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Effects of fungal liquid culture aqueous extract and phytohormones on *Striga hermonthica* (Del.) Benth. germination

Magdoline Mustafa Ahmed, Mohammed Mahgoub Hassan*, Migdam Elsheikh Abdelgani
Environment, Natural Resources and Desertification Research Institute, the National Center for Research, Khartoum, Sudan.

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Abstract: Laboratory experiments were conducted to assess the effects of some fungal isolates (supernatant and pellet) on germination of *Striga* seeds. Five fungal isolates were isolated from soil samples of *Striga*-infested or non infested sorghum fields in Sudan. Isolates differed in their effect on Striga seeds germination. Treatment with isolate Ai 41 resulted in seeds germination rates of 0.00 to 4.19% for pellet and culture filtrates, respectively. In comparison, seed germination rates were 44% and 51.96% for water and culture medium controls, respectively. The study further included investigation of the effect of isolate Ai 41 on germination of Striga seeds in the presence of two growth regulators, the auxin Indole-3-Butyric Acid (IBA) and the cytokinine Benzyle Amino Purine (BAP). High concentrations of IBA and BAP, reduced germination of *Striga* seeds conditioned in water significantly, in response to GR24 as compared to media control. The suppressive effects of isolate Ai 41 consistently increased with increasing both BAP and IBA concentration. In among all treatments, results indicated that IBA alone or in combination with fungal isolate Ai 41 completely inhibited *Striga* seed germination in response to GR24. This study indicates that some microorganisms could be employed as antagonistic to *Striga*. Their use as potential bioherbicides to control *Striga* appears promising.

Keywords: Sorghum, *Striga*, fungal, supernatant, pellet, fungi, phytohoromones

Introduction

Parasitic weeds specially *Striga hermonthica* (Del.) Benth., are major contributors to hunger, malnutrition and food insecurity across sub-Saharan and northern Africa by reducing crop yields varying from 20 to 100% (Kiriro, 1991). *Striga* spp. is extremely difficult to control. This is mainly due to their efficient mechanisms of seed production, dispersal and longevity. Furthermore, *Striga* are in intimate physiological relationship with their host plants, which makes it difficult to apply conventional weed control measures (Musselman, 1984). Several *Striga* control methods including mechanical, biological and chemical have been developed. Potentially, more effective methods can be developed if these are based on detailed knowledge of the host-plant parasite interaction. A number of currently existing strategies to limit *Striga* damage are based on the germination stimulant (Sun *et al.,* 2007).

Sorghum is a viable food grain for millions of people who live in marginal areas with poor and erratic rains and often poor soils of Africa, South Asia and Central America (AATF, 2011). Sorghum production is constrained by both biotic and abiotic factors, and one of the most serious threats is infestation by the witch weed *Striga* (Ejeta and Butler, 1993). Biological control of weeds by using microbial agents involves the utilization of microbial living organisms to manipulate, suppress, reduce or eradicate the weeds. Different micro-organisms such as fungi, actinomycetes and bacteria have a great inhibitory effect on *Striga* seed germination and their developmental stages. Many research workers reported that *Fusarium spp.*, *Alternaria spp.*, *Enterobacter sakazakii* and *Pseudomonas* reduced *Striga* seeds germination (Abbasher *et al.,* 1998; Babalola *et al*., 2007). Considering that the early stages of parasite development are key to the parasitic plants lifecycle, searching for natural compounds able to interfere with these early phases could be an attractive and environmentally friendly approach for management of parasitic weeds. This approach would be especially attractive if rhizosphere microbes could be selected to overproduce and excrete such natural compounds (Sands *et al*., 2003). Zonno and Vurro (2002) reported that some toxins produced by *Fusarium* were able to inhibit germination of *Striga* seeds and proposed their practical use in integrated strategies of

***Corresponding Author:**
Prof. Mohammed Mahgoub Hassan,
Environment, Natural Resources and Desertification Research Institute,
The National Center for Research,
Khartoum, Sudan.

parasitic plants management. Because of the seriousness of this parasitic weed (*Striga*), this study was conducted to fulfill the following: (i) To evaluate the effects of some fungal isolates, supernatant and pellet on *Striga* seeds germination (ii) To investigate the effects of the most effective fungal isolate's pellet and supernatant in presence of two different growth regulators on *Striga* seeds germination.

Materials and Methods

Isolation of soil-borne fungi

Soil samples were collected from two sites, Shambat in Khartoum state and Sinnar in Sinnar state. Ten samples were collected from each site, 5 of which were collected from *Striga*-infested Sorghum fields and the other 5 from non–infested Sorghum fields. Sampling was randomly made at a depth of 5-10 cm, at each location and were kept in plastic bags. Each site was designated by an initial referring to the place of collection viz: A for Shambat and S for Sinnar. The letters (F) and (i) were added to refer to fungi isolated from samples collected from fields free of *Striga*, and fields infested with *Striga*, respectively. Each isolate was given a numbr according to the arrangements of isolation. The spread plate method (Hartman, 2011) was used for isolation of the fungi. Ten grams of each sample were suspended in 90 ml of sterilized distilled water then shaken till completely dispersed. Serial 10 fold dilutions were prepared. Aliquots (0.2 and 0.3 µl) from dilutions 10^{-3} and 10^{-6} were added to Petri dishes containing Potato Dextrose Agar medium (PDA) and the antibacterial chloramphenicol. A glass rod spreader (alcohol- and flame-sterilized) was used to ensure even spreading of the fungal colonies within the medium. The Petri dishes, inverted, were incubated in the dark at 27 ºC for 7-9 days. Representative microbial colonies were purified by sub- culturing and were subsequently characterized morphologically. The isolated fungi were preserved in PDA media and kept at 5 ºC for further studies. Forty three fungal isolates were obtained from the soil samples.

Test solutions

1. GR24 stock solution: Stock solution of GR24, a synthetic *Striga* germination stimulant, was prepared by dissolving 1mg in 1ml of acetone and completed to volume 100 ml with sterile distilled water. The solution was kept in a fridge at 5ºC until used. Two concentrations of GR24 were used 0.1 and 0.01ppm.

2. Phytohormones: Two phyto-hormones were used during this study; auxin, Indole-3-Butyric Acid (IBA) and cytokinine, Benzyle Amino Purine (BAP). Stock solutions of these chemicals were prepared by dissolving 1mg in 1ml of distilled water. Two concentrations of IBA 0.5 and 1mg/ml and two concentrations of BAP 5 and 10 mg/ml.

Media preparation and sterilization

1. Potato-Dextrose Agar medium (PDA): The fungal isolates were cultured on PDA amended with chloramphenicol. The medium was prepared by boiling 200g of sliced potato in 1 liter distilled water until the potato was soft. Twenty grams of dextrose and 20g agar powder were added to the medium and the volume was adjusted to 1 liter then sterilized by autoclaving for 15 minutes at 121 ºC and left to cool. All fungal isolate were maintained on Potato Dextrose Agar (PDA). Cultures on solid medium were stored at 5°C until use.

2. Wheat flour medium preparation (WFM): Wheat flour medium was prepared as described by Hassan *et al*., (2013). Briefly, ten grams of wheat flour were placed in 500 ml conical flask and 400 ml distilled water were added followed by hand shaking for five minutes. From the resulting extract, 250 ml were placed in 500 ml conical flasks, then autoclaved for 15 minutes at 121ºC and left to cool for 24 h.

Striga seeds collection and surface disinfection

Striga seeds were collected from infested sorghum plants in Gezira Scheme, Sudan. *Striga* seeds were surface disinfection as described by Babiker *et al*., (1993). The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) solution for 1min with continuous agitation. Subsequently, the seeds were thoroughly washed three times with sterilized distilled water and plotted dry on Whatman filter paper No.1 and were allowed to dry under a laminar flow hood. The seeds were stored in sterile glass vials and kept at room temperature until used.

***Striga* seeds conditioning and germination**

Glass fiber filter papers (GF/C) discs were cut (4mm diameter), wetted thoroughly with water and sterilized in the Oven at 100 °C for 1 h. to be ready for use. The sterilized discs were placed in 9 cm. sterilized Petri dishes lined with Whatman filter papers, and moistened with 4 ml sterilized distilled water, or wheat flour medium inoculated or not inoculated with respective fungal isolate. About 25 - 50 surface disinfected *Striga* seeds were sprinkled on each glass disc in each dish, the dishes were then sealed with Parafilm, and kept in black polyethylene bags, then incubated at 28 °C in the dark for 10 days.

For germination, glass filter paper discs containing conditioned *Striga* seeds were plotted dry on filter paper (Whatman No.1) to remove excess water, and then transferred to sterile Petri dishes. Each disc was treated with 20 µl of the GR24 solution at a rate of 0.1 or 0.01 ppm. The seeds were re-incubated in the dark at 28 °C, and then examined for germination 24 h. later using a stereomicroscope.

Effects of fungal supernatant and pellet on Striga seeds germination

In all experiments, treatments were arranged in Randomized Complete Design with 4 replicates. Out of the 43 isolates obtained from soil samples, five fungal isolates and strains were selected, based upon a preliminary screening, for their ability to inhibit germination of GR24 induced *Striga hermonthica* seeds. Wheat flour medium was inoculated with 3 discs (collected by core borer 4mm diameter) of each of the 5 fungal isolates obtained from 7 - 9 days old cultures propagated on PDA medium. The flasks were incubated for 10 days at 27 °C, with intermittent hand shaking every two days to maintain better growth.

After 10 days, the culture was separated by centrifugation at 4000 rpm for 10 minutes to obtain the fungal supernatant metabolites and pellet suspension. Five ml of the crude culture (with pellet), or filtrate culture (without pellet and with toxins), un-inoculated liquid wheat medium and sterilized distilled water were added to each Petri dish. *Striga* seeds were sprinkled on the glass fiber discs in each Petri dish. The dishes, sealed with Parafilm, were incubated at 28 °C for 10 days. *Striga* discs were treated with GR24 at a rate of 0.00, 0.01 or 0.1 ppm and re-incubated and examined for germination. Each experimental run included two controls: *S. hermonthica* seeds conditioned in sterile distilled water and seeds conditioned in wheat flour medium.

Effects of fungal isolate Ai 41 (pellet and supernatant) and phytohormones on Striga seeds germination

In this experiment, the inhibitoriest fungal isolate Ai 41**(**pellet and supernatant**)** and two phytohormones IBA and BAP were evaluated for their ability to inhibit *Striga* germination in response to GR24. Ai 41 isolate was cultured in liquid wheat flour medium. After 10 days, the culture was separated by centrifugation at 4000 rpm for 10 minutes to obtain the fungal supernatant and pellet. Five ml of the crude culture (with pellet), or filtrate culture (without spores and with toxins), un-inoculated liquid wheat medium and sterilized distilled water were added to each Petri dish. *Striga* seeds were sprinkled on the glass fiber discs in each Petri dish. The dishes, sealed with Parafilm, were incubated at 28 °C for 10 days. Then discs were plotted dry on filter papers (Whatman No.1), and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with auxin IBA at a rate of (0.5, 1 mg/ml) and cytokinine BAP at a rate of (5, 10 mg/ml) alone or in combination with GR24 at a rate of 0.1ppm in ratio (1:1) to induce germination. The Petri dishes, sealed with Parafilm and kept in black polyethylene bags, were incubated in the dark at 28 °C for 24h. and then examined for germination using a binocular stereomicroscope.

Statistical analysis

Data of germination percentage were calculated for each disc, transformed to arc sine (Gomez and Gomez, 1984) and then subjected to analysis of variance (ANOVA). Means were compared with the least significant difference (LSD) at 5% level of significance.

Results and Discussion

Effects of fungal pellet on Striga seeds germination

Conditioning of *Striga* seeds in wheat flour medium reduced germination, albeit not significantly, as compared to water control in response to GR24. All fungal pellet inhibited *Striga* seed germination in response to GR24.

In among all fungal pellet , SF 18, AF 4 and Ai 41 reduced germination significantly, as compared to conditioning media in response to GR24 at 0.1ppm. They reduced germination by 51.4%, 71% and 100%, respectively (Table .1).

Effects of fungal supernatant on Striga seeds germination

Results displayed that all fungal supernatant reduced *Striga* germination. Among all fungal isolates, supernatant of isolates SF 18 and Ai 41 reduced *Striga* germination significantly, as compared to conditioning media, in response to GR24 at both concentrations (Table 2). They reduced germination by 51.6% and 100 %, respectively, as compared to the wheat flour medium.

Table 1: Effects of fungal isolates (supernatant) on *Striga* seeds germination %

Treatments / GR24 Conc (ppm)	Conditioning media		Fungal isolates (supernatant)					
	Water	WFM	SF 18	AF 4	Ai 41	Ai 42	Ai 45	Mean
0.1	48.66 (55.84)	44.00 (48.24)	23.53 (20.87)	39.41 (40.50)	04.19 (2.08)	37.67 (37.53)	47.64 (54.03)	35.02 (37.02)
0.01	51.96 (61.69)	47.98 (55.10)	23.23 (16.10)	34.91 (33.46)	0.00 (0.00)	41.98 (45.14)	37.41 (37.21)	33.93 (35.53)
Mean	50.31 (58.77)	45.99 (51.67)	23.38 (18.49)	37.16 (36.98)	2.10 (1.04)	39.83 (41.34)	42.53 (45.62)	

LSD of fungi 10.28; LSD of GR24 concentration 5.49; LSD of interaction 14.54. WFM: wheat flour medium. Data without brackets indicate arc sine transformed data

Table 2. Effects of fungal isolates (pellet) on *Striga* seeds germination %

Treatments GR24 conc (ppm)	Conditioning Media		Fungal isolates (pellet)					
	Water	WFM	SF 18	AF 4	Ai 41	Ai 42	Ai 45	Mean
0.1	48.66 (55.84)	44.00 (48.24)	21.41 (17.29)	12.76 (6.60)	0.00 (0.00)	45.14 (50.27)	35.91 (35.19)	29.69 (30.44)
0.01	51.96 (61.69)	47.98 (55.10)	4.67 (2.57)	27.28 (22.87)	9.56 (5.37)	38.19 (38.96)	39.22 (41.03)	31.27 (32.52)
Mean	50.31 (58.77)	45.99 (51.67)	13.04 (9.93)	20.02 (14.74)	4.78 (2.69)	41.66 (44.62)	37.56 (38.11)	

LSD of fungi 10.46; LSD of GR24 concentration 5.59; LSD of interaction 14.79. WFM: wheat flour medium. Data without brackets indicate arc sine transformed data

Mohamed, (2002) reported that the crude filtrate of a locally isolated *Fusarium* isolate reduced *Striga* germination by 50% after three days following conditioning *Striga* seeds with the filtrate but the germination rate increased with time. The same author proved that *Striga* seeds germination is strongly inhibited by the conidial suspension, which indicates that the effect of this isolate and its possible phytotoxicity on *Striga* seeds needs further investigations.

In the present study, results indicated that metabolites produced by fungal isolate Ai 41 may have the potential to be used as bioherbicides to control *Striga*. However, more pathogencity and ecological studies are essential before the isolate or its metabolites can be routinely released to the agricultural system. *F. nygamai* was found to produce phytotoxins such as fusaric acid, dehydrofusaric acid and their corresponding methyl esters in high levels (Capasso *et al.*, 1996). These substances proved to be very phytotoxic against *Striga* seeds even at lower concentrations. Also these results are in line with findings of (Sauerborn *et al*., 1996; Ciotola *et al*., 1995 and Abbasher *et al*., 1996) who found that application of *Fusarium nygamai* and *F. oxysporum* caused more than 90% reduction in *Striga hermonthica* emergence. This indicates that metabolites from fungi may curtail *Striga* germination.

Effects of fungal isolate Ai 41 (supernatant) and phytohormones on Striga seeds germination

Striga seeds conditioned in water or in wheat flour medium and similarly treated with GR24 at 0.1ppm exhibited comparable germination (Fig.1). *Striga* seeds conditioned in water and treated with BAP at 5 mg/ml induced *Striga* germination albeit not significantly, as compared to the water, in response to GR24. However, at the higher concentration of BAP and both concentrations of IBA, *Striga* germination was decreased significantly, as compared to media control, in response to GR24. Fungal isolate Ai 41 supernatant inhibited *Striga* seed germination significantly, as compared to controls. Moreover, supernatant of the fungus isolate Ai41 reduced *Striga* seeds germination in presence of synthetic stimulant GR24. The inhibition of *Striga* radicle growth by fungi could be caused by the fungal producing phytohormones that interfered with the hormonal balance of the weed. Isolate Ai 41 supernatant inhibited germination of *Striga* seeds completely at both concentrations of IBA in the absence of the germination stimulated GR24. On the other hand, germination of *Striga* seeds was reduced non significantly by the increase of BAP concentration in the absence of GR24.

Effects of fungal Ai 41 (pellet) and phytohoromones on Striga seeds germination

Striga seeds conditioned in water or in wheat flour medium and similarly treated with GR24 at 0.1ppm exhibited comparable germination (Fig.2). In the absences of the growth stimulant GR24, the growth horomon BAP at concentration 5 mg/ml was more suppressive than at 10mg/ml. similarly, IBA reduced *Striga* seed germination significantly at both concentrations. BAP at 10 mg/ml reduced germination significantly, in response to GR24. IBA reduced *Striga* seeds germination significantly, in response of GR24. Isolate Ai 41 pellet reduced *Striga* seeds germination at both concentrations of IBA significantly, in absence or presence of GR24. Fungal isolate Ai 41 pellet inhibited *Striga* seeds germination significantly, in absence or presence.

Striga damage is attributing to perturbations of the hormonal balance of its host which sets in at the very early stages of infestation. The parasites were found to decrease the levels of auxins (IBA) cytokinines (BAP) (Drennan and El Hiweris, 1979). Because parasitic weeds generally damage their host plants even before they emerge above ground, soil inoculation with soil borne pathogens might be of great advantage to hinder the growth of *S. hermonthica* at an early stage of development. In the present study only one fungal isolate (Ai 41) reduced the germination of *S. hermonthica* seeds by more than 90% irrespective of *Striga* seeds status when the fungus was applied during seed conditioning phase. Zonno and Vurro (1999) demonstrated that fungal toxins could cause 100% inhibition of *Striga* seeds germination, and different fungal toxins and concentrations resulted in different inhibition percentages. They concluded that the high activity shown by some fungal toxins suggests that they may have potential for use as more natural and safe herbicides to suppress parasite seed germination.

Ethylene is claimed to be the actual inducer of germination in *Striga* seeds and that all *Striga* germination stimulants act through promotion of ethylene biosynthesis in the parasite seeds. All germination stimulants known to date inhibit germination when applied to unconditioned seeds (Babiker, 2007). The ubiquitous nature of ethylene producing fungi in soil may explain the relative preponderance of fungi capable of suppressing *Striga* germination noted in the present study.

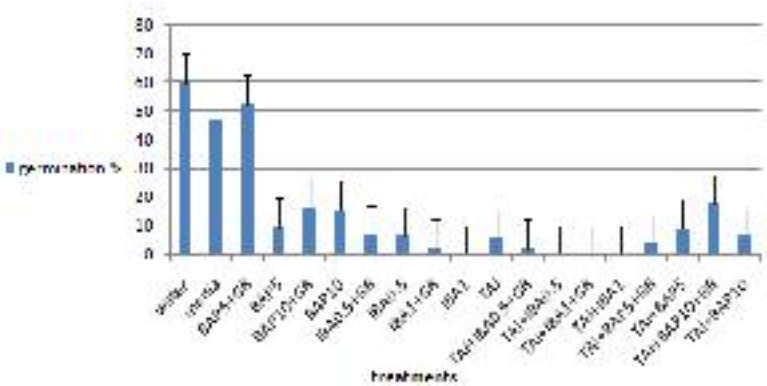

Fig. 1: Effects of fungal isolate Ai 41 (supernatant) and phytohormones on *Striga* seeds germination%. Vertical bar indicates S.E.

Medium: Wheat Flour Medium. BAP5: Benzyle Amino Purine at 5mg/ml; BAP10: BAP at 10 mg/ml; IBA0.5: Indole-3-Butyric Acid at 0.5 mg/ml; IBA1: IBA at 1mg/ml; GR24 concentration at 0.1ppm; TAi: Supernatant of isolate Ai 41

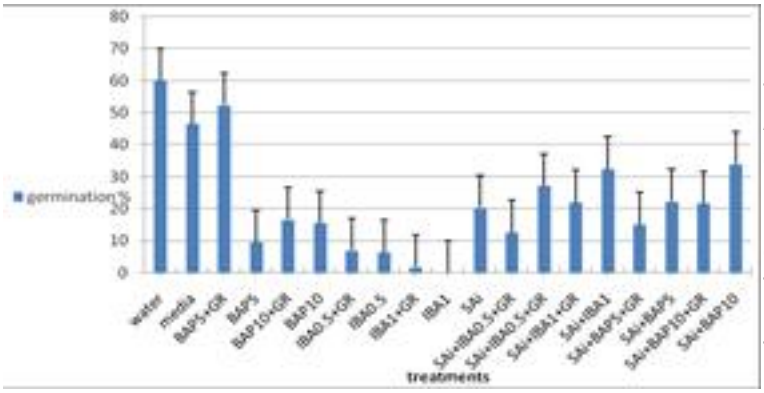

Fig. 2: Effects of fungal isolate Ai 41 (pellet) and phytohoromones on *Striga* seeds germination%. Vertical bar indicates S.E.
Media: Wheat flour medium. BAP5: Benzyle Amino Purine at 5mg/ml; BAP10: B A P at 10 mg/ml; IBA0.5: Indole-3-Butyric Acid at 0.5 mg/ml; IBA1: IBA at 1mg/ml; GR24 concentration at 0.1ppm; SAi: pellet of isolate Ai 41

Soil borne pathogens have a number of practical advantages in the control of root parasitic weeds such as Striga and Orobanche (Sauerborn *et al*., 2007). For one, this parasite spends most of its life cycle below ground and thus produces most damage to host plants before emergence. Therefore, fungal isolates (pellet) provide a good candidate as a Striga pathogen since it can grow and survive through the soil for a long time. The specificity of these fungal forms to infect only *Striga* spp. is an important attribute when considering a pathogen for biological control, since a Striga – specific pathogen may provide better environmental safety as a bioherbicide. Zonno and Vurro (2002) showed that some toxins produced by fungi of the genus Fusarium were able to inhibit germination of Striga seeds and proposed their practical use in integrated strategies of parasitic plant management. Bioherbicides can be integrated into intensively managed agriculture production systems because of their potential to yield quick and reliable levels of weed control. The use of phytopathogenic fungi and/or their toxic metabolites to control weeds is interesting (Benítez et al., 2004; Evidente and Abouzeid, 2006) since their biocontrol agents are highly specific and their toxic metabolites are possibly natural herbicides (Evidente and Motta, 2001). Therefore Biological control measures using fungi (especially Ai 41) could be integrated with other non-costly control methods to achieve an affordable control of Striga that can be adopted by the subsistence farmers.

Conclusions

Some fungal strains and isolates were found to perturb early growth stages of *S. hermonthica*. Circumstantial evidence suggests that the fungal action on *Striga* may be mediated through phytohormones. Future research should focus on re-screening of the effective fungi and fungal isolates and rank them according to their ability to suppress or promote specific stages in *Striga* life cycle at the green house and controlled field trials.

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