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ORIGINAL ARTICLE

EFFECTS OF SOME FUNGAL ISOLATES ON GERMINATION AND HAUSTORIUM INITIATION OF *STRIGA HERMONTHICA* (DEL.) BENTH

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ABSTRACT: Two laboratory experiments were conducted to assess the effects of fungal isolates on germination and haustorium initiation of *Striga hermonthica*. Forty three fungal isolates were isolated from infested and un-infested sorghum fields with *Striga hermonthica*. A preliminary screening was conducted to assess the effect of these isolates on germination of preconditioned *Striga* seeds. Sixteen fungal isolates in addition to a standard strain *Trichoderma viride* were selected according to their effects on *Striga* seeds germination and haustorium initiation. In the first experiment isolates and strain were assessed for their effect on germination of *Striga* seeds during conditioning in response to GR24. The second experiment was conducted to study the effects of fungal isolates and *T. viride* on *Striga* haustorium initiation. The results revealed that six fungal isolates had no effects; six isolates reduced germination (34-93%) significantly while other isolates significantly enhanced germination (15–59%) of *Striga* seeds. Moreover, isolate Ai 41 reduced germination over 90% as compared to the corresponding control. Similarly, few fungal isolates inhibited haustorium initiation while some had no effect. Isolate Ai 50 was the only isolate that completely (100%) inhibited haustorium in response to DMBQ. The study concluded that some fungi can be used as control agents against *Striga* and suggested that further studies had to be conducted in the green house and field to verify theses results and develop a strategy for the use of fungi as mycoherbicides against *Striga*.

KEY WORDS: Fungi, *Striga* Germination, Haustorium Initiation.

INTRODUCTION

Parasitic weeds are major contributors to hunger, malnutrition and food insecurity across sub-Saharan and northern Africa by reducing crop yields. The parasitic weed of the genus *Striga*, generally termed as ''witchweed'', is a noxious root parasite having a broad range of hosts including many important poaceous crops. *Striga* seed requires a period of after-ripening and so cannot germinate at the end of the rainy season in which it is produced (Scholes and Press, 2008). Subsequent to after-ripening period, seeds have to be subjected to warm moist conditions for 2-14 days (conditioning period) prior to exposure to an exogenous germination stimulant. The germination stimulant is exuded by root of host and some non-host plants (Parker and Riches, 1993). Several germination stimulants have been identified in root exudates most of these are collectively described as the strigolactones. It was discovered that the germination strategy in *Striga* is based on ethylene biosynthesis and action. Host-derived signals and other compounds act by eliciting the synthesis of ethylene and ethylene initiates the biochemical changes leading to germination (Logan and Stewart, 1991). Conditioning and Strigol remove restrictions on the ethylene biosynthetic pathway as both ACC-synthase and ACC-oxidase (the major enzymes in ethylene biosynthesis) were reported to be rate limiting (Babiker *et al.*, 2000). Subsequent to germination, which occurs in close proximity of the host roots, *Striga* germilings, in response to a second chemical signal from the host root, produce haustoria through which the parasite seedlings draw nutrients from the host. The haustorium attaches, penetrates and establishes connection with the host xylem (Parker and Riches, 1993).
Many control methods were developed against the parasite problem including physical, cultural,

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chemical and biological were developed to curb the parasite (Joel, 2000; Ejeta, 2005). So far, these methods however have only had a limited impact on controlling *Striga* and today there is no single control method that can effectively solve this problem (Ejeta, 2005; Oswald, 2005). Finding adequate methods to control parasitic weeds has been the subject of extensive research and several reviews have been published on this topic (Khan *et al.*, 2007; Hassan *et al.*, 2010; AATF, 2011). Biological control of weeds by using microbial agents means the utilization of microbial living organisms to manipulate, suppress, reduce or eradicate the weeds. Different micro-organisms including fungi, actinomycetes and bacteria have a great inhibitory effect on *Striga* seed germination and/or early developmental stages. Many research workers reported that *Fusarium* spp., *Alternaria* spp., *Enterobacter sakazakii* and *Pseudomonas* spp. reduced *Striga* seeds germination (Abbasher *et al.*, 1998; Babalola *et al.*, 2007). The present study was conducted to evaluate the effects of different fungal isolates on *Striga* seeds germination and haustorium initiation.

MATERIALS AND METHODS

A series of laboratory experiments were undertaken to investigate the effects of fungal strains and isolates on GR24-induced germination of *Striga hermonthica* seeds and haustorium initiation in response to DMBQ. In all experiments, treatments were arranged in Randomized Complete Design with 4 replicates.

2.1. GR24 stock solution

GR24, a synthetic *Striga* germination stimulant, was kindly provided by Professor B. Zwanenberg, University of Nimijhen, Netherlands. Stock solution of the stimulant was prepared by dissolving 1mg in 1ml of acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept in a fridge at 5°C till used.

2.2. DMBQ stock solution

DMBQ (2,6-dimethoxybenzoquinone), a chemical which induces haustorium initiation in *Striga*, was generously provided by Professor Sugimoto of Kobe University, Japan. Stock solution (100 ml) was prepared by dissolving 1.68 mg in 1ml of acetone and completing to volume (100 ml) with sterile distilled water. The solution was kept in fridge at 5 °C till used.

2.3. Media preparation and sterilization

2.3.1. Potato-Dextrose Agar medium (PDA)

The fungal isolates and strains 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 litre then sterilized by autoclaving for 15 minutes at 121 °C and left to cool. All fungal isolates and strains were maintained on Potato Dextrose Agar (PDA). Cultures on solid medium were stored at 5°C till used.

2.3.2. Wheat flour medium preparation

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. The mixture, allowed to standing for 2 h., squeezed to pass through cheesecloth and the resulting extract (250 ml) was placed in 500 ml conical flasks and autoclaved for 15 minutes at 121°C and left to cool for 24 h.

2.4. Collection and isolation of soil-borne fungi

Soil samples were collected from two sites, Shambat in Khartoum State and Sinnar in Sinnar State, Sudan. Ten samples were collected from each site, 5 of which were collected from *Striga* infested sorghum fields and the others from non–infested sorghum fields. Sampling was randomly made at a depth of 5-10 cm, at each location and the samples were kept in paper 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 *Striga* free and infested sorghum fields, respectively. Each isolate was given a number according to the arrangements of isolation.

The spread plate method (Hartman, 2011) was used for isolation of the fungi. Ten grams of each sample, suspended in 90 ml of sterilized distilled water, were shaken till completely dispersed and 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 PDA medium 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 °C for 7-9 days. Representative microbial colonies, purified by sub-culturing, were subsequently characterized morphologically. The isolated fungi were preserved in PDA medium and kept at 5 ºC for further studies. Fungal cultures were renewed every 2 months.

Forty three fungal isolates were obtained from the soil samples. In addition, a fungal strain, *Trichoderma viride* was obtained from the Environment and Natural Resources Research Institute (ENRRI), the National Center for Research, Khartoum, Sudan.

2.5. Propagation of fungi on wheat flour medium

Wheat flour medium, prepared as described above, was inoculated with 3 discs (collected by core borer 8mm diameter) of fungal isolates and strains obtained from 7-9 days old cultures propagated on PDA medium. The flasks were incubated for 10 days at 27 ^{o}C, with intermittent hand shaking every two days to maintain better growth.

2.6. Striga seeds collection and surface disinfection

Striga seeds were collected from infested sorghum plants in Gezira Scheme, Sudan. *Striga* seeds were cleaned by placement in 1000 ml measuring cylinder containing tap water. Tween 20 (an emulsifier disinfectant) was added and the seeds were agitated for 5 minutes. The seeds were washed 5 times with tap water to free them of sand and Tween 20. The seeds were then 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.

2.7. Striga seeds conditioning and germination

Glass fiber filter papers (GF/C) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven at 100 ^{o}C for 1 h. to dry and be sterilized and ready for use. The sterilized discs were placed in 9 cm. sterilized Petri dishes lined with Whatman filter papers were moistened with 4 ml sterilized distilled water, or wheat flour medium inoculated or not inoculated with the respective fungal isolate or strains. About 25 - 50 surface disinfected *Striga* seeds were sprinkled on each glass disc in each dish, sealed with Parafilm, and placed in black polyethylene bags, then incubated at 28 ^{o}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 aliquots of the GR24 solution at a rate of 0.1 or 0.01 ppm. The seeds were re-incubated in the dark at 28 ^{o}C, and then examined for germination 24 h. later using a stereomicroscope.

2.8. Effects of fungal isolates and Trichoderma viride on GR24-induced germination of S. hermonthica seeds (during conditioning)

A total of 43 fungal isolates and *T. viride* were evaluated for their effect on *S. hermonthica* seeds germination in response to GR24. Screening was done in two stages: a preliminary stage and a confirmatory stage. In the preliminary stage, the screening included all isolates and strains (44), while the confirmatory stage based on the results of the preliminary screening, included 16 selected isolates and *T. viride*. Three batches were conducted to the study the effect of fungal isolates and strains on *Striga* seed germination. Each experimental batch included two controls comprised of *S. hermonthica* seeds conditioned either in sterile distilled water or uninoculated wheat flour medium. In both preliminary and confirmatory screening, *Striga* seeds were treated with GR24 at 0.1 or 0.01 ppm, re-incubated and examined for germination. Only results of the confirmatory screening are presented in this study.

2.9. Effects of fungal isolates and Trichoderma viride on haustorium initiation

Three batches were evaluated for their effects of fungal isolates and strains on *Striga* haustorium initiation (based on the results of the confirmatory screening). Surface disinfected *Striga* seeds, were placed on 8 mm glass fiber discs and conditioned in presence and absence of fungal isolates or strains, were plotted dry on filter papers (Whatman No.1), and transferred to sterile Petri dishes. The discs containing *Striga* seeds were treated, each, with 25µl GR24 solution at a rate of 0.1 ppm to induce germination. The Petri dishes, sealed with Parafilm and placed in black polyethylene bags, were incubated in the dark at 28 ^{o}C for 48 hours. The discs containing the germinated seeds (*Striga* germilings) plotted dry on filter paper, were placed and inverted top - down on similar 8 mm discs without *Striga* seeds. The pairs of discs were treated, each with 40 µl of 20 µM DMBQ. *Striga* germilings resulting from seeds conditioned in water or in wheat flour medium similarly treated with DMBQ were included as controls for comparison. The Petri dishes, sealed with Para film and placed in polyethylene bags, were incubated in the dark at 28 ^{o}C for an additional 24 h. and subsequently examined for haustorium initiation using a binocular stereomicroscope.

2.10. Statistical analysis

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

RESULTS AND DISCUSSION

3.1. Effects Of Fungal Isolates And T. Viride Strain On GR24-Induced Germination Of S. Hermonthica Seeds (During Conditioning)

The results revealed that some fungal isolates and *T. viride* reduced, and others enhanced, germination significantly in response to GR24 in comparison with the media control. In all experiments, seeds conditioned in distilled water and treated with fungi in the absence of the germination stimulant (GR24) displayed negligible germination. In all batches, germination of seeds conditioned in wheat flour medium was less, albeit not significantly, than seeds conditioned in water. This indicates that the constituents of the wheat flour medium had no significant effect on germination of *Striga* seeds and hence its effect is excluded throughout the experiment. In the first batch, fungal isolates Si 23, Ai 42 and Si 20 decreased *Striga* seeds germination in response to GR24 at both concentrations, significantly, in comparison to the control (Table 1). Germination of *Striga* seeds treated with fungal isolate Si 23 was reduced by 35.9% as compared to the control in wheat flour medium. However, at the highest concentration of GR24 (0.1 ppm), fungal isolate Af 1 enhanced germination significantly, as compared to conditioning medium. Furthermore, fungal isolate Si 20 reduced *Striga* seeds germination significantly, as compared to the conditioning media in response to the lowest concentrations of GR24.

Table 1: Effects of fungal isolates on GR24-induced germination % of *Striga hermonthica* seeds (during conditioning) – (Batch 1)

Treatments	Conditioning medium		Fungal isolates					
GR24 Conc. (ppm.)	Water	WFM*	Si 23	Si 20	Ai 38	Af1	Ai 42	Mean
0.1	58.91 (72.73)	56.61 (69.65)	36.30 (41.86)	53.25 (63.00)	58.38 (71.32)	69.49 (85.89)	48.87 (56.64)	54.54 (76.05)
0.01	57.75 (71.45)	55.13 (66.23)	54.58 (65.54)	48.69 (56.40)	57.31 (70.78)	56.07 (68.63)	49.84 (58.14)	54.19 (65.31)
Mean	58.33 (72.09)	55.87 (67.94)	45.44 (53.70)	50.97 (59.70)	57.84 (71.05)	42.56 (77.26)	49.36 (57.39)	

LSD for fungi= 11.74, LSD for concentration =6.28, LSD for interaction =16.61

In the second batch, *Striga* seeds conditioned in water and wheat flour medium and treated with GR24 displayed comparable germination (Table 2). The fungal isolates SF 29, Si 29, Si 33 Si 34 and Ai 41, irrespective of GR24 concentration reduced germination significantly in comparison to the controls. Among the fungal isolates Ai 41 caused the highest inhibition as GR24 at both rates induced negligible germination (about1 and 4%). SF 13 enhanced germination significantly at both GR24 concentrations. Ai 39 on the other hand showed inconsistent performance as it had no effect on germination at the lowest GR24 concentration, but caused significant inhibition at the highest stimulant concentration.

Table 2: Effects of fungal isolates and strains on GR24-induced germination % of *Striga hermonthica* seeds (during conditioning) – (Batch 2)

Treatments	Conditioning medium		Fungal isolates and strain							
GR24 Conc. (ppm.)	Water	WFM*	Sf 29	Si 33	Ai 39	T. viride	Si 34	Ai41	S 13	Mean
0.1	45.12 (50.24)	45.84 (51.00)	31.18 (29.48)	36.14 (35.31)	34.88 (33.20)	32.08 (29.03)	52.56 (62.98)	11.34 (3.95)	58.19 (71.96)	38.60 (40.79)
0.01	42.02 (44.83)	38.15 (44.83)	38.52 (38.80)	29.26 (24.26)	42.70 (46.04)	36.78 (36.33)	46.59 (52.73)	2.51 (0.76)	52.13 (62.23)	36.52 (44.09)
Mean	43.57 (47.54)	42.00 (47.92)	34.85 (34.14)	32.70 (29.79)	40.46 (39.62)	34.43 (32.68)	49.57 (57.86)	6.97 (2.36)	55.16 (67.14)	

LSD for fungi =7.10, LSD for concentration= 3.35, LSD for interaction =10.04

* WFM: wheat flour medium. Data without brackets indicates arc sine transformed data.

In the third batch, in among all fungal isolates, isolate Ai 50 reduced germination significantly, as compared to conditioning media in response to GR24 at 0.1 and 0.01 ppm (Table 3). It reduced germination by 69.9 to 78.5% as

compared to corresponding control, at both concentration of GR24.

Table 3: Effects of fungal isolates on GR24-induced germination % of *Striga hermonthica* seeds (during conditioning) – (Batch 3)

Treatments	Conditioning medium		Fungal isolates					
GR24 Conc. (ppm.)	Water	WFM*	Ai 47	Af 11	Ai 50	Sf 10	Ai 49	Mean
0.1	49.65 (58.07)	46.34 (52.33)	41.16 (43.81)	45.83 (51.39)	9.96 (5.84)	50.52 (59.47)	41.14 (43.29)	40.66 (44.89)
0.01	46.76 (53.04)	45.33 (50.59)	39.80 (41.10)	44.00 (48.11)	13.68 (16.67)	45.05 (50.48)	25.43 (19.03)	37.15 (39.86)
Mean	48.20 (55.56)	45.83 (51.46)	40.48 (42.46)	44.92 (49.75)	11.82 (11.26)	47.79 (54.98)	33.28 (31.16)	

LSD for fungi =10.28, LSD for concentration= 5.49, LSD for interaction= 14.54

Striga seeds need special stimulant for germination (Babiker *et al.*, 2000). The inhibitory effects of the fungal isolates applied during conditioning could be attributed to a direct effect of fungal isolates or strains on *Striga* seeds or indirectly through production of chemical(s) (Imaseki, 1991; Babiker *et al.*, 1993). Both inhibition and promotion of *Striga* germination can be achieved by manipulation of ethylene biosynthesis, inhibition of ethylene biosynthesis, ethylene action, or by promotion of its metabolism (Imaseki, 1991). These results are in accordance with previous findings in which the fungal isolate Foxy 2 was able to reduce germination of *S. hermonthica* seeds by more than 90% when the fungus was applied during the seed conditioning phase, and it prevented the emergence by 98% when it was used as soil inoculums. Moreover, two isolates of *F. oxysporium* and one of *Fusarium solani* reduced emergence of *S. hermonthica* by 88%, 98% and 76%, respectively (Kroschel *et al.*, 1996). In conclusion, *Striga* bio-control based on *Fusarium* inoculums incorporation proved to constitutes an effective control method against *S. hermonthica* (Yonli *et al.*, 2012).

3.2. Effects Of Fungal Isolates And T. Viride Strain On Haustorium Initiation

Three *in vitro* experiments (batches) were undertaken to evaluate the efficacy of fungal isolates and *T. viride* on *Striga* haustorium initiation in response to DMBQ. In all batch, *Striga* germilings resulting from seeds conditioned in wheat flour medium enhanced haustorium, albeit not significantly as compared to the water.

In the first batch, out of the 5 tested isolates, 3 inhibited haustorium initiation, albeit not significantly, as compared to both conditioning media, in response to DMBQ (Table 4). Isolate Ai 38 was the inhibitory. It reduced haustorium initiation by 55.9% and 61.9% as compared to water and wheat flour medium, respectively.

Table 4: Effects of fungal isolates on haustorium initiation % in response to DMBQ – Batch (1)

Treatments	Conditioning medium		Fungal isolates				
	Water	WFM*	Si 23	Af 1	Ai 42	Ai 38	Si 20
DMBQ	44.84 (55.50)	51.79 (63.82)	37.83 (44.28)	31.20 (39.17)	54.23 (63.78)	19.77 (24.11)	52.95 (63.64)

LSD = 43.53

* WFM: wheat flour medium. Data without brackets indicates arc sine transformed data.

In the second batch, The effects of fungal isolates and *T. viride* strain on haustorium formation varied from non-significant to significant when compared with medium control (Table 5). Fungal isolates Ai 39 and Ai 41 reduced haustorium initiation significantly, as compared to medium in response to DMBQ. They reduced haustoria by 51 and 58.9%, respectively as compared to the wheat flour medium control.

Table 5: Effects of fungal isolates and strains on haustorium initiation % in response to DMBQ – Batch (2)

Treatments	Conditioning medium		Fungal isolates and strains						
	Water	WFM*	Sf 29	Sf 13	Si 33	*T.viride*	Ai 39	Ai 41	Si 34
DMBQ	58.15 (71.58)	73.84 (89.60)	72.34 (87.80)	59.85 (73.68)	69.55 (83.75)	66.31 (83.78)	30.40 (32.33)	36.19 (41.68)	66.43 (78.75)

LSD = 29.02

In the third batch, in among all fungal isolates, isolate Ai 50 was the only isolate that completely inhibited haustorium initiation in response to DMBQ (Table 6).

Table 6: Effects of fungal isolates on haustorium initiation % in response to DMBQ – Batch (3)

Treatments	Conditioning medium		Fungal isolates				
	Water	WFM*	Ai 49	Ai 47	Sf 10	Ai 50	AF 11
DMBQ	87.49 (99.24)	70.67 (85.12)	49.30 (56.96)	90.00 (100.00)	56.76 (62.44)	0.00 (0.00)	90.00 (100.00)

LSD = 27.68

* WFM: wheat flour medium. Data without brackets indicates arc sine transformed data.

These results are in line with some other reports (Thomas *et al.*, 1998; Mabrouk *et al.*, 2006). The inhibition of haustorium initiation by fungal isolates may be attributed to phytotoxic substances, inhibitors or extracelluar enzymes that degrade and/or curtail release of the haustorium factor from the host root. Auxin and auxin-like compounds have been reported to inhibit haustorium initiation in *Striga* and production of the haustorium factor (DMBQ) by intact sorghum roots requires production and release of H_2O_2 from the parasite root tip (Keyes *et al.*, 2000). H_2O_2 is critical for activation of host perioxidases and oxidative release of DMBQ from the host epidermal cells. Differential production of the enzyme catalase, which disproportionate H_2O_2 to H_2O and molecular oxygen, by fungal isolates would lead to differential production of DMBQ and hence differential reduction in haustorium initiation (Keyes *et al.*, 2000).

In the present study only two fungal isolates Ai 41 and Ai 50 proved to be suppressive to both *Striga* seeds germination and haustorium initiation. Inoculation of soil with a soil borne microorganisms which attacks the parasite at the early developmental stages is advantageous as it may hinder the growth of the parasite and curtails its deleterious effects on hosts (Kroschel *et al.*, 1996). Germination, radical elongation, haustorium initiation, attachment and penetration are the earliest developmental stages of *Striga*. These stages are especially fragile, can be modulated by phytohormones and are very likely targets for control methods (Babiker *et al.*, 1993).

CONCLUSION AND SUGGESTIONS

Fungal isolates were found to perturb early growth stages of *S. hermonthica*. 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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