RACE IDENTIFICATION AND BIOLOGICAL CONTROL OF *USTILAGO SCITAMINEA* THE CAUSAL ORGANISM OF SUGARCANE SMUT DISEASE IN UPPER EGYPT.

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Abstract

Four isolates of *Ustilago scitaminea Syd,* the causal organism of sugarcane smut disease were obtained from diseased samples showing symptoms of smut and collected from different localities of El-Minia, Qena and Aswan Governorates. These isolates were able to infect NCo310 sugarcane variety (highly susceptible) with variable degrees of infection. Two of these isolates were highly pathogenic; one isolate was moderate, while other isolate had no effect. The enzymatic patterns of esterase and phosphatase in addition to the proteolytic enzymes protein were used to determine the genetic variability among the four tested isolates of *Ustilago scitaminea.* The results of the dimeric esterase zymogram for the four tested isolates revealed that esterase was controlled by two loci, Est-1 and Est-2. The locus of Est-1 was dimeric and represented by two alleles, one was fast ($Est\text{-}1^F$) and one slow ($Est\text{-}1^S$) allele. The four isolates were heterozygous at the Est-1 locus in which three bands were detected. The acid phosphatase pattern showed no differences between the four isolates of *Ustilago scitaminea.* However, all of tested isolates exhibited only one band at RF= 0.45 in their zymogram. The electrophoretic pattern of enzymatic protein revealed 6 enzymatic bands at RF 0.6, 0.73,077,0.80,0.84 and 0.96. No differences were observed among the four isolates of *Ustilago scitaminea* in the enzymatic protein pattern. Testing antagonistic capability of *Trichoderma spp.* against *Ustilago scitaminea in vitro,* revealed that *Trichoderma viride* had a mycoparasitic effect on the growth of smut disease pathogen. Biological control of the smut disease by dipping healthy cane setts in *T. viride* suspension (2×10^6 propagules / ml) before and after inoculation with the pathogen gave a noticeable control of the disease. The efficiency of *Saccharomyces cervisiae* in bio-control of the smut disease of sugarcane was also studied under greenhouse condition. Results indicated that using *S. cervisiae* as a bio-agent agent was effective in controlling the disease. A complete control of the disease was achieved by spraying cane plants with the tested yeast at the same time of the pathogen addition.

INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is considered one of the most important field crops in Upper Egypt for sugar production. There are many diseases affecting the productivity of this crop. Sugarcane smut disease causes by *Ustilago scitaminea Syd.* seriously induced tremendous qualitative and quantitative losses of sugarcane production. This disease has been recorded in Egypt since 1930 (Fahmy , 1930). The occurrence of smut (*Ustilago scitaminea Syd)* in sugarcane has been reported from different sugar producing countries, (Seshadri *et al.,* 1984). Since, (Olufolaji 1985) mentioned that sugarcane planters all over the world fear the arrival in their cane fields of smut, caused by *Ustilago scitaminea* ,owing to the losses it causes, which may be as high as 40 % or more. Peros (1985) stated that electrophoresis of three enzyme systems (esterase, phosphatase and leucineamino peptidases) of *Ustilago scitaminea* isolates which taken from Guadeloupe,Reunion and four African countries revealed that there was no differences between sugarcane smut isolates in the course of tested enzymes. In recent years, several investigators have tried to control certain plant diseases by biological methods(Fokkema and Lorbeer, 1974., Fokkema *et. al.*, 1979 ., Revathi *et. al.*, 1997 and Chowdhury, 1998). The aim of the present research was to study race identification and biological control of *Ustilago scitaminea* the causal organism of sugarcane smut disease in Upper Egypt.

MATERIALS AND METHODS

Isolation and Identification of the causal organism:

Teliospores of the diseased sugarcane plants bearing whips were collected from El-Minia, Qena and Aswan Governorates. Spores were suspended in 1.5 % solution of copper sulfate for 24 hours for spore sterilization as described by (Christensen and Stakman, 1926). To identify fungal isolates, teliospores were microscopically examined and measured according to their morphological characteristic using Carl Zeiss eyepiece micrometer as described by (Mundkur and Thirumalachar, 1952).

Race identification:

Single spore isolates of the fungus were grown on PDA medium at 30° C for 7 days and then were taken for electrophoretic analysis.

Polyacrylamide gel electrophoresis:

The electrophoresis was carried out in vertical polyacrylamide gels,using the slab gel apparatus" SE600 vertical slab gel, Hoffer". Polyacrylamide gel electrophoresis was carried out according to Laemmli, 1970 with 7.5 % acrylamide for isozyme and enzymatic protein analysis.

Biological control :

A .Using *Trichodermas pp.*

Two isolates of *Trichoderma viride and T. harzianum,* obtained from the Department of Plant pathology, University of Assiut were tested for their antagonistic capability against *Ustilago scitaminea in vitro* by using Gliotoxin fermintation liquid medium (GFM) at 25 ° C as described by Brain and Hemming, 1945. Observation of antagonism and / or parasitism of the tested fungi was recorded after 10 days of inoculation.

B. Using yeasts :

Four isolates of yeasts were isolated from sugarcane during the determination of microbial load of syrup on malt agar medium. These four isolates were identified according to their morphological and physiological characteristics as reported by (Lodder, 1970 ,Barnett and Pankhurst, 1974). They were identified as *Saccharomyces cerevisia, Torulopsis spp., Schizosaccaromyces spp.* and *Hansenula spp.*

RESULTS

Race identification of *Ustilago scitaminea :*

The two-enzymatic patterns of esterase and acid phosphatase in addition to the enzymatic protein were used as criteria to determine the genetic variability among the four tested isolates of *Ustilago scitaminea.* Results of the dimeric zymogram (Fig.1) reveal that estrase was controlled by two loci, Est-1 and Est-2, in the four tested isolates. The locus of Est-1 was dimorphic and represented by two alleles, one fast ((Est-1^F) and the other allele slow (Est-1^S). The four tested isolates were heterozygous at the Est-1 locus in which three bands were detected.These three bands were, the homodimeric fast band (Est-1^F , RF = 0.43), the heterodimeric one (Est-$1F^S$, RF= 0.31) and the homodimeric slow band (Est-1^S , RF=0.26). Meanwhile, the locus of Est-2 was monomorphic, in which only one band at RF=019 had observed in all isolates. The acid phosphatase pattern (Fig. 2) show no differences

between the four isolates of *Ustilago scitaminea*. However, all the tested isolates exhibited only one band at RF= 0.45 in their zymogram.

The electrophoretic pattern of enzymatic protein reveal 6 enzymatic bands at RF 0.6, 0.73,077,0.80,0.84 and 0.96(Fig. 3) No differences were observed among the four isolates of *Ustilago scitaminea* in the enzymatic protein pattern.

Biological control:

A. Using *Trichoderma spp*

Antagonistic capability of *Trichoderma viride*,(one isolate) and *Trichoderma harzianum* (one isolate) was tested against *Ustilago scitaminea* the causal organism of sugarcane smut disease, using (isolate IV, highly pathogenic). Results show that *T. viride* exhibited mycoparasitism on the mycelium of the tested pathogen and completely inhibited its growth. However, the other tested antagonistic organism (*T.harzianum*) had no effect.

Effect of *Trichoderma viride* on the incidence of smut disease:

Setts of Nco310 sugarcane variety were treated by dipping in spore suspension concentration ($2x10^6$ propagules /ml.) before or after the artificial inoculation with smut pathogen. The untreated setts were used as control. Percentage of smut was recorded after 8 months from planting. Results in Table (1) and Figure (4) show that treating sugarcane setts with *Trichoderma viride* spore suspension before inoculation with the smut fungus exhibited the highest reduction in the percentage of disease incidence, followed by treating setts with *T. viride* after inoculation with the pathogen. However, the least reduction in disease incidence was observed when *T. viride* spore suspension was added to the soil .

B. Using yeasts:

Antagonistic efficiency of four isolates namely; *Saccharomyces cerevisia*, *Torulopsis spp.*, *Schizosaccaromyces spp.* and *Hansenula spp.* against the smut pathogen isolate IV (highly virulent) was tested *in vitro.*

Data obtained are presented in Table (2) It was found that all tested yeasts inhibited the growth of the causal organism of sugarcane smut *in vitro* .Tested yeasts varied in their antagonistic effect against the fungal growth. However, raising yeast dilution reduced such effect .*Saccharomyces cerevisia* caused a complete growth inhibition of *Ustilago scitaminea in vitro* at the concentration of 10 %. The least percentage of growth inhibition was obtained by using Hansenula *spp* at the concentration of $1:10^{6.}$

Data presented in Tale (3) indicate that a spray cane plant with *Saccharomyces cerevisia* was effective in controlling the disease. Spraying the yeast at time of *Ustilago scitaminea* inoculation gave complete disease control. However, smut infection percentage reached 5.6,5.6 and 16.7 when inoculation with the pathogen was after 7,14 and 21 days from yeast application respectively. Percentage of smut disease of untreated control plants was 44.4%.

DISCUSSION

Smut disease of sugarcane caused by *Ustilago scitaminea Syd* is considered one of the most important diseases in Egypt and other countries. This disease was recorded for the first time in Egypt by Fahmy (1930) and caused great losses in the susceptible sugarcane varieties. The biochemical differences in the banding patterns of esterase and acid phosphatase isozymes in addition to the enzymatic protein were used to study the variability among the tested four isolates of *Ustilago scitaminea.*

The analysis of esterase, acid phosphatase and enzymatic protein revealed no differences between the four isolates of *Ustilago scitaminea.* These results are in agreement with that reported by Peros (1985). As theoretically, proteins and isozymes provide a direct measure of gene homology, the results indicated a clear similarity between the tested isolates of *Ustilago scitaminea* at the tested loci. Testing the antagonistic capability of *Trichoderma spp.* against *Ustilago scitaminea* showed that *T. viride* had a mycoparasitic effect on the fungal growth of the smut pathogen. Such results are in the line with those reported by Sampang (1989), Revathi *et.al.*,(1997) and Singh *et al.*,(1997). However, *T. harzianum* had no effect. Biological control of the smut disease by dipping healthy cane setts in *T. viride* suspension ($2x10^6$ propagules /ml.) before and after inoculation with the pathogen gave a noticeable control of the disease. Application of *T. viride* before artificial inoculation with the pathogen gave high reduction on disease incidence than application of the bio-agent after inoculation. These results are in agreement with those concluded by Pandmanaban and Alexander (1984); Miranda *et.al.*,(1996)., Revathi *et. al.,* (1997) and Chowdhury (1998). The use of *Saccharomyces cerevisiae, Torulopsis spp., Schizosaccaromyces spp.* and *Hansenula spp.* as biological agents for controlling sugarcane smut pathogen was tested. Laboratory evaluation of the above mentioned yeasts against mycelial growth of *Ustilago scitaminea in vitro* showed that all the tested yeasts were antagonistic to the smut fungus. *Saccharomyces cerevisiae* at concentration of 10 % caused a

complete inhibition of fungal growth *in vitro*. The efficiency of the yeast *Saccharomyces cerevisiae* in the bio-control of smut disease was also studied under the greenhouse conditions. The obtained results showed that spraying cane plants with suspension of the tested yeast successfully controlled the disease. A complete control of smut was achieved by spraying plants with the tested yeast suspension at the time of the pathogen inoculation. However, efficacy of the treatment was less when inoculation with the pathogen followed yeast application. These results are in the line with those reported by Fokkema *et. al.,* (1979)., Blakeman and Fokkema (1982) and Punja (1997). The reduction of smut incidence might be due to colonization of the yeast to plant surface and consequently protect the bud from the fungus invasion.

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Table (1): Effect of *Trichoderma viride* on the incidence of smut disease on Nco310 sugarcane variety.

Treatment	Infection%
Adding *T.viride* suspension to soil before planting	21.66
Treating setts with *T. viride* after inoculation with smut spores	15.00
Treating setts with *T. viride* before inoculation with smut spores	5.00
Control (untreated)	41.66

L.S.D. 5 % 24.00

Table (2) Antagonistic capability of four isolates of yeasts on the growth of *Ustilago scitaminea Syd. In viro.*

Yeasts	Yeast Concentrations / mL					
	1:10	$1:10^2$	$1: 10^3$	$1: 10^4$	$1: 10^5$	$1: 10^6$
Saccharomyces cerevisia	*100	92.69	83.67	73.68	70.75	58.14
Torulopsis spp.,	91.12	88.89	79.10	65.56	59.43	56.43
Schizosaccaromyces spp.	83.92	80.41	76.20	74.27	56.88	42.90
Hansenula spp.	*80.66*	*79.82*	*71.67*	*53.07*	*49.14*	*40.17*

* Percentage of Ustilago scitaminea mycelial growth weight reduction.

Table (3): Effect of yeast application on the percentage of smut disease incidence of Nco310 sugarcane variety.

Time of pathogen inoculation after yeast application (days)	%of infection
0	0.0 *
7	5.55 *
14	5.56 *
21	16.67 *
Control (untreated with yeast)	44.44 *

*Average of 18 plants

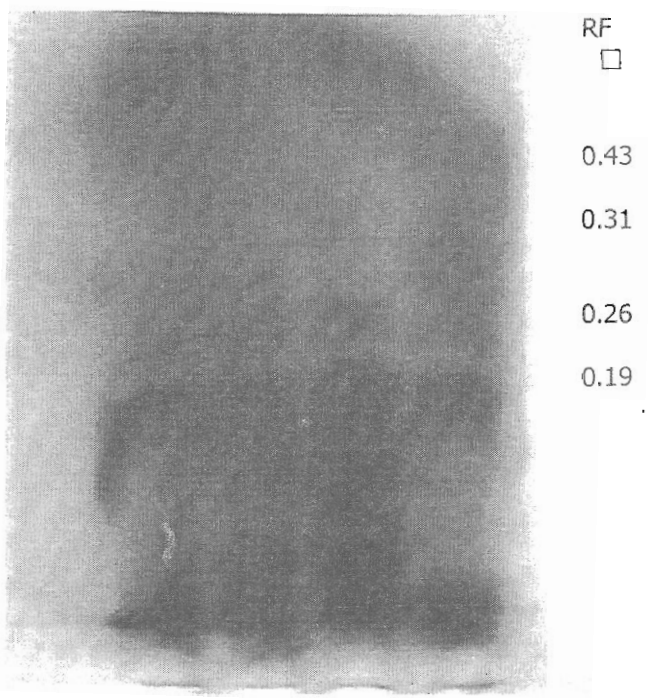

Figure (1) The esterase isozyme in mycelial growth of *U. scitaminea*

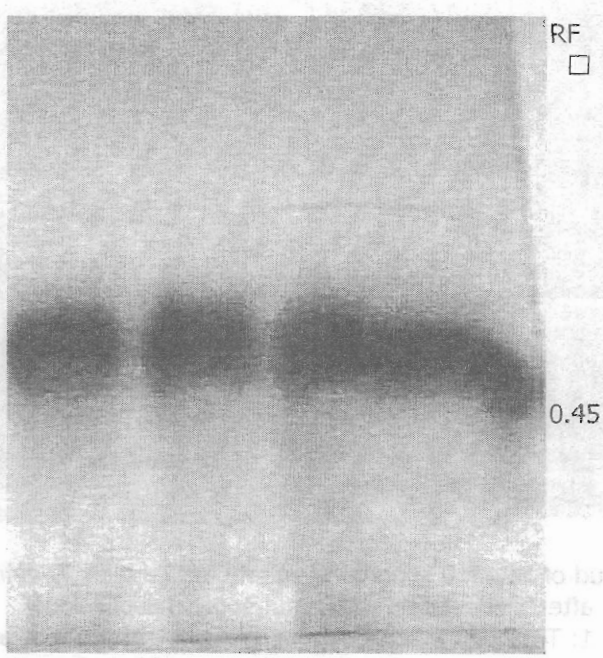

Figure (2) The acid phosphatase isozyme in mycelial growth of *U.scitaminea*

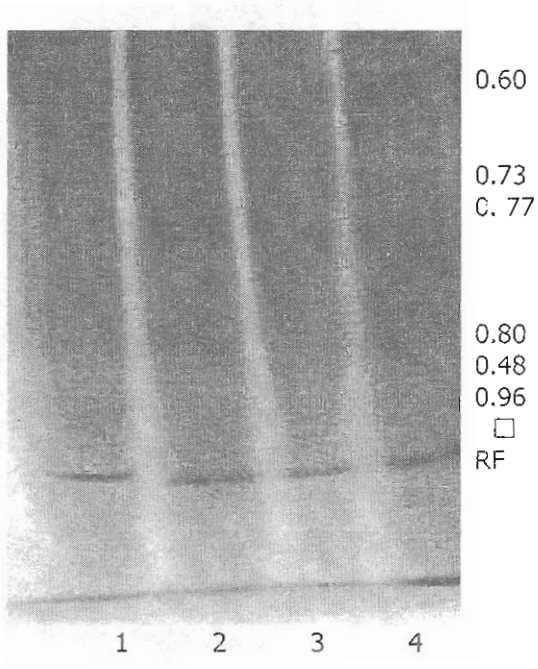

Figure (3) The enzymatic protein isozyme in mycelial growth of *U.scitaminea*

Figure (4) Bud of NCo310 sugarcane variety teated with *T. viride* bio-agent before and after inoculation with smut spore suspension.
1: Treatment with *Trichoderma viride* after inoculation with smut spores.
2: Treatment with *Trichoderma viride* before the inoculation with smut spores.
C: Untreated (Control).

تعريف السلالات والمقاومة الحيوية لمسبب تفحم قصب السكر ***Ustilago scitaminea***

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استخدم التحليل الكهربي لتحديد الاختلافات الوراثية بين عزلات التفحم الأربعة ، وذلك لثلاثة طرز أنزيمية (الأستيريز ، أسد فوسفاتيز وأنزيمات البروتين وتبين ما يلى:

يتحكم في أنزيم الأستيريز موقعين هما (Est-1، Est-2) وذلك في الأربعة عزلات المختبرة و أتضح أن الموقع الخاص بال (Est-1) له مظهرين مما يدل على أنه يتحكم فيه أثنين من الأليلات أحدهما ($Est\text{-}1^f$) ، والآخر ($Est\text{-}1^S$) . وكانت عزلات التفحم الأربعة خليطة وراثيا في الموقع Est-1 ، حيث تبين وجود ثلاثة حزم فيه ، بينما أنزيم حمض الفوسفاتيز قد أظهر عدم وجود اختلافات بين عزلات التفحم الأربعة ، بينما أظهر حزمة واحدة في الموقع Rf = 0.45 فى الزيموجرام. بينما فى أنزيمات البروتين فقد أظهرت ستة حزم في المواقع Rf = 0.6,0.73,077,0.8,048,0.96 . بينما لم تظهر أي اختلافات في العزلات الأربعة.

باختبار تأثير التضاد لفطر الترايكودرما على فطر يوستلاجو سكيتامينا أظهر الفطر تريكودرما فيردى تطفلا على النمو الفطري لمسبب تفحم القصب في المعمل ، بينما أدى استخدام عزلات الخمائر التالية في المعمل : *Saccaromyces cervisiiae,Schizosacchacromyces sp.,Torulopsis sp. And Hansenula sp.* إلى تثبيط نمو فطر تفحم القصب يوستلاجو سكيتامينا ، واختلفت تلك الخمائر في مدى هذا التضاد ، وتبين أن زيادة تخفيف الخميرة يؤدى إلى نقص في تأثيرها على الفطر المسبب للمرض. هذا ولقد سببت الخميرة *Saccaromyces cervisiiae تثبيطا كاملا لنمو المسبب فى المعمل عند التركيز ١٠% . بينما كان اقل تثبيط قد تم الحصول عليه بواسطة الخميرة Hansenula sp. وذلك عند تركيز ١: 10^{-6}* وأمكن التوصل إلى مقاومة مرض تفحم القصب وذلك بغمر عقل القصب في معلق تريكودرما فيردى (٢X ١٠ $^{-٦}$ وحدة عدوى / مل) قبل وبعد إجراء العدوى الصناعية بجراثيم الفطر فطر التفحم ، وانخفضت نسبة الإصابة بالمرض بدرجة كبيرة بمعاملة العقل بفطر تريكودرما قبل إجراء العدوى بالمسبب المرضى مقارنة بحدوث العدوى بالفطر الممرض بعد المعاملة بالتريكودرما . كما أمكن التوصل إلى مقاومة المرض بصورة كاملة في الصوبة وذلك برش سكارومايسس سيرفسيا في وقت إحداث العدوى بفطر تفحم قصب السكر .