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Virulence and Control of *Sporisorium ehrenbergii* Vánky Races Attack Sorghum in Sohag Regions of Upper Egypt

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Abstract: ***Sporisorium ehrenbergii Vánky is the causal agent of long smut (LS) on sorghum in several African and Asian countries. For effective breeding programs to evolve LS resistant varieties to control this important disease, information on the current status of physiological races of S. ehrenbergii is most essential. In this study, when teliospores of 22 collected isolates were cultured on PDA medium at 30°C for 15 days, three distinct and frequent morphological colonies were observed and they were designated as form No. 1, 2, and 3. Virulence of these isolates and their forms was tested on certain sorghum genotypes/varieties which could serve as a set of differentials for evaluating resistance to LS in field experiments to characterize the putative virulent races. All 7 isolates of the form No. 2 were highly virulent (HV) on all 13 tested sorghum genotypes/varieties, and they were designated race No. 2. In contrast, all 6 isolates of the form No. 1 were only HV on 3 tested sorghum genotypes/varieties (race No. 1) and all 9 isolates of the form No. 3 were HV on 9 tested sorghum genotypes/varieties (race No. 3). Following in vitro screening test, water extracts of rheum (Rheum rhabarbarum) and common walnut (Juglans regia) at 1% exhibited full inhibition of teliospore germination of S. ehrenbergii. Moreover, spraying inoculated sorghum plants with 1% rheum and common walnut extracts twice during panicle emergence significantly reduced the incidence of LS compared with untreated control plants.***

Keywords: Control; Long smut; Plant extracts; Sorghum; *S. ehrenbergii*; Virulent races.

1. Introduction

Grain sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world after wheat, rice, corn and barley. It is grown in more than 100 countries all over the world. In 2012, Egypt planted 160 thousand ha of sorghum with an average yield of 5625 kg ha^{-1} [6]. More than 80% of the area devoted to sorghum lies within El-Fayoum, Assiut and Sohag governorates of Upper Egypt. In the areas where sorghum is traditionally grown, plants may be attacked by different smut diseases. Among them, LS caused by *Sporisorium ehrenbergii* Vánky (syn. *Tolyposporium ehrenbergii* (Kühn) Pat.) is a limiting factor in sorghum productivity in several African and Asian countries [19, 18, 5, 25, 33]. Teliospores of *S. ehrengergii* often stick together to form balls, which can survive in the soil for many years. These teliospore balls can also adhere to the sorghum seeds and with other infective propagules serve as the primary source of inocula [17, 33]. The infection takes place only during the booting stage of sorghum, when teliospores germinate and produce sporidia that can be carried through wind currents to flag leaves and washed down into the boots to initiate infection of individual spikelets [32, 24, 17, 33]. Airborne teliospores may also settle on the flag leaf and germinate in water droplets accumulated at the flag leaf sheath to produce sporidia, which can infect the opening florets in the panicle [43]. In addition to cultivated grain sorghum, many wild sorghum species are also attacked by *S. ehrenbergii* [41, 1, 24, 12, 8]. During survey of LS in growing successive seasons 2010 and 2011 of Sohag regions, symptoms of LS (Figure 1) were observed on the cultivated sorghum cultivars and two other related species broomcorn (*S. vulgare* Pers.) and sweet sorghum (*S. saccharatum* Linn. Moench). For effective breeding programs to evolve LS resistant varieties, information on the current status of physiological races of *S. ehrenbergii* in Egypt is most essential. Existence of at least two races of *S. ehrenbergii* has been suggested before [41]. As far as available of information there are no reports to date on the existence of races or pathotypes worldwide of *S. ehrenbergii* and 9 morphological forms were identified of 150 collected isolates from Assiut, Sohag and Qena governorates of Upper Egypt where the disease was earliest reported [5]. Otherwise, existence of the physiological forms and races of other related fungi causing smut diseases on sorghum were reported [7, 14, 34, 23, 3, 4, 22, 8, 45]. *Sporisorium sorghi* and *S. reilianum*, the causal organisms of kernel and head smut of sorghum, respectively, are known to produce distinct forms, which differ in their color, surface, consistency, margin, and rate of growth.

Host plant resistance is considered the best strategy for control LS [19, 17], as a result of searching for resistant genotypes, no immune genotypes to the disease have been identified [31, 17, 33]. However, several sorghum lines have exhibited high levels of resistance to LS in different parts of the world; these include Regular Hegari, Redlan, and Spur Fetertia [40], Impi fodder [35], four Pakistani cultivars C-45, AUS-6, NK-125, and NK-263[27], SC630-11E, QL-3 (India), and SC326-6 [31] and two American cultivars B.9612 and R.9645 [33]. Control of LS mainly depends on fungicide treatment [20, 11]. As a fungicidal application causes hazards to human health and increases environmental pollution. Hence, alternatives and eco-friendly approaches for the control of plant diseases are needed. Several plant

extracts such as neem, garlic, henna, rheum, goldenrod and lemon grass were applied effectively to control various plant pathogens [16, 15, 39, 36]. Limited information on the efficacy of emulsified neem (*Azairachta indica*) seed oil as foliar application to control LS was reported [2]. Successful application of various plant extracts with antifungal activity for the control kernel smut of sorghum caused by *S. sorghi* under greenhouse or field trials were earlier reported [30, 45, 42, 2, 28, 38]. The objectives of this research were intended to (1) survey LS in Sohag regions, (2) explore and characterize the distinct and virulent races attacking the cultivated sorghum based on the vulnerability of some available genotypes/varieties to the collected isolates and their forms, and (3) investigate the potential of several plant extracts for control of *S. ehrenbergii* in *vitro* and in field trails.

2. Materials and Methods

Survey of sorghum LS disease in Sohag regions

A field survey of LS disease on sorghum was conducted in the summer growing seasons 2010 and 2011 in eleven counties of the Sohag governorate, Upper Egypt listed in (Table 1). Five villages from each county and five sorghum fields' from each village were randomly chosen. Fields were only assessed if the sorghum crop was in the milk or later maturity stage. In total, 500 plants were randomly selected from each field using a W-pattern of zigzag method described by [35]. Heads of growing plants were examined for LS infection, and then the percent of infected plants and the average number of sori per infected head was calculated.

Source of *S. ehrenbergii* isolates and their identification

Sori of smutted panicles from certain cultivars of grown sorghum were collected in paper bags and sampled through the 2010 growing season in three regions (Nord, Middle and South of Sohag), which represent all eleven counties surveyed. Sori were crushed to collect the teliospores. The resultant smut masses were passed through a sieve (100-mesh screen) to eliminate plant debris, and stored at ambient temperature in the laboratory for further studies. Identification of *S. ehrenbergii* was carried out according to the morphological characteristics of teliospores and their germination on water agar as described previously [41, 21, 37, 8].

Morphological differences between the isolates and frequency of morphologic forms

Teliospores (100 mg) of each collected sample was immersed in 1.5% CuSO4 solution, centrifuged at 10,000 rpm for 5 min, washed twice with sterile distillated water (SDW) through centrifugation and then re-suspended with 100 ml SDW. A small quantity of teliospore suspension (10 µl) was transferred immediately to test tubes containing 10 ml of liquid potato dextrose agar (PDA) at 50°C. The agar was poured into Petri plates before solidification, and solidified plates were incubated at 30°C. Growing colonies were checked daily for a few days, and pure cultures were transferred onto new PDA plates. After 15 days of incubation on plates at 30°C, the distinct and frequent pure colonies were examined for diameter, color, consistency, topography, and margin to define the morphological forms. Pure cultures of all isolates were coded, transferred on PDA slants in test tubes and maintained at 5°C for further studies.

Used plant materials

Sorghum genotypes and varieties used in this study to evaluate their vulnerability to the collected isolates of *S. ehrenbergii* are listed in (Table 2 and 3). The commonly grown varieties in Egypt (Giza 3, Giza 15, Giza 54, Giza 114, Dorado and Shandawel 2) of *S. bicolor*, five breeding accessions (PI 574555, PI 574560, PI 574580, PI 574598, PI 574610) kindly supplied by Plant Genetic Resources Conservation Unit (Georgia, USA), and two unknown varieties of the commonly grown *S. saccharatum* and *S. vulgare* in Sohag regions of Upper Egypt were used to test virulence of the isolates. Plant species from which extracts were prepared for testing growth inhibition against *S. ehrenbergii* in this study are listed in (Table 4).

Preparation of LS inocula and inoculation method

Teliospore suspension (1×10^6 spores ml^{-1}) of each tested LS isolate was made with SDW. After an incubation period of 16-20 h at room temperature, the suspension was filtered through a double-layered muslin cloth [33]. On other hand, the aqueous suspension of sporidia (1×10^6 sporidia ml^{-1}) was also prepared from a 7-day-old growth of *S. ehrenbergii* incubated at 30°C on PDA for each tested form [43]. For inoculation of the plants at boot stage, 10 ml of spore suspension was placed between the flag leaves and the panicles using a pipette. Inoculated boots were labeled and covered with paper bags at the time of inoculation. Following full appearance of panicles, bags were removed.

Virulence of the collected isolates and their forms to sorghum genotypes after artificial inoculation

Field experiments on several sorghum genotypes and varieties were conducted in the successive growing seasons 2011 and 2012 at the Experimental Farm, Fac. of Agric., Sohag Univ., Egypt, to assess virulence of the collected isolates and their designated forms, and the tests were also undertaken to characterize the putative races of *S. ehrenbergii*. The field soil was sandy-loam with organic matter (2.3%), sand (73.2%), silt (16.4%), clay (8.5%) and a pH of 7.7. The sowing date in both seasons was 15th of May. In each trial, sorghum seeds sterilized with 1% sodium hypochlorite solution of each genotype or variety were sown in plots in a randomized complete block design with three replicates. Plots were two rows, each 3.5 m long with 0.6 m row and 0.3 m plant spacing. Following full emergence, hills were thinned to two plants per hill in each row. All cultural practices were applied as recommended for sorghum production. At boot stages, twenty plants per plot were inoculated with spore suspensions of each tested LS isolate or mixtures of different forms, and then boots with the bases of flag leaves were covered as described above. Any plant exhibiting LS sori on the panicle was scored as infected. The number of infected plants per plot was counted at maturity and expressed as percent incidence [33]. In this study, isolates with a disease incidence (DI) of 1-20% are considered weakly virulent (WV), 20.1-40% as moderately virulent (MV) and >40% as highly virulent (HV). Any genotype/variety with a disease incidence 0% was recorded as immune (I), with 1-9.9% smutted heads as highly resistant

(HR), with 10-20% as resistant (R), with 20.1-40% as susceptible (S), and with >40% as highly susceptible (HS).

In vitro* screening test for bioefficacy of some plant extracts on growth of *S. ehrenbergii

Plant extracts of tested plants (Table 3) were prepared by stirring 10 g of the plant powder in 100 ml heated (50°C) distillated water (DW) for one hour, followed by centrifugation for 10 min at 10,000 rpm and 4°C. The supernatant was collected and used as stock solution (10%), which was added to warm, sterilized PDA to obtain final concentrations of 1, 3 and 5%. PDA amended with DW instead of plant extracts served as control. After pouring the medium in Petri plates (9 cm) and solidification of the agar, each plate was inoculated with 0.5 ml teliospores suspension (1 mg ml^{-1}) of virulent race No. 2 (mixture of isolates 2, 6, 9, 13, 15, 19 and 22) and four plates were used for each treatment. Then plates were incubated at 28°C for 18 hours, and the germination of teliospores was evaluated microscopically by checking 100 teliospores per plate. Teliospores were rated as germinated if germ tubes (young promycelia) were visible. Percent inhibition of spore germination was computed in relation to the percentage of germinated spores on control plates. In another experiment, stock solution (10%) of each tested plant extract was diluted with DW to obtain final concentrations of 1, 3 and 5%. PDA plates were sprayed with 0.5 ml teliospore suspensions. Filter paper discs (1cm in diameter) were immersed in each tested concentration for 5 min. Then treated discs were placed in the middle of inoculated plates and four plates were used for each tested concentration. Discs immersed in DW and placed in middle of inoculated plates were used as control. Three days after incubation of the plates at 30°C, inhibition zones (cm) of *S. ehrenbergii* growth were measured.

Control of LS by foliar application of plant extracts

Two field trials were conducted during the successive growing seasons 2012 and 2013. The experiments were laid out in spilt plot design with three replicates of each treatment. The sorghum cultivars Giza 15, Dorado and Shandawel 2 were randomly assigned to the main plots, separately and independently for each of the three replications. Plots were 8 rows, each 3.5 m long with 0.6 m row and 0.3 m plant spacing. Each row consisted of 20 hills, two plants per hill making a total of 40 plants per subplot. The sowing date in both seasons was 15th of June. At boot stages, all plants of main plots were inoculated with spore suspension of race No. 2 (mixture of isolates 2, 6, 9, 13, 15, 19 and 22), and then boots including bases of flag leaves were covered as described before. Water extracts of 1% common walnut, 3% cowslip and 1% rheum were sprayed separately using a hand sprayer machine (Shandong Wish Plant Protection Machinery Co., Ltd., China) on panicles in subplots twice starting at emergence of the panicles (the second spray was done 3 later days than first spray). Some inoculated plants of each sorghum variety remained untreated in subplots and served as control. Disease incidence of infected plants per subplot was calculated at maturity as described before.

Statistical analysis

All collected data were statistically analyzed using analysis of variance with the MSTAT-C program and the least significant difference (L.S.D.) was used at 0.05 and 0.01 levels of probability [9].

3. Results

Survey of sorghum LS disease in Sohag regions

A field survey for the occurrence of LS disease on sorghum was conducted during the growing summer seasons 2010 and 2011 in 11 counties of Sohag governorate. Collected data indicate that sorghum was infected with LS with various degrees of infection (Table 1). Disease was 28.37 and 7.71% in 2010 and 2011 with an average number of sori per infected head of 22.74 and 10.5, respectively. The highest infection was observed in El-Maragha and Gehena in both seasons 2010 and 2011 (36.68, 11.92%) and (36.24, 11.04%), respectively. While the lowest infection was 18.87 and 4.14% in Dar El-Salam in 2010 and 2011, respectively. On average, the LS infection and the average number of sori per infected head were higher in 2010 than in 2011.

Figure 1: Sori of long smut on sorghum (*Sorghum bicolor*) Shandawel 2 cultivar.

Morphological differences between the isolates and frequency of morphologic forms

Twenty-two isolates of *S. ehrenbergii* were obtained from diseased sorghum samples collected from different fields in three regions (Nord, Middle and South) of the Sohag governorate representing all eleven counties surveyed in 2010 season (Table 2). Colonies of the collected isolates varied in their morphological characteristics of diameter, color, consistency, topography and margin after culturing on PDA medium at 30°C for 15 days. On the basis of morphological characteristics, it could be concluded that the 22 collected isolates exhibited 3 distinct and frequent forms of *S. ehrenbergii* and they were named form No. 1, 2 and 3 (Fig. 2). The designated forms differed in their frequency. The most frequent form was form No. 3 with nine (40.9%) of all isolates. Form No. 1 occurred in six (27.3%) and form No. 2 in seven (31.8%) isolates (Table 2).

Virulence of the collected isolates and their forms in response to sorghum genotypes

The collected isolates and their forms of *S. ehrenbergii* obtained from different resources (Table 2) were tested on certain sorghum genotypes in field trials of the two successive seasons 2011 and 2012 to demonstrate the differences in their pathogenic capabilities. The tests were also undertaken to determine and characterize the putative races of the pathogen based on the reactions of certain sorghum genotypes to the 22 collected isolates. Data presented in Table 3 and 5 indicate that isolates of *S. ehrenbergii* varied significantly in their virulence on the tested sorghum genotypes. Isolates No. 2, 6, 9, 13, 15, 19 and 22 of the designated form No.2 were highly virulent on all tested sorghum genotypes. Therefore this form was defined as race No. 2. Isolates No. 1, 3, 4, 7, 8 and 20 of form No.1 were only highly virulent on Giza 3, Giza 114 and an unknown variety of *S. vulgare*. Therefore this form was defined as race No. 1. Isolates No. 5, 10, 11, 12, 14, 16, 17, 18 and 21 of form No.3 were highly virulent on Giza 15, Dorado, Shandawel 2, American accessions PI 574555, PI 574560, PI 574580, PI 574598, and PI 574610 and an unknown variety of *S. saccharatum*. Therefore this form was defined as race No. 3.

Quantitative reactions of certain sorghum genotypes to the 22 collected isolates and/or their forms (Table 3 and 5) indicated that none of the tested genotypes was immune. All tested sorghum genotypes were highly susceptible (HS) to the isolates of designated form (race) No.2. The genotypes Giza 3 and Giza 114 were HS to race 1 and R to race 3, *S. vulgare* was HS to race 1 and HR to race 3, Giza 15 was S to race 1 and HS to race 3, Giza 54 was S to race 1 and R to race 3, and Dorado, Shandawel 2, PI 574555, PI 574560, PI 574580, PI 574598, PI 574610, and *S. saccharatum* were HR to race 1 and HS to race 3.

Bioefficacy of tested plant extracts on growth of *S. ehrenbergii*

To test the effect of some plant extracts on teliospore germination and growth inhibition of *S. ehrenbergii* (Table 6), *in vitro* screening test was done. Results indicate that water extracts of the tested plants significantly varied in their effects at all tested concentrations 1, 3, and 5%. Extracts of common walnut and rheum were the most effective ones and they completely inhibited teliospore germination at all tested concentrations. Moreover, rheum and common walnut caused the highest inhibition of colony growth with average inhibition zones of 1.27 and 0.45 cm, respectively (Fig. 3). Extract of cowslip at 3 and 5% completely suppressed teliospore germination and resulted in inhibition of colony growth with an average inhibition zone of 0.39 cm. On the other hand, tested extracts of datura, goldenrod, mugwort, nettle, purple coneflower, salvia and soapwort did not alter teliospore germination.

Control of LS by foliar application of plant extracts

Spray of water extracts of the tested plants twice starting from panicle emergence of treated varieties significantly reduced the incidence of LS as compared with untreated control in both successive seasons 2012 and 2013 (Table 7). Results also show that 1% rheum extract was the most effective in both seasons, where it reduced the infection with LS of Giza 15, Dorado and Shandawel 2 in the 2012 and 2013 (Table 7). Whereas, 3% cowslip extract showed the lowest inhibitory effect on LS development in both seasons. Moreover, 1% common walnut extract reduced the infection with LS of Giza 15, Dorado and Shandawel 2 moderately in both seasons.

4. Discussion

LS of sorghum caused by *S. ehrenbergii* was first reported in Egypt in 1887 and was discovered later in many African and Asian countries [17]. LS has gained prominence as a serious problem in certain countries of Africa and Asia [10, 13, 18, 5, 25, 33, 43]. The disease is reported both in cultivated and wild sorghum species. In some cultivars under favorable conditions grain damage could be 5% or more, thus causing substantial economic loss to farmers [43].

In this study, a field survey of LS disease of sorghum was conducted in the successive growing seasons 2010 and 2011 of Sohag regions, Upper Egypt. Based on the obtained results, infection with LS varied in both seasons of all surveyed regions. Infection and the average number of sori per infected head were higher in 2010 than those of 2011. Such results may be due to the differences between the environmental conditions of the two seasons in surveyed areas as well as the replacement of cultivars cultivated by farmers which could vary in their susceptibility and resistance to LS disease and may be due to the existence of various races of the pathogen.

Because of the fact that pathogen has various physiological forms, which differ greatly in their cultural characteristics and virulence, it seemed highly desirable to characterize the forms of *S. ehrenbergii* that exist at Sohag regions. During this study, twenty-two isolates of identified *S. ehrenbergii* were obtained from certain sorghum cultivars and two other related species (broomcorn and sweet sorghum) growing in different locations of Sohag. Three distinct and frequent forms based on their morphological characteristics were distinguished from the 22 isolates, when teliospores of these isolates were cultured on PDA at 30°C for 15 days. They are designated as form No. 1, 2, and 3 that occurred in slightly different frequency, with form No. 3 occurring most frequently. Such results are in accordance with those reported by [5] who defined nine forms from a total of 150 isolates in Sohag, Assiut and Qena Governorates of Upper Egypt, and their designated form No. 6 exhibited the highest frequency among all isolated forms.

This investigation also showed that all twenty-two isolates of *S. ehrenbergii* and/or their isolated forms varied significantly

Table 1: Incidence of LS disease of sorghum observed in eleven counties of Sohag governorate, Upper Egypt during survey in 2010 and 2011 growing seasons.

County (Abbreviation)	*2010*		*2011*		*Average*	
	Infection (%)[a]	*Sori per head*[b]	*Infection (%)*[a]	*Sori per head*[b]	*Infection (%)*[a]	*Sori per head*[b]
El-Baliana (ElB)	24.96	24.75	7.78	10.61	**16.37**	**17.68**

Girga (Gir)	20.30	19.84	5.07	9.80	**12.68**	**14.82**
Dar El-Salam (Dar)	18.87	13.02	4.14	8.14	**11.50**	**10.58**
El-Monshah (ElMo)	21.21	19.71	7.86	9.83	**14.53**	**14.77**
Sohag (Soh)	33.83	25.77	5.94	9.59	**19.88**	**17.68**
El-Maragha (ElMa)	36.68	31.41	11.92	12.33	**24.30**	**21.87**
Akhmem (Akh)	33.67	23.53	9.09	10.61	**21.38**	**17.07**
Sakolta (Sak)	28.26	20.55	8.43	9.54	**18.34**	**15.04**
Tahta (Tah)	28.73	22.95	7.46	10. 13	**18.09**	**22.95**
Tema (Tem)	29.35	24.66	7.84	10.76	**18.59**	**17.71**
Gehena (Geh)	36.24	27.87	9.28	11.04	**22.76**	**19.45**
Average	**28.37**	**23.09**	**7.71**	**10.22**	**18.04**	**16.66**
L.S.D. at 5%	1.78	1.41	1.09	1.11	1.27	1.21

[a] Out of 25 fields representing of five villages based on sample of 500 plants in each field.
[b] Average number of sori per infected head.

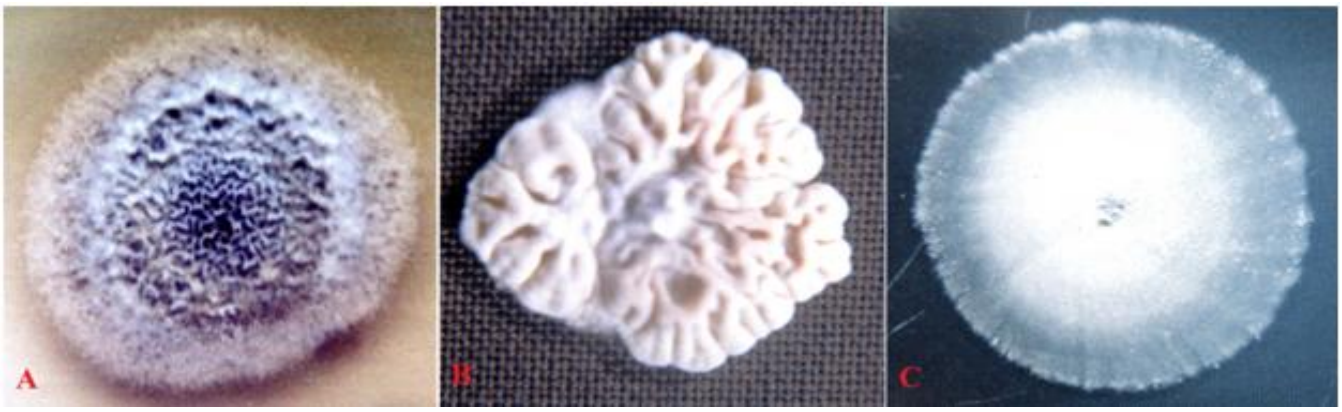

Figure 2: Colony morphologies of 22 single teliospore isolates of *S. ehrenbergii* after 15 days of culture at 30° C on PDA medium; (A) form No. 1, (B) No. 2 and (C) No. 3.

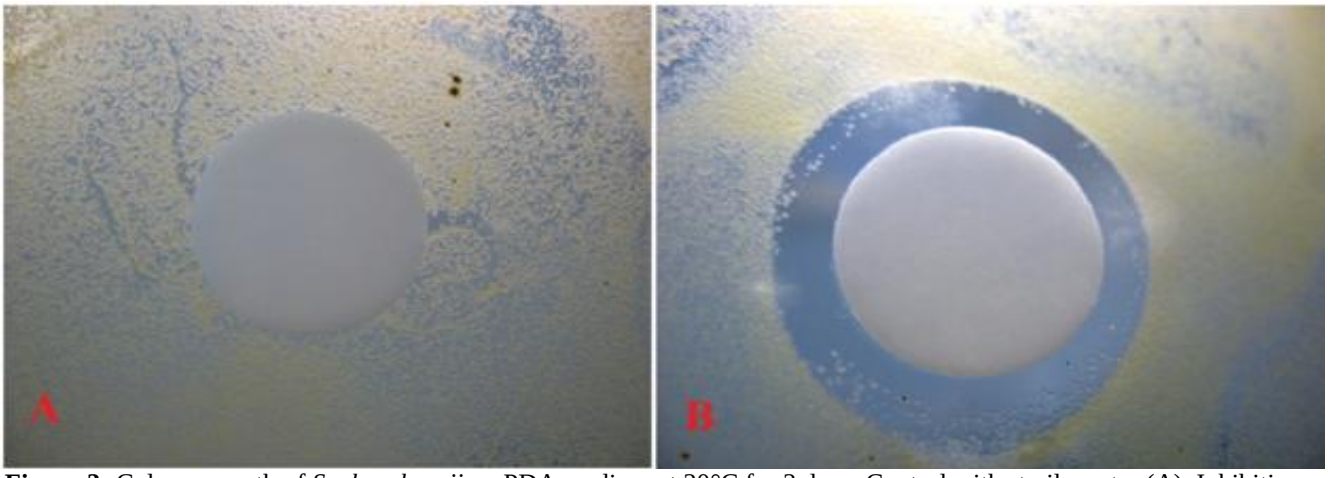

Figure 3: Colony growth of *S. ehrenbergii* on PDA medium at 30°C for 3 days; Control with sterile water (A), Inhibition zone of growth with 1% rheum extract (B).

Table 2: Morphological characteristics of *S. ehrenbergii* forms isolated from Sohag governorate.

Isolate No.	Source			Code[a]	Colony morphological characteristics[b]					**Designated form No.**
	Region (Abbr.)	County (Abbr.)	Sorghum (cultivar)		Diameter (cm)	Color	Consistency	Topography	Margin	
1	North (N)	Geh	*S. bicolor* (Giza 3)	Sb-N-Geh-G3-1	3.4	Grey white	Cottony leather	Flat and creased	Irregular circle	1
2	Middle (M)	Soh	*S. bicolor* (Giza 3)	Sb-M-Soh-G3-2	2.3	White	Leathery	Raised and creased	Pyriform regular	2
3	South (S)	ElMo	*S. bicolor* (Giza 3)	Sb-S-ElMo-G3-3	3.5	Grey white	Cottony leather	Flat and creased	Irregular circle	1
4	N	Tem	*S. bicolor*	Sb-N-Tem-G15-4	3.4	Grey white	Cottony	Flat and	Irregular	1

			(Giza 15)				leather	creased	circle	
5	M	ElM	*S. bicolor* (Giza 15)	Sb-M-ElMa-G15-5	3.7	White	Chalky	Flat, beaded center	Regular circle	3
6	M	Akh	*S. bicolor* (Giza 15)	Sb-M-Akh-G15-6	2.2	White	Leathery	Raised and creased	Pyriform regular	2
7	S	ElB	*S. bicolor* (Giza 15)	Sb-S-ElB-G15-7	3.3	Grey white	Cottony leather	Flat and creased	Regular circle	1
8	N	Tah	*S. bicolor* (Giza 114)	Sb-N-Tah-G114-8	3.4	Grey white	Cottony leather	Flat and creased	Irregular circle	1
9	S	Dar	*S. bicolor* (Giza 114)	Sb-S-Dar-G114-9	2.1	White	Leathery	Raised and creased	Pyriform regular	2
10	N	Tem	*S. bicolor* (Dorado)	Sb-N-Tem-Do-10	3.8	White	Chalky	Flat, beaded center	Regular circle	3
11	M	ElM	*S. bicolor* (Dorado)	Sb-M-ElMa-Do-11	3.7	White	Chalky	Flat, beaded center	Regular circle	3

Table 2: Continued.

12	M	Sak	*S. bicolor* (Dorado)	Sb-M-Sak-Do-12	3.6	White	Chalky	Flat, beaded center	Regular circle	3
13	S	ElB	*S. bicolor* (Dorado)	Sb-S-ElB-Do-13	2.1	White	Leathery	Raised and creased	Pyriform regular	2
14	S	Gir	*S. bicolor* (Dorado)	Sb-S-Gir-Do-14	3.6	White	Chalky	Flat, beaded center	Regular circle	3
15	N	Tah	*S. bicolor* (Shandawel 2)	Sb-N-Tah-Sh2-15	2.2	White	Leathery	Raised and creased	Pyriform regular	2
16	M	Soh	*S. bicolor* (Shandawel 2)	Sb-M-Soh-Sh2-16	3.5	White	Chalky	Flat, beaded center	Regular circle	3
17	N	Tem	*S. saccharatum*[c]	Ss-N-Tem-17	3.7	White	Chalky	Flat, beaded center	Regular circle	3
18	M	ElM	*S. saccharatum*[c]	Ss-M-ElMa-18	3.6	White	Chalky	Flat, beaded center	Regular circle	3
19	S	Dar	*S. saccharatum*[c]	Ss-S-Dar-19	2.1	White	Leathery	Raised and creased	Pyriform regular	2
20	N	Geh	*S. vulgare*[c]	Sv-N-Geh-20	3.4	Grey white	Cottony leather	Flat and creased	Irregular circle	1
21	M	Sak	*S. vulgare*[c]	Sv-M-Sak-21	3.5	White	Chalky	Flat, beaded center	Regular circle	3
22	S	ElMo	*S. vulgare*[c]	Sv-S-ElMo-22	2.1	White	Leathery	Raised and creased	Pyriform regular	2

[a] Isolate code: Abbreviation of sorghum type (Sb, *Sorghum bicolor*; Ss, Sorghum *saccharatum*; Sv, *Sorghum vulgare*), abbreviation of Sohag governorate regions (N, north; M, middle; S, south), abbreviation of counties (see Table 1), number of isolate.

[b] A total of 274 colonies of the 22 isolates of LS were investigated . Of these, 75 (27.3%) displayed form No. 1 of 6 isolates, 112 (40.9%) form No. 3 of 9 isolates, and 87 (31.8%) colonies displayed form No. 2 of 7 isolates.

[c] Unknown cultivated variety

Table 3: Virulence of collected *S. ehrenbergii* isolates on different sorghum genotypes.

Isolate code	**Form No.**	***S. bicolor* (genotype/variety)**											***S. saccharatum*[b]**	***S. vulgare*[b]**
		Giza 3	Giza 15	Giza 54	Giza 114	Dorado	Shandawel 2	PI 574555	PI 574560	PI 574580	PI 574598	PI 574610		
Sb-N-Geh-G3-1	1	53.33[a]	21.67	25.00	41.67	5.00	1.67	3.33	5.00	3.33	1.67	5.00	5.00	51.67
Sb-M-Soh-G3-2	2	51.67	46.67	45.00	48.33	46.67	56.67	46.67	46.67	41.67	45.00	43.33	43.33	58.33
Sb-S-ElMo-G3-3	1	56.67	23.33	26.67	41.67	8.33	8.33	3.33	5.00	3.33	5.00	3.33	5.00	56.67
Sb-N-Tem-G15-4	1	41.67	38.33	21.67	41.67	6.67	8.33	5.00	3.33	1.67	3.33	5.00	3.33	48.33
Sb-M-ElMa-G15-5	3	18.33	45.00	18.33	16.67	48.33	53.33	43.33	41.67	41.67	43.33	41.67	41.67	3.33
Sb-M-Akh-G15-6	2	41.67	41.67	53.33	46.67	43.33	55.00	45.00	43.33	43.33	43.33	45.00	41.67	56.67
Sb-S-ElB-G15-7	1	51.67	21.67	16.67	41.67	5.00	5.00	3.33	3.33	3.33	3.33	3.33	3.33	51.67
Sb-N-Tah-G114-8	1	51.67	21.67	25.00	41.67	6.67	6.67	3.33	5.00	3.33	3.33	3.33	5.00	51.67
Sb-S-Dar-G114-9	2	43.33	45.00	56.67	48.33	46.67	56.67	46.67	48.33	45.00	43.33	41.67	43.33	55.00
Sb-N-Tem-Do-10	3	16.67	43.33	16.67	16.67	43.33	51.67	45.00	43.33	41.67	43.33	43.33	41.67	3.33

Sb-M-ElMa-Do-11	3	18.33	41.67	18.33	18.33	45.00	56.67	41.67	43.33	41.67	45.00	41.67	43.33	3.33
Sb-M-Sak-Do-12	3	16.67	41.67	18.33	18.33	46.67	51.67	43.33	41.67	43.33	43.33	41.67	41.67	3.33
Sb-S-ElB-Do-13	2	41.67	43.33	53.33	46.67	43.33	56.67	43.33	46.67	45.00	45.00	43.33	45.00	56.67
Sb-S-Gir-Do-14	3	15.00	41.67	18.33	16.67	43.33	53.33	41.67	41.67	41.67	41.67	45.00	41.67	3.33
Sb-N-Tah-Sh2-15	2	41.67	43.33	55.00	46.67	46.67	56.67	45.00	46.67	43.33	43.33	43.33	43.3 3	55.00
Sb-M-Soh-Sh2-16	3	16.67	43.33	18.33	18.33	45.00	53.33	41.67	41.67	41.67	43.33	41.67	41.67	3.33
Ss-N-Tem-17	3	18.33	45.00	16.67	18.33	46.67	51.67	43.33	45.00	43.33	41.67	43.33	41.67	3.33
Ss-M-ElMa-18	3	16.67	41.67	16.67	18.33	43.33	51.67	41.67	41.67	41.67	45.00	41.67	43.33	3.33
Ss-S-Dar-19	2	45.00	46.67	56.67	45.00	43.33	55.00	46.67	45.00	46.67	46.67	45.00	43.33	56.67
Sv-N-Geh-20	1	51.67	21.67	21.67	43.33	5.00	5.00	5.00	5.00	3.33	3.33	5.00	3.33	51.67
Sv-M-Sak-21	3	18.33	41.67	18.33	18.33	45.00	56.67	45.00	43.33	41.67	43.33	41.67	45.00	3.33
Sv-S-ElMo-22	2	43.33	43.33	55.00	43.33	43.33	53.33	43.33	46.67	43.33	46.67	43.33	45.00	56.67
L.S.D. at 5%		1.73	1.50	1.57	1.44	1.07	0.92	0.87	0.89	1.13	0.87	0.90	0.94	0.95
1%		2.31	2.01	2.10	1.93	1.42	1.24	1.17	1.19	1.51	1.16	1.21	1.26	1.28

[a] The values are the means of disease incidence (DI) of total 120 inoculated plants representing 3 replicates in 2011 and 2012 growing seasons.
[b]Unknown variety.

Table 4: List of plant species from which extracts were used for testing growth inhibition against *S. ehrenbergii*.

Name of plant	Part used*
Common walnut (*Juglans regia*)	leaves
Cowslip (*Primula veris*)	Roots
Datura (*Datura stramonium*)	leaves
Goldenrod (*Solidago canadesis*)	stem
Nettle (*Urtica dioica*)	leaves
Purple Coneflower (*Echinacea purpurea*)	stem
Rheum (*Rheum rhabarbarum*)	stem
Salvia (*Salvia officinalis*)	leaves
Soapwort (*Saponaria officinalis*)	stem

* Powdered material sold by alfred Galke GmbH, Gittelde, Germany for pharmaceutical or other uses; in this study used for preparation of plant extracts.

Table 5: The putative races of *S. ehrenbergii* described on the base of reactions of certain sorghum genotypes to the designated forms.

Genotype or variety	LS percentages (2011-2012) average					
	Race (form) No. 1[a]		Race (form) No. 2[b]		Race (form) No. 3[c]	
	Incidence %[d]	Class[e]	Incidence %[d]	Class[e]	Incidence %[d]	Class[e]
S. bicolor						
Egyptian varieties						
Giza 3	51.67	HS	45.00	HS	16.67	R
Giza 15	26.67	S	53.33	HS	43.33	HS
Giza 54	25.00	S	61.67	HS	18.33	R
Giza 114	41.67	HS	46.67	HS	16.67	R
Dorado	6.67	HR	45.00	HS	46.67	HS
Shandawel 2	5.00	HR	56.67	HS	51.67	HS
American accessions						
PI 574555	3.33	HR	45.00	HS	43.33	HS
PI 574560	5.00	HR	46.67	HS	41.67	HS
PI 574580	3.33	HR	45.00	HS	43.33	HS
PI 574598	3.33	HR	45.00	HS	43.33	HS
PI 574610	5.00	HR	51.67	HS	41.67	HS
S. saccharatum[f]	3.33	HR	45.00	HS	45.00	HS
S. vulgure[f]	51.67	HS	56.67	HS	3.33	HR

L.S.D. Genotype or variety (G) at. 5% = 1.03 at. 1% = 1.45

Races (Ra) at. 5% = 2.05 at. 1% = 4.73

G × Ra at. 5% = 0.98 at. 1% = 1.33

[a] Form No. 1 is a mixture of the isolates 1, 3, 4, 7, 8 and 20 in which was used for inoculating plants.
[b] Form No. 2 is a mixture of the isolates 2, 6, 9, 13, 15, 19 and 22.
[c] Form No. 3 is a mixture of the isolates 5, 10, 11, 12, 14, 16, 17, 18 and 21.
[d] The values are the means of total 120 inoculated plants representing 3 replicates in growing seasons 2011 and 2012.
[e] Genotypes were classified; 1-9.9% smutted heads as highly resistant (HR), 10-20% as resistant (R), 20.1-40% as susceptible (S), and >40% as highly susceptible (HS).
[f] Unknown variety.

Table 6: Effect of plant extracts on teliospore germination and growth inhibition of *S. ehrenbergii in vitro*.

Plant extract	Teliospore germination (%)[a]			Growth inhibition (cm)[b]		
	1%	3%	5%	1%	3%	5%
Common walnut	0.00	0.00	0.00	0.34	0.41	0.62
Cowslip	77.00	0.00	0.00	0.16	0.45	0.58
Datura	33.00	16.75	7.50	0.21	0.32	0.47
Goldenrod	54.00	31.50	29.50	0.06	0.09	0.14
Mugwort	63.00	52.00	31.00	0.04	0.05	0.08
Nettle	96.75	71.50	43.00	0.01	0.02	0.03
Purple coneflower	92.25	76.75	63.50	0.00	0.03	0.05
Rheum	0.00	0.00	0.00	1.12	1.28	1.42
Salvia	86.25	73.25	55.25	0.04	0.06	0.09
Soapwort	98.25	96.75	92.50	0.00	0.01	0.03
Control (Sterile water)	99.25	99.00	99.25	0.00	0.00	0.00
Average	**63.61**	**47.04**	**38.31**	**0.18**	**0.24**	**0.31**

[a] Values are the means of 400 teliospores per four plates.
[b] Values are the means of inhibition zones (cm) per four plates.
For teliospore germination:
L.S.D. Plant extracts (PE) at. 5% = 1.38 at. 1% = 2.54
Concentrations (C) at. 5% = 1.87 at. 1% = 4.32
PE × C at. 5% = 0.90 at. 1% = 1.23
For growth inhibition:
L.S.D. PE at. 5% = 0.12 at. 1% = 0.17
C) at. 5% = 0.23 at. 1% = 0.54
PE × C at. 5% = 0.11 at. 1% = 0.15

Table 7 : Effect of foliar spray of plant extracts on incidence of LS during the growing seasons 2012 and 2013.

Plant extract (conc.)	Disease incidence (%)								
	Giza 15 variety			Dorado variety			Shandawel 2 variety		
	2012	2013	Average	2012	2013	Average	2012	2013	Average
Common walnut (1%)	11.67[a]	13.33	12.50	16.67	18.33	17.50	15.00	16.67	15.83
Cowslip (3%)	23.33	26.67	25.00	25.00	23.33	24.17	31.67	26.67	29.17
Rheum (1%)	8.33	10.00	9.17	13.33	11.67	12.50	10.00	13.33	11.67
Untreated control	51.67	55.00	53.34	43.33	46.67	45.00	55.00	58.33	56.67
L.S.D. at. 5%	2.59	6.83		1.49	2.48		7.46	2.48	
1%	4.77	12.54		2.75	4.57		13.70	4.57	

[a] The values are the means of total 60 inoculated plants representing 3 replicates

in virulence on certain sorghum genotypes when they were tested in field trials. Based on the obtained results, all seven isolates of the form No. 2 were HV on all tested sorghum genotypes. In contrast, all 6 isolates of the form No.1 were HV on the Egyptian varieties Giza 3, Giza 114 and (an unknown variety of *S. vulgare*), and all 9 isolates of the form No. 3 were HV on the Egyptian varieties Giza 15, Dorado, Shandawel 2, American accessions PI 574555, PI 574560, PI 574580, PI 574598, and PI 574610, and (an unknown variety of *S. saccharatum*). Differences between *S. ehrenbergii* isolates and forms in virulence were recorded by [5] and were also reported for *S. sorghi* and *S. reilianum* [7, 14, 3, 4]. To date no races or pathotypes of the fungus are recognized worldwide, therefore the present study was started to characterize the putative physiological races of *S. ehrenbergii* in Sohag regions of Upper Egypt on the basis of reactions of the tested sorghum genotypes, which could serve as a set of differentials for evaluating resistance to LS isolates. It is evident from the results that the Egyptian varieties Dorado, Shandawel 2, American accessions and an unknown variety of *S. saccharatum* are identified as potential sources of LS resistance, where they showed to be HR to all isolates of form No. 1. As a result, this form could be characterized as a race No. 1. Whereas, all tested genotypes are HS to all isolates of form No. 2, and this form could be characterized as a race No. 2. The Egyptian varieties Giza 3, Giza 54, Giza 114, and (an unknown variety of *S. vulgare*) are R and HR, respectively, to all isolates of form No. 3, and this form could be characterized as a race No. 3. Similarly, other sorghum genotypes exhibited different levels of resistance to LS [40, 35, 27, 31, 5, 33]. Based on the previous studies, two fungi *S. sorghi* and *S. reilianum* causing smut diseases on sorghum are known to produce physiological races [7, 14, 34, 3, 22, 8, 44]. Since only a limited number of LS collections was analysed, no conclusions about the distribution and frequency of large amount of races in the country can be drawn at present. It is therefore suggested that these studies should be continued to determine the range of physiological races of LS caused by *S. ehrenbergii* in all areas of cultivated sorghum and to assess changes in the population structure of the fungus in order to better manage LS disease. However, present knowledge about the races would be useful in breeding for resistance, and prove better understanding of the genetic host resistance.

Various higher plants are known to have antifungal properties due their ability to produce some fungitoxic compounds. The role of these plants in controlling processes against invading fungi can be fungicidal, where the fungus will be killed or fungistatic where growth will be checked [38]. Water extracts of common walnut, cowslip, datura, goldenrod, mugwort, nettle, purple coneflower, rheum, salvia and soapwort at concentration 1, 3 and 5% based on inhibition of teliospore germination and colony growth of *S. ehrenbergii in vitro* was conducted. Based on the obtained results, the tested extracts varied significantly in their effect at all tested concentrations. Extracts of rheum and common walnut at 1% were the most effective plant extracts, where they completely inhibited teliospore germination and caused the highest inhibition zones of growth compared to the other extracts. Cowslip extract at 3% completely suppressed germination and resulted in significant inhibition zones. It was found that water extracts of 1% rheum and 3% common walnut and cowslip completely inhibited teliospore germination of *S. sorghi*, the causal pathogen of kernel smut of sorghum, and these extracts did not exhibit toxic effects on sorghum seed germination and growth [28].

Based on data obtained from field trails, spray of fresh water extracts of 1% rheum and common walnut and 3% cowslip twice during panicle emergence of treated varieties significantly reduced the incidence of LS as compared with untreated control in both successive seasons 2012 and 2013. The efficacy of emulsified neem seed oil as foliar application for reducing the infection with LS of sorghum was reported [2]. Several investigators had tested the same or different plants in controlling kernel smut of sorghum and they found a similar positive effect [30, 45, 42, 28, 38, 26]. Further studies are required to determine the mechanisms of control and the chemicals responsible for such activity and their properties.

Results of this study have provided essential information about the existing virulent races of *S. ehrenbergii* that attack the growing sorghum in Sohag regions of Upper Egypt. The research efforts on LS disease need to remain focused on monitoring and characterizing races of the pathogen. The maintenance of reference of isolates and their forms representing the various races of the *S. ehrenbergii* is critical to gaining a better understanding of host resistance to this important LS pathogen. From assessment tests for the susceptibility of certain sorghum genotypes to LS isolates, it can be concluded that the Egyptian cultivars Dorado, Shandawel 2 and American accessions PI 574555, PI 574560, PI 574580, PI 574598, and PI 574610 could be identified as potential sources of LS resistance. The present study has also identified rheum, common walnut and cowslip as effective botanical plants to control LS of sorghum. Therefore, in areas where LS infection is high, the relatively cheap, non-polluting and environmentally safe water extracts of these plants can be used effectively to reduce disease.

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