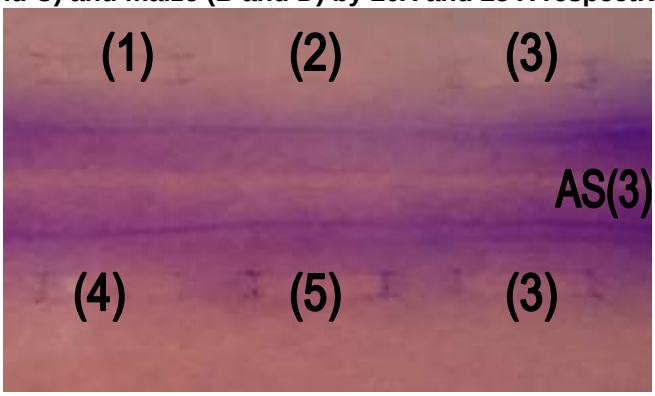

Fig.3. Modified ouchterlony double diffusion patterns of *E. turcicum*. Trough contains antiserum of forage sorghum isolate SK.TS-3 (3). Upper wells contain antigens of SD.TS-1 (1); SD.TS-2 (2); and SK.TS-3(3). Bottom wells contain antigens of GM.TC-1(4); GM.TC-2(5) and SK.TS-3(3).

At 20 and 23°C, maize isolate GM.TC-2 was significantly smaller in radial growth than the other three isolates (SD.TS-1 & SD.TS-2 from sorghum and GM.TC-1 from maize). Meanwhile, colonies of sorghum isolates were significantly larger (90 and 84mm) at 27°C than maize isolates (58 and 70mm). However, the radial diameters growth of the sorghum isolates was not significantly different comparing with the maize isolates at 35°C. Generally, radial diameters growth mean of the two sorghum isolates was more than those of the maize isolates at 27°C.

Greenhouse test: Reaction of ten sorghum and maize varieties against the two sorghum isolates of *E. turcicum* from Sids showed in Table, 5. The two sorghum isolates, SD.TS-1 and SD.TS-2, infected only the local varieties of grain sorghum (G115, G113 and G123) as well as the Sudan grass (forage sorghum).

Table 5: Disease reaction on a set of different hosts against two sorghum isolates of *E. turcicum* under greenhouse conditions.

Sorghum and maize Va	Genus & species	SD.TS-1 [y]	SD.TS-2
G-15 (local)	***Sorghum bicolar***	+	+
G-113 (local)	***Sorghum bicolar***	+	+
G-123 (local)	***Sorghum bicolar***	+	+
Dorado (short)	***Sorghum bicolar***	-	-
Horas (short)	***Sorghum bicolar***	-	-
Izeas (short)	***Sorghum bicolar***	-	-
Sudan grass (tall)	***Sorghum sudanens***	+	+
SC-122 (maize)	***Zea mays***	-	-
SC-129 (maize)	***Zea mays***	-	-
Giza-2 (maize)	***Zea mays***	-	-

[y] **Disease assessed based on infected or non infected varieties where; + = infected and - = non infected.**

Serological test: A modified ouchterlony double diffusion test (Fig 3) show that antigen of sorghum and maize isolates gave the same reaction, one precipitin line, with the antiserum of sorghum isolate SK.TS-3 (3). Meanwhile the homologous reactions of the sorghum isolate which capable to infect both maize and sorghum in previous study, showed two precipitin lines.

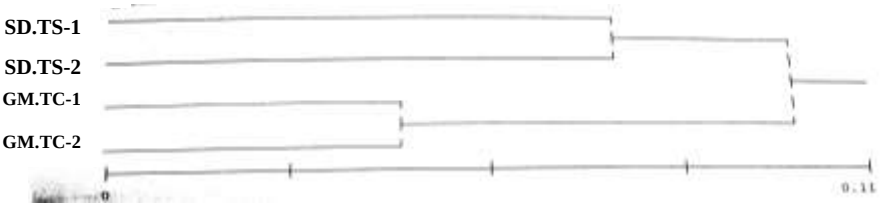

Fig.4: Dendrogram generated from cluster analysis of 60 conidia (length, width and septate mean; maximum & minim length, width and septate number) of four isolates of *E. turcicum* obtained from Sids and Gemmeiza. Sids isolates (SD.TS-1 and SD.TS-2) from sorghum and Gemmeiza isolates (GM.TC-1 and GM.TC-2) from maize, using Bray and Curtis (1957) Distance Matrix.

DISCUSSION

One colony type was clearly distinguishable between sorghum isolates from nature at Sids Agricltural Research Station (SARS). The colonies of sorghum isolates were identical and could be distinguished from maize isolates on the basis of colony morphology, physiology and conidial characters. Sids isolates of *E. turcicum* from sorghum attacked only local grain sorghum and Sudan grass under natural and artificial infection. These obtaining results confirm with the result of Lefebver & Sherwin, 1945. They reported that two cultures of *E. turcicum* isolated from common Sudan grass and one from Atlas sorghum were pathogenic on Sudan grass and Gooseneck sorghum, but all failed to infect corn. Also, previous reported of EL-shafey *et al*. (1982) and El-naggar, A., (2006) suggested that some of the collected *E. turcicum* isolates from sorghum or maize from Delta-Egypt caused heavy infection only on its own host.

Our results reveled that sorghum isolates of *E. turcicum* had a fast radial growth in culture at 27C than maize isolates. Also, there were considerable differences in length and septate number of conidia among sorghum and maize isolates grown on LCHA after 15 days at 27°C which resulted in a distinct separation of isolates into two clusters according to its own host. These results are consistent with some extent to those obtained and discussed by Bergquist and Masias (1974) who reported that a slight more rapid rate of growth was observed with *E. turcicum* from sorghum at 31°C than maize isolates but there were no differences in conidial morphology among isolates. Modified ouchterlony double diffusion test failed to differentiate the sorghum isolates from maize ones. These results refer to a strong similarity (had the same antigenic determinants) between them and similar with the results obtained by Rataj-Guranowska *et al.* (1984) on *Fusarium oxysporum* f. sp*. lupini.* They compared between 2 races of the fungus by tandom-crossed immunoelecrtophoresis and found that antiserum of one of them could not differentiate between them.

The occurrence of distinct fungal colony type of *E. turcicum* isolates from sorghum along with host-specificity provides basic characters for classifying isolates from sorghum as forma specialis and this contrary with Masias and Bergquist (1974). They used forma specialis to designate isolates of *E. turcicum* pathogenic to a single host species while the term race was used for those isolates of the fungus, which were virulent to specific cultivar within a host species that carries a specific gene for resistance. Such information would be valuable in sorghum disease resistance breeding programs. In these programs, breeding materials of grain sorghum and Sudan grass should be screened for northern leaf blight resistance by using pathogenic isolates of *E. turcicum* f.sp. *sorghi* which recovered from these hosts or the breeding materials should be evaluated under field conditions where the forma specialis occurs naturally.

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ظهور الطراز المتخصص فى إصابة السورجم من الفطر إكسيروهيلم تيرسيكم المسبب للفحة أوراق الذرة الرفيعة فى جنوب مصر

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قسم بحوث امراض الذرة الشاميةوالمحاصيل السكرية - معهد بحوث أمراض النباتات- مركز البحوث الزراعية - الجيزة – مصر

فى عام 2005 لوحظ بمحطة البحوث الزراعية بسدس – بنى سويف (جنوب مصر) إصابة شديدة بمرض لفحة الأوراق المتسبب عن الفطر إكسيروهيلم تيرسيكم على صنف الذرة الرفيعة جيزه-15 وحشيشة السودان(جنس السورجم) بجوار أصناف من الذرة الشامية بدون إصابة. لذلك أجريت خلال الأعوام 2006, 2007, 2008 ثلاث تجارب تحت كل من الظروف الطبيعية فى الحقل بسدس والصناعية (لعزلتين فطريتين من السورجم المنزرع بسدس) بالصوبة على 10 أصناف من الذرة الرفيعة والشامية لتحديد التخصص النوعى للفطر إكسيروهيلم تيرسيكم. كما تم مقارنة الشكل المزرعى وصفات الجراثيم ومعدل النموعلى 4 درجات حرارة مختلفة و التفاعل السيرولوجى لعزلتى السورجم المعزولة من سدس (وجه قبلى) بأخريتين معزولتين من الذرة الشامية من محطة البحوث الزراعية بالجميزة (وجه بحرى).

أظهرت عزلات السورجم طراز مزرعى واحد حيث أمكن تمييزها بلون زيتونى غامق ، حافة محددة وميسليوم هوائى منضغط قليلا بينما تميزت مزرعة عزلات الذرة الشامية بحافة محددة، وميسيليوم هوائى غزير فى منتصف مزرعة لونها رمادى إلى أبيض مخضر. أظهرت النتائج فى الحقل والصوبة أن عزلات السورجم لم تصب إلا أصناف الذرة الرفيعة المحلية وحشيشة السودان (العوائل المعزولة منها). كما بينت النتائج أن عزلات السورجم تنمو بسرعة أكبرمعنويا على البيئة من عزلات الذرة الشامية على درجة الحرارة 27، فى نفس الوقت وجدت فروق معنوية فى صفات الجراثيم الكونيدية ولم توجد فروق سيرولوجية بين عزلات العائلين (الذرة الرفيعة والشامية) حيث وجد أن المدى الطولى للجراثيم الكونيدية لعزلات السورجم أكبر منه لعزلات الذرة الشامية (66-145 ،40-92ميكرون) وكذلك عدد الجدر العرضية بالجرثومة (6 ،4 جدر) على التوالى.

بناء على وجود طراز مزرعى محدد للفطر إكسيروهيلم تيرسيكم المعزول من الذرة الرفيعة وحشيشة السودان (جنس السورجم) بجانب التخصص العائلى فإن عزلات الفطر المتحصل عليها من سدس صنفت كطراز متخصص منفصل.

قام بتحكيم البحث

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