J. Agric. Sci. Mansoura Univ., 29 (10): 5835 - 5846, 2004

SORGHUM GRAIN-ASSOCIATED MYCOFLRA WITH SPECIAL EMPHASIS ON THE VIRULENCE OF *Acremonium strictum*

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ABSTRACT

A survey for fungi associated with non sterilized grains of two grain sorghum cultivars (Dorado and Giza 113) using standard blotter method indicated that Giza 113 grains had more inocula of associated pathogenic fungi than those of Dorado grains. Three seed health testing methods (agar plate, blotter and freezing) were evaluated for detecting the most prevailing pathogenic fungi associated with sterilized Giza 113 grains. Freezing method was the most sensitive for detection of *F. moniliforme, F. roseum* and *A. strictum* which are considered the most important pathogens as they cause wilt diseases.Special emphasis was given to the fungus *Acremonium strictum*, the causal microorganism for the widespread Acremonium wilt disease in grain sorghum. Eighteen isolates of *A. strictum* were collected from 7 governorates in middle and upper Egypt and evaluated under greenhouse conditions for virulence on two sorghum genotypes EGH-2 (resistant) and Giza 114 (susceptible). Isolate No. 9 was the most aggressive one. Reactions of twenty commercial and promising grain sorghum cultivars were evaluated for resistance to the isolated *A. strictum* at Giza and Sids Stations using soil infestation technique. Dorado was the highest resistant cultivar and Giza 113 was the highly susceptible one.

Keywords: Acremonium wilt,grain sorghum,grain-associated fungi

INTRODUCTION

Fungi associated externally or internally with sorghum grains are responsible for many plant diseases, reduction of grain viability and various kinds of spoilage (Bressan, 2003; Prom *et al.*, 2003). Numerous reports have listed a large number of fungi which could be isolated from sorghum grains in the field as well as during storage (Randhawa *et al.*, 1998; Prom *et al.*, 2003).*Acremonium strictum* has been considered as one of the frequent fungal species in stored sorghum grains (Ibrahim and El-Menchawy., 2001) and sunflower (Kushal and Saharan., 1994).

Acremonium wilt in maize,corn and grain sorghum caused by *Acremonium strictum* W. Gams (*Cephalosporium acrermonium* Corda) was studied by many investigators. The pathogenicity of the fungus has been found to be readily established and confirmed on sorghum more than the other crops.(Koehler, 1960; Zaher, 1974). The disease was first described in Egypt by El-Shafey and Refaat (1978) as a stalk-rot disease. Later, it was described as a true vascular wilt disease by El-Shafey *et al.* (1979).The fungus grows superficially on seedling roots, spreads after penetrating the xylem vessels of the roots and reaches the vascular bundles of the stalk. The

root length and leaf area of the infected plants were drastically decreased as the fungus filled the wood vessels causing vascular wilt to the plants (Khalefa, 2000).

The severity of the disease depends mainly on the pathogenicity of the fungal isolate and plant genotype as well as the environmental conditions (El-Shafey *et al.*,1999; Zein El-Abedeen et *al.*,2000). Isolates from maize or sorghum were more virulent on sorghum than maize as they produced black bundle in maize and wilt symptoms in sorghum (El-Assiuty, 1982). On the other hand, grain sorghum varieties exhibited different degrees of resistance against infection with some soil-borne fungi (Ali and Warren 1987) .Unfavourable conditions enhanced infection of maize with *A. strictum* (Sabet *et al.*,1970) .

The present work was planned to investigate the mycoflora associated with non-sterilized sorghum grains. A comparative study was carried out among three different methods for isolation of endophytically associated fungi with surface-sterilized grains. A special emphasis was given to the fungus *Acremonium strictum*,the causal agent of Acremonium wilt in grain sorghum plants.

MATERIALS AND METHODS

Sorghum grains

Twenty two grain sorghum genotypes (ICSR 89028, Giza 113, ICSR-21, Giza 15, CS 3541, Selection-1007, ICSV 138, M 36565, ICSR 93001, Local 162, Local 129, Giza 54, Assiut 14, Local 29, Line C, Local 44, Dorado, ICS 3548, Shandaweel 6, Local 245, Giza 114 and EGH-2) obtained from Sorghum Res. Dept., Field Crops Res. Inst., ARC, Giza were used in this study.

Isolation of fungi associated with sorghum grains

The most prevalent pathogenic fungi associated with grains of a resistant (Dorado) and a susceptible (Giza 113) sorghum cultivars were isolated, purified and identified.

The standard blotter method (Neergaard, 1979) was applied for isolation of fungi associated with 100 grains of each cultivar. Grains were rinsed twice with sterilized distilled water, dried between two sterilized filter papers and plated in Petri dishes on three layers of well moistened blotters (10 grains/plate). Plates were incubated under alternating cycles of 12 hr. light and darkness at 25 °C for 7 days. Growing fungi were isolated, subcultured , purified and identified according to cultural, morphological and microscopical characteristics compared with the descriptions given by Barnett (1960), Booth (1971), Chidambaram *et al.* (1973), and Ellis (1976). Identification was confirmed at Mycol. and Dis. Survey Dept., Plant Pathol. Res. Inst., ARC, Giza.

Three modified methods namely, agar plate, standard blotter and deep-freezing (Limonard, 1966) were applied for isolation of fungi associated

with surface-sterilized grains of Giza 113 cultivar.Three representative samples each contained 100 grains were examined as follows :

Agar plate method

A grain sample was treated with sodium hypochlorite solution (5%) for 2 min., rinsed with sterile distilled water, dried and plated on PDA medium containing 200 ppm streptomycin (10 grains/plate). The plates were then incubated at 25°C and observations were recorded daily up to day 7. Seed-borne fungi were isolated, purified and identified.

Standard blotter method

A surface-sterilized grain sample was sown on moistened blotters treated with streptomycin (10 grains/plate) and incubated under alternating cycles of 12 h. artificial day light (ADL) and darkness at 25 °C for 7 days. Seed-borne fungi were isolated, purified and identified.

Freezing method

A surface-sterilized grain sample was sown on moistened blotters treated with streptomycin (10 grains/plate) and incubated at 28 °C for 2 days to induce germination. The germinated grains were then frozen overnight at – 20 °C to prevent the emberyo growth and incubated at 25 °C for 6 days. Isolated seed-borne fungi were subcultured, purified and identified.

Isolation of *A. Strictum*

Samples of grain sorghum plants showing Acremonium wilt disease symptoms were collected from different governorates in Middle and Upper Egypt during 1998 growing season.The lower (3^{rd} to 5^{th} above ground) internodes of rotted stalks of the wilted plants were throughly washed with running water and left to dry. Then, they were surface sterilized with ethyl alcohol (95%), flamed and peeled under aseptic conditions. Small pieces of the interal tissues were cut out and plated on sterilized potato dextrose agar (PDA) medium (Booth, 1971). Plates were incubated at 28 ± 2 °C for 3-7 days and examined daily for the occurrence of fungal growth. The growing fungi were examined microscopically and purified using single spore technique.

Identification of *A. strictum* isolates

The obtained fungal isolates were identified by morphological characteristics and microscopic examination according to Barnett (1960) and Sabet *et al.* (1966), and confirmed by comparing these isolates with the Culture Collection of Maize, Sugar and Foliage Crops Res. Dis. Dept., Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt. Isolates were maintained on PDA slants under mineral oil in a refrigerator for the further studies.

Selection for the most virulent isolates of *A.strictum* under greenhouse conditions

The pathogenicity of eighteen *A. strictum* isolates, collected from Beni-Suef, Menia, Giza, Fayoum, Souhag, Assuit and Kena governorates

during 1999 growing season, was tested under greenhouse conditions using soil infestation technique. Two susceptible grain sorghum cultivars *i.e.*,Giza114 and EGH-2 were examined.

Fungal inocula were prepared by growing the obtained isolates separately in sterilized glass bottles (500 ml) each containing 100 g sorghum grains moistened with 50 ml distilled water for 15 days at 28 °C. Bottles were shaken every 2 days for homogenity.

Soil infestation was carried out by adding inocula of each *A. strictum* isolate at a rate of 50 g fresh weight/pot to sterilized pottery pots (25 cm dia.), filled with autoclaved loamy sand soil from Giza Exper. Stat. ARC. just before sowing as described by Sabet *et al.*(1970b).Five sorghum grains were planted in each pot and the experiment was replicated four times. Plants were fertilized with urea (46% N) 21 days after sowing at the rate of 3 g pot^{-1} and watered regularly with tap water. The temperature was 28 ± 2 °C. Disease percentages were recorded after 90 days of sowing.

Susceptibility of grain sorghum cultivars to the most virulent isolate of *A. strictum* under field conditions

Twenty commercial and promising cultivars of grain sorghum were evaluated for resistance to *A. strictum* at Giza and Sids Stations, Agric. Res. Center during 2000 growing season. An inoculum of the most virulent *A. strictum* isolates (No. 9), prepared as mentioned in the greenhouse experiment, was distributed in hills at a rate of 50 g fresh weight/hill before sowing. Four grains were planted in each hill and thinned later to one plant. Four replicates were planted with each cultivar.Eeach replicate contained two rows either with 20 drills at 30 cm apart. Plants were supplemented with ammonium nitrate (33.5 % N) at the rate of 150 $kg.fed^{-1}$ 21 days after sowing. Data were recorded after 90 days of sowing as percentage of wilted plants and transformed to arc sine.

Data were subjected to statistical analysis according to Snedecor and Cochran (1980). Means of the percentages of infection were transferred to arc sine degrees of all treatments and compared.

RESULTS AND DISCUSSION

Isolation of fungi associated with sorghum grains

The most prevalent fungi associated with non-sterilized sorghum grains are shown in Table (1). Up to 12 pathogenic fungi were successfully isolated from Giza 113 and 9 from Dorado cultivar. However, the saprophytic fungi associated with sorghum grains such as *Rhizopus* sp. and *Penicillium* sp., which are recognized for their little role in causing diseases were not considered during this study.The obtained results revealed that grains of Giza 113 accommodated more pathogenic fungi than Dorado. Fungi infected Giza 113 at frequencies ranged between 3 and 21 % and Dorado cultivar of up to 0 – 9 %. This is more likely due to certain characteristics displayed by the resistant cultivars such as grain hardness and high level of inhibitory phenolic compounds (Ghorade and Shekar, 1997). Similarly, Audilakshmi *et al.* (1999)

found that harder grain of sorghum, higher levels of seed phenols and darker glumes contributed to grain fungus resistance.

On the other hand, the highest incidence was recorded for the fungus *Alternaria* sp. and *Fusarium semitectum* being 21.0 and 21.0 % for Giza 113 and 9.0 and 3.0 % for Dorado cultivars, respectively. Both isolates represented 36 % of total infection rate followed by *Alternaria alternata* and *Fusarium roseum* recording frequencies of 12 and 11 % with the former cultivar and 6 % and 1 % for the latter. On contrary, Osman *et al.* (1988) found that *A. alternata* was the most dominant pathogenic fungus associated to sorghum grains representing up to 30 % of the total isolated fungi whereas *Alternaria* sp. was the lowest comprising only 1 %. The authors added that both type and frequency of infection were greatly influenced by geographical differences.

Table (1) :The most prevalent fungi associated with non-sterilized sorghum grains

Associated fungi	% infected grains[a]	
	Giza 113 (susceptible)	Dorado (resistant)
Alternaria alternata	12.0	6.0
Alternaria sp.	21.0	9.0
Fusarium moniliforme	10.0	0.0
F. roseum	11.0	1.0
F. semitectum	21.0	3.0
Acremonium strictum	3.0	0.0
Bipolaris bicolor	7.0	1.0
Cephalosporium sp.	4.0	0.0
Aspergillus flavus	8.0	2.0
A. niger	4.0	1.0
Curvularia lunata	8.0	4.0
Curvularia sp.	8.0	2.0
Total	117.0	29.0

a, Number of fungus infected grains among hundred tested grains.

As for the fungi *A. strictum* and *F. moniliforme*, they were detected only in Giza 113 but not in Dorado cultivar. Recently, *F. moniliforme* was reported as the most common pathogen in sorghum causing the disease in more than 88 % of infected grains with an average infection rate of 9.2 % (Ibrahim and El-Menchawy, 2001). This finding is inconsistent with the obtained results which showed *Alternaria* sp. as the dominant pathogen. Moreover,the same authors found *C. acremonium* to be the second dominant pathogen with Dorado cultivar being the best host at an average infection rate of 14 % compared to only 1 % with Giza 113 cultivar.This discrepancy could be attributed to variations in cultivar type, environmental conditions and methods of determination.

Isolation of fungi endophytic to sorghum grains

Due to the exceptional high grain infection rate recorded for almost all the isolated pathogenic fungi with the cultivar Giza-113 compared to Dorado (Table 1), the former was selected to carry out a comparative study adopting three diffrernt methods for isolation of the endophytic fungi.

It is obvious from data presented in Table(2) that the infection rate of sorghum grains and consequently the growth and development of all internally isolated fungi except *A. alternata*, was lower than that found for each respective isolate associated with non-sterilized grains using the same method of determination. An equal incidence for *A. alternata* was recorded under both conditions, whereas *Curvularia lunata* was not detected with sterilized grains.

The I/T ratio for all remaining isolates ranged between 12.5 - 66.7 % (Table, 3) with the causal pathogen of the Acremonium wilt (*A. strictum*) recording the 2nd highest I/T ratio of 66.7 %, followed by *Aspergillus niger* (50.0 %) and *F. moniliforme* (40.0 %). Thus *C. lunata* can simply be considered as the only superficially associated pathogen in sorghum grains under such conditions.The rest including *A. Strictum* as internally associated pathogens in sorghum grains.On the contrary, Osman *et al.* (1988) found that out of 21 tested pathogenic fungi including *C. lunata, Trichothecium roseum* was the only superficially associated and most frequent pathogen. *A. strictum* was not included in their study, and *T. roseum* was not isolated in the present study. Such discrepancy is likely due to differences in locality of application, cultivar used and method of determination. The authors used agar plate method and Giza 114 as cultivar.

In conformity with Khattak *et al.* (1993), investigating pathogenic potentiality in soybean seeds, the obtained results showed agar plate method as the most suitable and reliable seed health testing method as it produced the highest rates of internal infection for almost all isolates followed by standard blotter and finally deep freezing method . This is most probably due to presence of readily available nutrients in PDA medium that might have supprted fungal growth and cultivar infection. . Also, superiority of agar plate method confirmed greater numbers of fungal species compared to the same other two methods. Moreover, agar plate method was sensitive enough to detect internally the fungus *C. lunata*, which was never detected by standard blotter or deep freezing techniques (Table 2).

In accordance with Osman *et al.* (1988) the fungi *Alternaria* sp. and *A. alternata* were the most prevalent, internally-borne pathogens with the highest average infection rate recovered by all the three above-mentioned methods. It was worthy noting that despite its failure to detect internally associated *Cephalosporium* sp., *Curvularia lunata* and *Curvularia* sp., deep freezing technique was more sensitive compared to the other techniques in detecting efficiently the pathogenic fungi, *A. strictum, F. moniliforme* and *F. roseum* internally associated to sorghum grains. Similar observation was reported by other investigators and was attributed to a possible leakage of certain nutrients by freezing the germinated grains and subsequent incubation at 25°C that might specifically stimulated the pathogenic fungi compared with the others (Limonard, 1966) or to a direct suppression of the growth of non-

pathogenic fungi but not to pathogens by freezing process (El-Shafey *et al.*, 1991).

Table (2): The most prevalent fungi internally associated with surface - sterilized sorghum grains (Giza 113 cv.).

Associated fungi	% infected grains[a]		
	Seed health testing methods		
	Agar plate method	Standard blotter method	Deep freezing method
Alternaria alternata	16.0	12.0	8.0
Alternaria sp.	18.0	6.0	4.0
Fusarium moniliforme	2.0	4.0	5.0
F. roseum	2.0	2.0	4.0
F. semitectum	3.0	4.0	2.0
Acremonium strictum	2.0	2.0	4.0
Bipolaris bicolor	1.0	2.0	2.0
Cephalosporium sp.	1.0	1.0	0.0
Aspergillus flavus	1.0	1.0	1.0
A. niger	4.0	2.0	1.0
Curvularia lunata	4.0	0.0	0.0
Curvularia sp.	2.0	2.0	0.0
Total	56.0	38.0	31.0

a, number of fungus infected grains out of one hundred tested grains

Table (3) : The internal : total ratio (I / T) of pathogenic fungi associated with sorghum grains

Associated fungi	(I / T) ratio
Alternaria alternata	100.0
Alternaria sp.	28.6
Fusarium moniliforme	40.0
F. roseum	18.2
F. semitectum	19.0
Acremonium strictum	66.7
Bipolaris bicolor	28.6
Cephalosporium sp.	25.0
Aspergillus flavus	12.5
A. niger	50.0
Curvularia lunata	0.0

Isolation and identification of A. Strictum

In an attempt to study the virulence of *A. strictum* , a wide range search was conducted in a large area of sorghum planted fields.The most prevalent fungi were isolated from samples of grain sorghum plants, showing Acremonium wilt disease symptoms, collected from different governorates in middle and upper Egypt during 1998 growing season. All isolates exhibited the same identical characteristics. Their growth on PDA medium supplemented with 0.2% yeast extract at 28 ± 2 °C was moderately rapid covering the plate surface in about 15 days. They produced a dense mycelial mat with a distinctly raised smooth margin. The hyphae were hyaline, septate and profusely branched. The conidiophores were septate, slender and simple varying in length. The conidia were hyaline, single-celled, oval, straight or slightly curved produced

successively at the tip of the conidiophore and collected to form spore heads embedded in a slime matrix. When compared with those reported by Barnett (1960) and Sabet *et al.* (1966) for *A. strictum*, they were in conformity. Therefore, the isolates were all identified as *Acremonium strictum*, the well known causal fungus of Acremonium wilt disease in grain sorghum. The isolated fungus is very similar, if not identical, to *Cephalosporium acremonium* which was isolated from diseased grain sorghum (El-Shafey *et al.*, 1979) and *Acremonium strictum* which was reported to cause vascular wilt of Shastadaisy (Chase and Munnecke, 1980) and was the causal fungus of Acremonium wilt for an American cultivar of sorghum (Natural *et al.*, 1982).

Cultural variations among *A. strictum* isolates

Isolates of *A. strictum* varied in their mycelial colours (white, pink and buff), growth texture (soft, hard and leathery), colony margins and rate of growth on PDA medium. There was no correlation between colour and growth texture. Sectors of triangular shape-like were occurred in some isolates as was observed by Abdel-Al *et al.* (1981) who classified the wild type of *Helminthosporium teres* based on growth texture and color. Similarly, Cullen *et al.* (1982) found that isolates of *Gibberella zeae* which infected maize kernel differed in the production of red pigments, aerial mycelium, production of zearalenone or rate of growth during their cultivation on PDA medium. A cultural variation among *Cephaosporium acremonium* isolates found in sorghum and maize over wide geographical areas was also reported by Mansour *et al.* (1986). The author found that sectors frequently occurred in wild and monospore isolates and anastomosis was observed between hyphae and germ tubes. Moreover, more than one wild cultural types existed in the same governorate of locality.

Similar variations in *Cephalosporium maydis*, the causal fungus of late wilt disease of maize, isolates were observed by Awad (2002) who found that the mycelial colours of 46 isolates were ranged between whitish gray to gray and dark gray.These variations in colour and growth texture may represent different physiological races of the fungus *A. strictum* as concluded by El-Shafey *et al.* (1999).

Selection for the most virulent isolates of *A.strictum* under greenhouse conditions

Data illustrated in Figure (1) show that all the tested isolates varied in their virulence. It was clear that, infection was cultivar dependent. The cultivar EGH-2 was less susceptible to *A. strictum* isolates (0.0 - 35.0 % infection), whereas, Giza 114 was more susceptible (25.0 - 90.0 %). However, 14 isolates were avirulent to EGH-2 (0.0 % infection) and virulent to Giza 114 (25.0 % - 80 % infection). Isolates No. 4, 9, 13 and 17 were virulent to both tested grain sorghum genotypes. Isolate No. 9 isolated from Fayoum governorate (Tameya) was the most aggressive isolate, causing the highest infection percentage on both cultivars (90 and 35 % on Giza 114 and EGH-2, respectively). Differences in virulence between *A. strictum* isolates indicate that different pathotypes of this pathogen may exist. Similarly, El-Assiuty (1982) found that some isolates of *C. acremonium* causing stalk-rot disease of grain

sorghum were more virulent than others. On the other hand, sorghum and maize varieties showed a wide susceptibility to *C. acremonium* .Also, El-Shafey *et al.* (1999) recorded highly significant variations in virulence of large number of *A. strictum* isolates when their pathogenicity was tested on four grain sorghum cultivars *in greenhouse*. Dorado cultivar, on the other hand, was the most resistant whereas Giza 15 was the most susceptible.

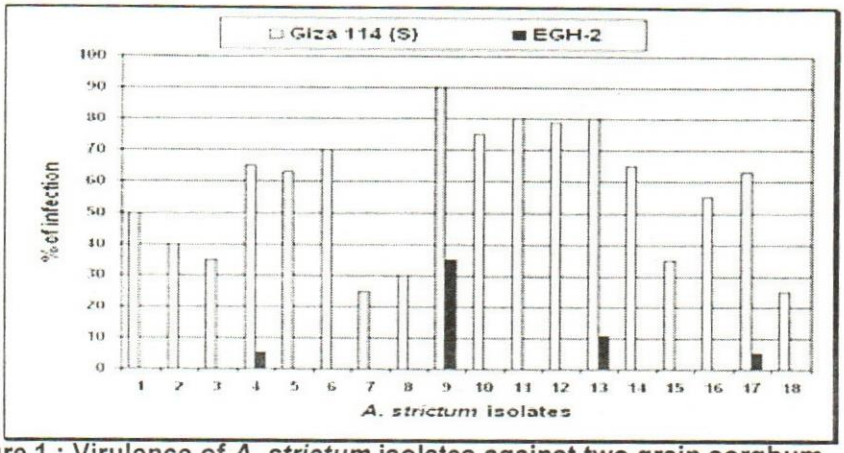

Figure 1 : Virulence of *A. strictum* isolates against two grain sorghum cultivars under greenhouse conditions.

For the superiority of isolate No (9) amongst all the tested *A. strictum* isolates, regarding its infection of both susceptible and resistant cultivars, it was selected for further studies.

Susceptibility of grain sorghum cultivars to the most virulent *A. strictum* isolate under field conditions

Table (4), show that grain sorghum cultivars had various responses to infection. Giza 113, Giza 15, local 44 and CS 3541 exhibited 81.87, 67.21, 67.21 and 60 infection percentage at Giza station and 80.02, 67.21, 73.57 and 60 at Sids station with mean infections of 80.95, 67.21, 70.39 and 60%, respectively. On the other hand, and similar to the findings of El-Shafey *et al.* (1999) Dorado cultivar showed the highest degree of resistance whereas Giza cultivars were highly susceptible to *A. strictum*. El-Assiuty (1982) showed that grain sorghum cvs. NES 1818 and NES 1324 displayed the highest resistance to the same fungus while cvs. Local 102 and 129 were the lowest.

Dorado cultivar exhibited the lowest infection rate (12.25% and 15.34 % at Sids and Giza station respectively, with an average of 13.8 %) and Giza 113 showed an infection of more than 80 %. The remaining cultivars displayed various degrees of susceptibility to the pathogenic fungus with average values between 13.8 and 81 %. Certainly, such difference in susceptibility is due to physiological and/or genetical differences among cultivars. Geographical differences could also be considered but with much less influence. Differences in infection rates for a given cultivar grown in Giza and Sids stations were much less pronounced compared to those for

different cultivars grown at the same station. Moreover, differences between Giza 113 and Dorado cultivars were highly significant and their interaction with localities showed also significant ($p<0.05$) differences. Accordingly, Giza 113 was selected as the most susceptible and Dorado as the most resistant cultivars and used through out the following part of the investigation.

Table (4) : Susceptibility of certain cultivars of grain sorghum to *A. strictum* isolate No. (9) under field conditions.

Grain sorghum cultivars	% Infection (arc sine)			% Infection (arc sine)			
	Giza Station	Sids Station	Mean	Grain Sorghum Cultivar	Giza Station	Sids Station	Mean
ICSR 89028	27.28	19.37	23.33	Giza 54	53.73	47.87	50.58
Giza 113	81.87	80.02	80.95	Assuit 14	50.77	49.60	50.19
ICSR-21	36.27	27.28	31.78	Local 29	50.77	45.57	48.17
Giza 15	67.21	67.21	67.21	Line C	30.00	26.56	28.28
CS 3541	60.00	60.00	60.00	Local 44	67.21	73.57	70.39
Sel.1007	36.27	36.27	36.27	Dorado	15.34	12.25	13.80
ICSV 138	30.00	27.97	28.99	ICS 3548	33.21	30.00	31.61
M 36565	30.00	28.66	29.33	Shanda-weel 6	16.95	15.34	16.15
ICSR 93001	35.06	32.58	33.82	Local245	53.73	53.73	53.73
Local 162	36.27	30.00	33.14	Local129	60.00	57.10	58.55
Means					43.60	41.05	42.33

L.S.D. ($P< 0.05$) : Cultivar (V) = 6.75 , Location (L)= 1.7, L x V= 4.64

Work is in progress concerning isolation of microorganisms prevailing in the rhizosphere of sorghum plants and soil apart as well. Subsequently, selection for potent strain(s) with promissing capabilities in biocontrolling of Acremonium wilt disease will be undertaken.

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الفطريات المصاحبة لحبوب الذرة الرفيعة ومدى قدرة فطر الاكريمونيوم ستريكتم على إصابة نباتات الذرة الرفيعة بمرض الذبول الاكريمونى.

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تم دراسة الفطريات المصاحبة لحبوب الذرة الرفيعة لكلا الصنفين بطريقة البلوتر فكان عدد الفطريات المصاحبة لحبوب جيزة ١١٣ أكبر من المصاحبة لحبوب صنف دورادو. وبتقييم ثلاث طرق مختلفة (آجار البطاطس والدكستروز والبلوتر وطريقة التجميد) فى إظهار الفطريات الممرضة المصاحبة لحبوب معقمة من صنف جيزة ١١٣ إتضح أن طريقة التجميد هى أكثر هذه الطرق حساسية فى إظهار فطريات فيوزاريوم مونيليفورم وفيوزاريوم روزيم و اكريمونيوم ستركتم والتى تعتبر من أهم الفطريات الممرضة المسببة لأمراض الذبول.وقد تم التركيز فى هذه الدراسة على فطر اكريمونيوم ستريكتم المسبب لمرض الذبول الاكريمونى للذرة الرفيعة . فتم الحصول على ١٨ عزلة من الفطر المسبب للمرض تم عزلها من نباتات مصابة جمعت من سبع محافظات فى وسط وجنوب مصر وقد وجد أن هذه العزلات تختلف عن بعضها فى لون الميسليوم وقوام المزرعة وحوافها على بيئة آجار البطاطس والدكستروز بالإضافة إلى إختلاف قدرتها على إصابة صنفين من الذرة الرفيعة بالمرض وكانت العزلة رقم (٩) هى أشد العزلات قدرة على إصابة كلا الصنفين (المقاوم والقابل للإصابة) وعند تقييم حساسية ٢٠ صنفا من الذرة الرفيعة لهذه العزلة فى الحقل إختلفت الأصناف فى مدى قابليتها للإصابة بالمرض وجاء الصنف جيزة ١١٣ أكثرها قابلية للإصابة (متوسط نسبة الإصابة ٨١%) بينما جاء الصنف دورادو أكثرها مقاومة للإصابة (متوسط نسبة الإصابة ١٣,٨%) . لذلك فقد تم إختيار العزلة رقم (٩) من الفطر أكريمونيوم ستركتم وصنفى الذرة الرفيعة دورادو ، جيزة ١١٣ لإجراء بقية التجارب.