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KERNEL SMUT OF BARNYARD GRASS (ECHINOCLOA CRUS-GALLI) IN EGYPT

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Abstract

Echinocloa crus-galli (Barnyard grass) is considered one of the most serious weeds in rice fields. Three years ago some smutted kernels of *E.crus-galli.* were observed. The causal fungus was isolated in Egypt and identified as *Ustilago trichophoron* (Link) Konicke det by Dr. J.E.M. Mordue (International Mycological Institute, U.K). The fungus produces sori in scattered flowers and on stems as bullate bodies from a few mm in diameter to 10 cm long swellings covered by a hispid membrane composed of an inner fungal layer and an outer layer of host origin. This study included different methods of inoculation to find the techniques to control or to minimize the seed residuals of this kind of weed in the fields and to avoid using herbicide as possible.

In the greenhouse, seeds of *E.crus-galli* were soaked either in suspensions of ustilospores or sporidia or incorporated directly with dry spores prior to seeding. Low disease incidence and severity of infection were observed which did not exceed 3.0 and 3.5% respectively. In case of injection at booting stage or spray the sporidial suspension just after heading (flowering) or one week later, the highest disease severities of 47.5%, 56% and 31 respectively were observed. However, no smutted kernels appeared in case of spraying spore suspension at either tillering or maturity stages.

Under field conditions injected plants at booting or plants sprayed at flowering stage resulted in a disease severity of 25.5% and 44% respectively. No symptoms were observed on the tested four rice cultivars namely, Giza 171, Giza 176, Giza 177 and Giza 181 subjected to the same treatments, indicating the specificity of the pathogen to this weed.

INTRODUCTION

Weeds are one of the most serious constraints to rice production in Egypt as well as in other countries. Among the weed species that affect rice is barnyard grass (*Echinochloa crus-galli*). It grows under varied climates and in many agronomic cropping systems and is one of the worst weeds in the world (Holm et al., 1977). Singh et al., (1985) reported that 1 and 2 barnyardgrass seedlings/hill reduced paddy yield by 20.2 and 37.5% respectively. The increase in the number of barnyardgrass from 0 to 12 plants/m^2 resulted in gradual and significant decrease in height,

number of tillers/hill, leaf area index and panicle weight of rice (Hassan and Rao, 1994). Hand weeding is becoming increasingly expensive and rural labors are unavailable when needed most. This often delays weeding and the crop suffers.

It has been found that *Echinochloa* spp. is attacked by a smut disease. The causal fungus was identified as *Ustilago trichophoron* and recorded in some countries (Fullerton & Longdon, 1969; Duran, 1973 & 1987 and Vanky, 1994). Fullerton & Langdon (1969) in demonstrating structural and developmental similarities in sori of *Ustilago* species attacking *Echinochloa* spp. concluded that a number of *Ustilago* on *Echinochloa* could be referred to as one species, namely *Ustilago trichophora.* They also indicate the need for further studies emphasizing soral ontogeny in the classification of the smut fungi. This study was carried out to identify the causal fungus of kernel smut of *E.crus-galli* and to obtain more information about the best inoculation method in order to use it as a mycoherbicide to eradicate or at least minimize the seed production of *Echinochloa* spp. in the soil and to avoid pesticide pollution.

MATERIALS AND METHODS

Preparation of the inoculum:

Samples from smutted seeds (sori) were collected, sterilized and opened under aseptic conditions. The ustilospores (Fig. 1-B) were distributed on the surface of Petri dishes containing potato dextrose agar (PDA). The inoculated dishes were left in the incubator at 28°C for one week. Mass of secondary sporidia (Fig. 2A) were collected by brushing in tap water and a sporidial suspension was prepared for artificial inoculation ($5x10^6$ sporidia/ml). On the other hand, chlamydospores (ustilospores) were prepared by suspending them from sterilized sori in tap water ($2x10^5$ spores/ml) and used for artificial inoculation.

Artificial Inoculation Techniques.

1. Greenhouse Conditions:

Trays (30x20x12 cm) filled with loamy soil were used to grow treated seeds of barnyard grass as follows:

A. Seeds of barnyardgrass were soaked in ustilospore or sporidial suspension for 24 hrs, then transplanted after germination.

B. Barnyardgrass seeds were incorporated with powders of ustilopores or collected sporidial spores from Petri-dishes and planted immediately after incorporation.

C. Healthy seeds were soaked in water for 24 hrs and transplanted after germination, then the following inoculation methods were carried out using sporidia or ustilospore suspension as follows:

a) Sprayed at tillering stage using plastic sprayer.

b) Injected at booting stage by a syringe.

c) Sprayed just after complete heading at anthesis.

d) Sprayed one week after heading.

e) Sprayed after complete heading (ripening).

f) Plants were left free from any inoculation as control.

Four rice cultivars, namely, Giza 171, Giza 176, Giza 177 and Giza 181 were tested as host plants using the same above mentioned inoculation methods under the same environmental conditions and the same period of incubation.

II. Field Experiment:

Four plots (each 4 x 5 m) were transplanted with *E.crus-galli*, while four other plots were transplanted with the rice cultivar Giza 177 alternatively. Labelled plants in the first plot of *E.crus-galli* were injected at booting stage with sporidial suspension, while ustilospore suspension was injected in the plants of the second plot. In the third plot, the inoculation were carried out at the evening in the middle two square meters kept under polyethylene cover, during spraying the sporidial suspensions, just after panicle emergence. The fourth plot was sprayed with ustilospore suspension. The same inoculation methods were adopted in case of rice cultivar Giza 177 at both booting and just after panicle emergence. Two plots were left free from inoculation as control, one for *E.crus-galli*, and the other for the rice variety Giza 177.

The infection was estimated as a percentage representing the number of smutted panicles compared with the healthy ones, whereas the severity of infection was estimated as a number of smutted grains/panicle.

RESULTS AND DISCUSSION

The causal fungus of smutted kernels of *E.crus-galli* was isolated and identified as *Ustilago trichophoron* (Link) Kornicke det by Dr. J.E.M. Mordue (IMI, UK). The ustilospores are single, globose to subglobose or ovoid 7-14 x 6-12 (mean 9.2 x

8.4) µm diam (Fig. 1B). Ustilospore wall is thin (0.5 µm), echinulate ornamentation, the spines somewhat unevenly distributed up to 1 µm long but usually less. The tapered spines (frequently bent at the apex) rarely interspersed with a few minute warts. This identification agrees with the findings of Vanky (1994).

The disease symptoms (Fig. 3) is characterized by the presence of sori or a black, sooty mass of spores which erupts from the inside of the kernel and adheres to the surface of the hull. Sori develop and form in seeds (Fig.3A), culms and nodes (Fig. 3 C) as much swolen galls. They range in size from small pustules 0.5 mm across by confluence to large locular, often cerebriform, structures 10 cm or more in length and 1-2 cm in diameter, in the floral axis or more usually confined to a few scattered florets (in ovaries, anthers or lodicules) and at the lower end of the size range. They are first covered by a firm membrane, later the spore mass is exposed. Sorus covering is thick, comprising an outer layer formed from host tissues with trichomes and an inner layer of fungal tissue. The covering eventually splits irregularly either apically or laterally to expose the spore mass. Spore mass developed around a variable but often distinct central columella of host and unpigmented fungal cells. They are dusty, dark olivaceous, brown, homogeneous composed of ustilospores only. Some buds on the nodes (late tillering from the axillary buds sprouting) exhibit galls, while most of the symptoms appear on the panicles.

In the greenhouse, data presented in Table (1) revealed that both percentage and severity of smutted panicles were low when seeds were soaked in sporidial suspension or incorporated with either sporidia or ustilospores. There was no distorted or smutted panicles when sporidial spore suspension was sprayed at tillering stage or at complete heading, whereas the highest percentage of smutted panicles were obtained when sporidial suspension was sprayed just after heading followed by injection at booting and sprayed one week from heading. The obtained data also showed that the highest number of distorted panicles were obtained from injection of sporidial suspension at booting, while very few developed in case of soaked or incorporated seeds with the fungal spores (Fig. 3B). No distorted panicles were observed with the rest of the inoculation methods. These results confirm those reported by Mordue (1995) who mentioned that symptoms vary from relatively inconspicuous infection of a few ovaries in an inflorescence through node and culm infection with normal inflorescence production, to infection that result in production of rosetts of short leafy axillary shoots and no inflorescence or distortion of the entire shoot system. The affected panicles showed different percentages of smutted grains which appear at random positions in the head depending on the severity of infection or the level of inoculum.

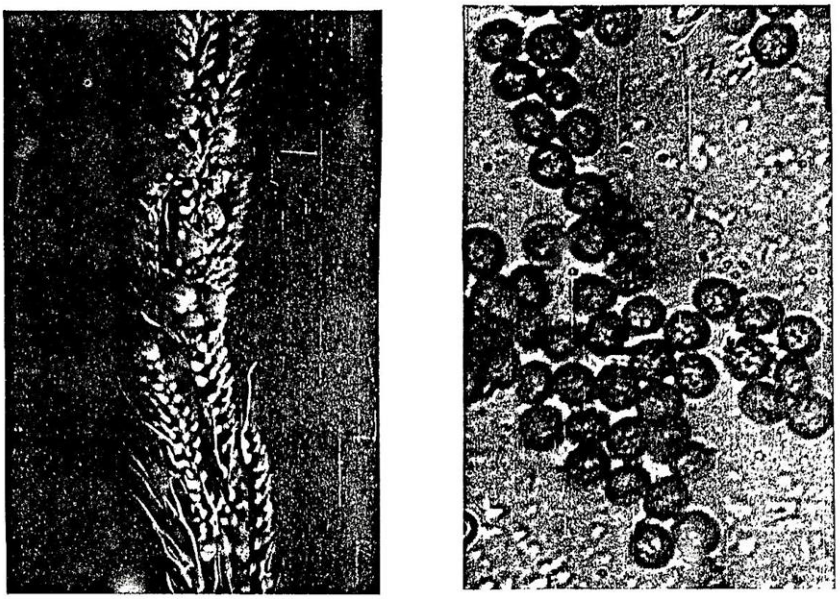
Table 1. Effect of different methods of inoculation with sporidia or ustil or ustilospores of *Ustilago trichophoron* on *Echinochloa crus-galli* under greenhouse and field conditions.

Treatments	Sporidial spore suspension					Utilospore suspension				
	No.of healthy panicles	No.of smutted panicles	No.of distorted panicles	% of infection	Severity of infection	No.of healthy panicles	No.of smutted panicles	No.of distorted panicles	% of infection	Severity of infection
I. Greenhouse:										
Seed were soaked for 24 hrs	115	1	2	2.50	3.5	135	0	1	0.7	0
Seeds were incorporated before growing	127	3	1	3.10	2.5	95	1	0	1.1	1.5
Plants were sprayed at tillering stage	136	0	0	0.0	0	104	0	0	0	0
Plants were injected at booting stage	157	49	26	32.3	47.5	135	0	0	0	0
Plants were sprayed just after heading	116	52	0	44.8	56	122	0	0	0	0
Plants were sprayed one week from heading	130	16	0	12.3	31	105	0	0	0	0
Plants were sprayed after complete heading	118	0	0	0.0	0	97	0	0	0	0
Control (free from inoculation)	150	0	0	0.0	0	150	0	0	0	0
II. Field:										
Plants were injected at booting stage	220	31	4	15.9	25.5	185	0	0	0	0
Plants were sprayed just after heading	190	38	0	2	44	206	0	0	0	0
Control (free from inoculation)	200	0	0	0	0	200	0	0	0	0

The same trend was clear when plants were injected at booting stage or sprayed just after heading. However, percentage or the severity of infection were lower in the field than in the greenhouse. This may be due to the higher relative humidity in the greenhouse than in the field. Neither infected panicles nor other mentioned symptoms were observed on the four tested rice cultivars. These result indicated that the fungus *Ustilago trichophoron* is specific to *Echinocloa* spp. and support the findings of Duran (1973).

It is obvious that infection is affected by the time and method of inoculation. Under field conditions, spraying inoculum at booting stage and just after heading led to an infection that may reach 20%. Therefore, this could be utilized as a biocontrol method of barnyard grass in providing some effect against the reproduction of the weed and reducing the number of seeds produced.

However, the presence of spores mixed with rice grains and its subsequent efficiency in controlling the weed in the field depends on its survival and other factors which need to be identified and studied further. Trials employing soil infestation with smut spores be may be useful.



A B

Fig. 1. A: Smutted swolen seeds (Sori)

B: Ustilospores (x 700)

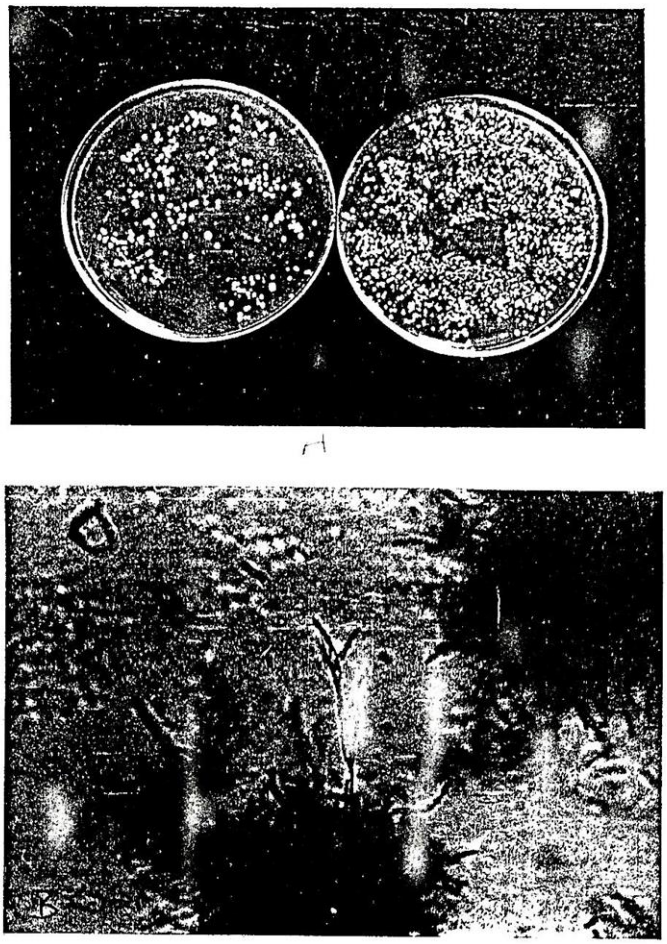


Fig. 2. A: Mass of secondary sporidia in Petri-dishes inoculated with ustilospores.

B: Secondary sporidia from Petri-dishes under the microscope (x 200).

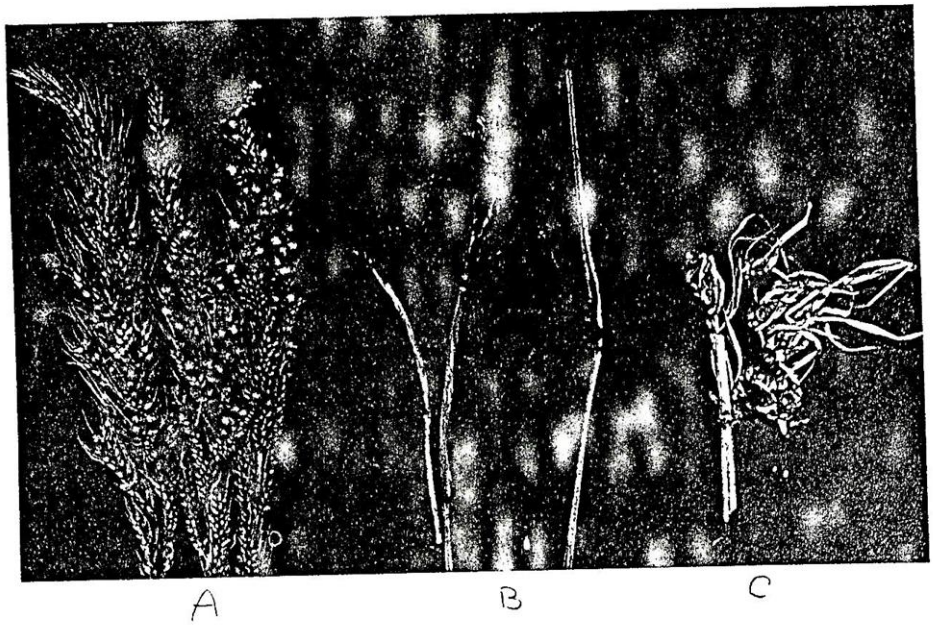


Fig. 3. A: Developed sori in seed
B: Distorted panicles
C : Swollen galls on culms and nodes

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تفحم الدنيبة فى مصر

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تعتبر حشيشة الدنيبه واحدة من أخطر الحشائش التى توجد فى حقول الأرز والتى تستخدم العديد من مبيدات الحشائش فى مقاومتها سنويا. منذ حوالى ثلاث سنوات لوحظ بعض التفحم فى الحبوب على سنابل الدنيبه حيث تم عزل الفطر المسبب وتنقيته، وتم تعريفه للمره الأولى فى مصر بواسطه معهد إنجلترا الدولى لتعريف الكائنات الدقيقة وهو فطر (يوستيلا جوتريكوفورون) والفطر يسبب تفحمات وتشوهات فى بعض الأزهار بالنوره، وعلى السيقان عادة ماتكون صغيره الحجم (عدة مليمترات فى القطر)، وقد تصل إلى ١٠ سم فى الطول. وهذه البثرات المتفحمه تكون مغطاه بغشاء داخلى من الفطر نفسه وغشاء خارجى يتكون من النبات العائل.

أجريت هذه الدراسة بغرض معرفة أكفأ طريقة فى إحداث العدوى لإستخدامها فى مقاومة حشيشة الدنيبة أو التقليل من كمية البذور الناتجة منها والتى تبقى فى الأرض للموسم القادم.

تمت الدراسة فى الصوبة بإستخدام نقع التقاوى فى معلق من الجراثيم التيلتيه أو الأسبوريدية أو خلط التقاوى مباشرة قبل الزراعة مع مسحوق كل من النوعين على حده. وقد كانت أعراض الإصابة بهذه الطرق قليلة الحدوث فقد تراوحت شدة الإصابة من صفر إلى ٣,٥٪ فقط. بينما فى حالة الحقن فى مرحلة قبل طرد السنابل مباشرة (Booting) أو الرش بالجراثيم الأسبوريديه مباشرة بعد الطرد فى مرحلة التزهير زو بعد أسبوع من الطرد، أظهرت النتائج أن شدة الإصابة عالية حيث وصلت إلى ٤٧,٥ ، ٥٦، ٣١٪ على التوالى. بينما لم تلاحظ أعراض تفحم فى حالة الرش بمعلق جراثيم النوعين على النباتات فى مرحلة التفريع أو مرحلة النضج الكامل.

كما أجريت دراسة حقليه حيث تبين أن حقن النباتات فى مرحلة قبل الطرد أو الرش فى مرحلة التزهير بالجراثيم الأسبوريديه قد أعطت إصابة تصل إلى ٢٥,٥ – ٤٤٪ على التوالى. لذا يتضح أن طريقة الرش بالجراثيم الأسبوريديه فى مرحلة التزهير أعطت أعلى إصابة يليها الحقن فى مرحلة قبل الطرد تحت ظروف كل من الصوبة والحقل.

كما تم إختبار أربعة أصناف من الأرز جيزة ١٧١ ، جيزة ١٧٦ ، جيزة ١٧٧ وجيزة ١٨١ حيث تم عدواها بنفس الطرق السابقة تحت نفس الظروف للتأكد من عدم قدرة الفطر على إصابة المحصول الرئيسى حيث لم يلاحظ أى تفحمات على أى جزء منها مما يدل على تخصص الفطر على حشيشةة الدنيبه فقط