



Germination Inducing Activity of Cotton (*Gossypium hirsutum* L.) Genotypes on *Striga hermonthica* (Del.) Benth

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Striga hermonthica, an Orbanaceae, is a devastating root parasitic weed on Poaceae crop. The infection by *Striga* starts with germination of its seed, which is induced by germination stimulant secreted by the host and non host root. Most of germination stimulant are strigolactones SLs,

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which have been discovered as plant hormones that regulate the development of different plant parts. Amount and composition of SLs may vary among different plant species as well as among different cultivars of the same species. The first natural SLs were, isolated from the root exudates of cotton (*Gossypium hirsutum* L.) and given the name strigol. Cotton is major crop in the Sudan and recently cotton production in the rain-fed area, where *Striga* is most prevalent, is receiving much attention. The present investigation was set to study genotypic variability in efficacy of cotton (*Gossypium hirsutum* L.) as a trap crop for *Striga*, was match with objective to developing cost effective control in prevalent *Striga* endemic area. Laboratory experiments of three cotton genotypes were grown in a greenhouse, harvested, severed into roots, stems, leaves, flowers, bolls and seeds, air dried and powdered and tested for germination inducing activity (GIA). The GIA for the powder varied with plant part and crop cultivar. Irrespective of cultivar GIA was highest for roots (31.9-49.9%) and lowest for bolls (16.59-26.52%). Among cultivars Hamid showed the highest overall GIA (48.9% germination) while Barac (c) 67 showed the lowest (16.5% germination). GIA declined with plant age. Further, GIA of Hamid was superior to that of Barakat and Barac (c) 67. GIA of cotton cv Hamid root powder increased with powder amount, reached a maximum (67.5% germination) and subsequently declined. GIA of cotton cv Hamid root exudates increased with volume and time, reached a peak (38.45% germination) at 15 μ L 30 DAS and subsequently declined. GIA of roots exudates of cotton cv Hamid collected *in situ* from potted plants, were tended to increase by plants density. Cotton shoots and roots powder and roots exudates effectively induced germination of *Striga* seeds.

Conclusion: The present study result cotton cv. Hamid shoots and roots powder and roots exudates the most effective to induced germination of *Striga* seeds.

Keywords: Cotton; germination; *Striga hermonthica*; stimulant; root powder; trap crop; root exudates.

1. INTRODUCTION

Most plants are autotrophic and fix carbon through photosynthesis. However, some are parasitic that obtain their resources (assimilates, water, nutrients) partly or completely from (host) plants to which they are attached by haustoria. Parasitism originated independently several times during angiosperm evolution, and the life style of parasitic plants varies greatly across taxa (Nickrent et al., 1998). Parasitic plants have a wide environmental tolerance and are represented by about 4100 species in approximately 19 families of flowering plant (Press & Phoenix, 2005). Some species are facultative parasite that they are able to survive in the absence of host, but, normally they grow better when attached to hosts, while others are obligatory parasitic and cannot develop independently (Parker, 2009). Although some parasitic plants are still photo- synthetically active (hemiparasitic), other depends entirely on a host (holoparasitic). Depending on which host organ is infected parasitic plants are grouped into stem or root parasites (Mayer, 2006). The most widely spread and important parasitic angiosperms belong to the genera *Striga*, *Orbanche*, *Phelepanche* and *Cuscuta* of the family Orobanchaceae. Most of the hosts belong to the Poaceae, Asteraceae, Solanaceae, Cucurbiteaceae and Fabaceae. The genus

Striga, which consists of obligate hemiparasitic root parasites (Parker, 2009), comprises 35 species of which more than 80% are to be found in Africa. Of the species at least 11 parasitize crops and pose one of the several biological constraints to agriculture in low input farming system especially in the African Savanna (Parker & Riches, 1993). *Striga* one of the major genera of parasitic weeds, infest about 40% of cereal production area of Africa, resulting in crop losses estimated at US\$ 1 billion annually, affecting the livelihood of approximately 300 million people (Ejeta, 2007). The tremendous impact of parasitic plants on world agriculture has prompted much research aimed at preventing infection and infestation. Many potential control methods including physical, cultural, chemical and biological were developed (Joel et al., 2007). So far these methods however have only had a limited impact on controlling the menace and today there is no single control measure that effectively solves the problem (Oswald, 2005). Trap crops, false hosts, are those crops that induce germination of *Striga* Seeds, but are not parasitized, and consequently result in suicidal (Botanga et al., 2003). Trap crops are usually non-host species that produce germination stimulants, sometimes in high amount, and hence induce massive germination of the parasite (Khan et al., 2002). For example root exudates of non-hosts crop such as sunflower

(*Helianthus annuus* L.) stimulated 16.7%, Roselle (*Hibiscus sabdariffa* L.) elicited 25.5%, (*Abelmoschus esculentus* (L.) Moench.) induced 56.2%, (*Lablab purpureus* (Jacq.) Verdc.) and cotton (*Gossypium* spp.) affected 75% germination compared to 48.2% induced by the root exudates of the highly susceptible sorghum variety Daber-1 (Dawood et al., 2015). The efficacy of trap crops could possibly be increased if overproduction of germination stimulants can be achieved through selection or molecular breeding. The latter can be achieved by over expression of one or more of the limiting genes in the strigolactones biosynthetic pathway. Over expression of strigolactones production could possibly improve mycorrhizal colonization efficiency and hence confers an additional benefit. The practice of rotating susceptible cereal crops with trap crops has been adopted to reduce *Striga* soil seed bank level in many countries in West Africa with rewarding outcomes (Aly, 2007). Strigolactones (SLs) have been agreed upon as the major natural parasitic weed germination stimulants (Zwanenburg & Posipisil, 2013). Further, SLs are produced by a wide variety of plant species. The first natural SLs was, interestingly, isolated from the root exudates of cotton (*G. hirsutum* L.) a non-host plant, (Cook et al., 1972). In 1972 the structure of a stimulant exude from cotton root was finally determined and given the name strigol and its absolute stereochemistry was established by x-ray diffraction analysis in 1985 (Cook et al., 1972; Butler, 1995). Later strigol was also detected in the root exudates of genuine hosts viz maize (*Zea mays*) and proso millet (*Panicum miliaceum* L.) and in lower amounts in sorghum. Amount and composition of strigolactones may vary among different plant species as well as among different cultivars of the same species (Xie et al., 2010). Strigol is extremely active biologically, it promotes germination of seeds of *S. asiatica* at a concentration as low as 10^{-16} M (Heather & Mittal, 1974). The present investigation, which includes six cotton cultivars collected from Sudan was carried out to study the influence of genotypic variation in the cotton germination inducing activity powder and root exudates in *Striga* infection.

2. MATERIALS AND METHODS

Seeds of cotton cultivars Hamid, Barakat and Barac (c) 67, Abdeen, Sene1 and Wager were obtained from the Agricultural Research Corporation (ARC) Wad Medani,

Sudan. *Striga hermonthica* seeds were collected in 2013 season from plants growing under sorghum in the Gadariief State. Agar nutrient less low melting agar was purchased from Nacalai Tesque Japan.

2.1 Germination Inducing Activity of Cotton as Influenced by Cultivars and Plant Parts Powder

Three cotton cultivars were screened, based on *Striga* germination result selected cultivars Hamid, Barac (c) 67 and Barakat, grown in pots in a greenhouse, were harvested at 150 days after sowing (DAS). Cotton shoots were cut at the soil surface and the roots were retrieved by careful and through washing with tap water. The plant shoots were severed into stems, leaves, flowers, bolls, and seeds. All plant parts including roots, each placed in a paper bag were allowed to dry under ambient conditions and grounded into fine powder using a kitchen grinder. A 2 mg from each plant part was assayed for germination inducing activity (GIA) using the sandwich techniques as described by Fujii, (1992) with minor modifications. Briefly the sandwich method resides on diffusion of germination stimulant, into agar. Three grams nutrient less agar were added to 1000 mL of distilled water and autoclaved for 20 minute at 15 bars and 121°C. The agar was allowed to cool in a water bath set at 40 °C. Aliquots of the agar solution (5 ml) were pipette, each, into each well of a multi-well-plate and allowed to solidify prior to placement of the test sample on top, then another 5 mL of the agar solution were placed on top, allowed to solidify, and subsequently discs containing conditioned *S. hermonthica* were placed on top. The discs were gently pressed to ensure contact with the agar. The multi-well-plates, cover in place, sealed with parafilm and warped in aluminum foil, were incubated at 30 °C in the dark for 24 h prior to examination for germination using a stereomicroscope.

2.2 Distribution of *Striga* Germination Stimulants in Cotton Plants as Influenced by Cultivar

Cotton (cv Hamid, Barac (c) 67 and Barakat), were sown, irrigated and harvested at 30, 60 and 90 day after sowing DAS, severed, dried and powdered as described in above. Test sample from each cultivar, were prepared and their germination inducing activity was assayed as previously described in above.

2.3 Activity and Persistence in Soil of *Striga* Germination Stimulants in Cotton Root Powder

Cotton, seeds (10/pots) were sown, irrigated and thinned to 5/pots 5 DAS. The seedlings, allowed to grow for 30 days after emergence, were harvested and severed into roots and shoots. The roots were placed in paper bags and subsequently air-dried and ground to fine powder as described in 3.2.2.1. Soil collected from the College experimental farm, mixed with sand (2:1v/v), was air-dried, crushed and sieved through a two mm mesh. Different amounts 0.3125, 0.6525, 1.25, 2.5 and 5 mg of cotton roots powder were mixed, each, with 10 g of the sieved soil, placed, each, in a glass Petri-dish. The soil in each Petri-dish was brought to field capacity (40% v/w) The Petri-dishes sealed with parafilm and weighted, were incubated for one day in the dark at 30°C. Water loss was replenished every 2 days by bringing each Petri-dish back to original weight. Activity of the stimulant(s) was monitored by determining germination inducing activity. Discs 8 mm containing conditioned *Striga* seeds were placed on the soil surface, in each Petri -dish and gently pressed. *Striga* seeds germination was assessed 24 h later. Ten gram samples of the soil/sand mix were mixed with root powder 1.25 and 5 mg each, placed in Petri-dishes and brought to 40% field capacity. The Petri-dishes were incubated for 0, 6, 12, 18, and 24 hour prior to placement of glass fiber discs contains conditioned *Striga* seeds and then seeds germination was assessed 24 h later.

2.4 *Striga* germination stimulants in cotton cv Hamid root exudates as influenced by time after sowing

Seeds of cotton (cv. Hamid), placed on a filter paper (Whatmann No 1) were wrapped in aluminum foil and subsequently dipped in sterilized distilled water for 3 days. The seedlings were transferred to 50 mL glass tubes containing 40% Long Ashton nutrient solution and allowed to grow for 5, 15, 30, 45 and 60 days at ambient temperature (23.5-29°C). Water loss was replenished every two days. Root exudates were collected 5, 15, 30, 45 and 60 days. At 3 days prior to sample collection the seedlings, the roots of which were thoroughly washed with tap water, were transferred to a new set of tubes filled with distilled water, re-incubated and allowed to grow for 3-days prior to sample collection. Water samples (50 mL each) containing the roots

exudates were extracted with ethyl acetate (3 x20) The ethyl acetate extracts, pooled, were allowed to stand overnight at 40 °C on anhydrous sodium sulphate. The extracts, filtered through Whatman No.1 filter paper, were subsequently evaporated to dryness at 40 °C using a rotary evaporator. The residues were re-dissolved, each, in 2 ml ethyl acetate. Aliquots of the ethyl acetate solution (0, 5, 15, 25µL) were applied, each to an 8 mm glass fiber discs and allowed to stand for 2 h to dry in a laminar flow cabinet. The treated discs, overlaid by disc containing conditioned *Striga* seeds, were moistened, each pair, with 40µL distilled water. The seeds were re-incubated in the dark at 30 °C for 24 h and subsequently examined for germination.

2.5 Effects of Cotton cv Hamid Population Density on Germination Inducing Activity of Root Exudates

Plastic pots 15 cm in diameter and 30 cm in height, perforated at the bottom, were filled with river sand up to 13 cm. A ceramic filter attached to a syringe barrel by a Teflon tube equipped with a 3 way tap lock, was inserted and sand was added to fill each pot to 4 cm below the rim. Cotton (cv Hamid) seeds (10) were sown in each pot. The pots were irrigated immediately. Subsequent irrigations were made at 2-days intervals. The emerged seedlings were thinned to 3, 6 and 9 plants per pot 2 days after emergence. Fifteen days after seedling emergence air was sucked out of the syringe barrel and the 3 way tap lock was open to suck water through the ceramic filter. The process was carried over night to lessen break down of the stimulant. The extracted samples containing root exudates were subsequently loaded on activated solid phase extraction (SPE) C18 Sep-pack column. The stimulant(s) were eluted off the column with two successive methanol washes (2+3 ml). Aliquots (0, 5, 15, 25µL) of the combined elute were applied, each, to an 8 mm glass fiber discs and allowed to stand for 1h in a laminar flow cabinet to ensure evaporation of methanol. Each of the treated discs was overlaid by a disc containing conditioned *Striga* seeds. Each pair of discs was moistened with 40 µL distilled water. The seed were re-incubated in the dark at 30°C and examined for germination.

2.6 Statistical Analysis

Data collected from all experiments were analyzed by analysis of variance (ANOVA), using

Statistic 8, complete software (2008), and Gen Stat (PC/windows 7), VSN, International Ltd., UK statistical packages. Laboratory data on germination were transformed to arcsin prior to analysis. Means were separated for significance using the Duncan Multiple Range Test (DMRT) and less significance difference (LSD) on factorials experiment, $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Germination Inducing Activity of Cotton as Influenced by Cultivar and Plant Part Powder

Generally germination inducing activity (GIA) of powder from cotton cultivars harvested at 150 days after sowing (DAS), varied with variety and organs (Table 1). Leaves powder from Barac (c) 67, Barakat and Hamid induced 28.2, 36.1 and 43.7% germination, respectively. The corresponding germination figures for stem powder were 30.1, 43.2, and 45.4%. Powders from roots of the respective cultivars were 31.9, 48.9, and 46.1%, respectively. Powder from flowers of the respective cultivars elicited 21.3, 31.9 and 29.6%, respectively. Powder from bolls of three cultivars Barac(c) 67, Barakat and Hamid induced 16.59, 22.38 and 26.52% germination, respectively. Within cultivars GIA was maximal and minimal for cv Hamid and Barac (c) 67. Within organs GIA was highest for powder from roots followed in descending order by stem, leaves, seeds, flowers and bolls (Table 1).

The finding that cultivars of the same crop vary in their ability to induce *Striga* germination stimulants, revived interest in trap cropping and/or intercropping as a component of an integrated management strategy for combating the parasite (Kapulin & Koltai, 2014). The introduction of new cotton varieties of different genetic background and the fact that strigolactones (SLs), albeit synthesized in the roots, are translocate to the shoots (Yoneyama et al., 2012) and that the natural haustorium factor 2, 6-dimethoxy-p-benzoquinone is a lignin breakdown product (Cui et al., 2018) instigated the research reported in the present study. Further, SLs are, produced by a wide variety of plant species (Xie et al., 2010), have been discovered as plant hormones (Umehara et al., 2008) that regulate the development of different plant parts (Kapulin & Koltai, 2014).

3.2 Distribution of *Striga* Germination Stimulants in Cotton Plants as Influenced by Cultivar and Time after Sowing

A general trend of the germination inducing activity (GIA) of cotton powder at 2mg, irrespective of cultivar, was an increase in the order of leaves, stems and roots (Table 2). Further, within organs the GIA decreased with time and was invariably maximal and minimal at 30 and 90 day after sowing emergence (DAS), respectively. Leaf powder from Barac (c) 67 induced 43.7, 35.9 and 31.2% germination 30, 60 and 90 DAS, respectively. Leaf powder from Barakat elicited 49.6, 39.9 and 28.5% germination at 30, 60 and 90 DAS, respectively. The corresponding germination figures for Hamid leaf powder were 58.0, 47.0 and 35.4%. Stem powder from Barac (c), elicited 61.1, 40.3 and 41.7 % germination at 30, 60 and 90 DAS, respectively. Stem powder from Barakat, on the other hand, provoked 65.0, 48.1 and 48.2 % germination at 30, 60 and 90 DAS, respectively. Stem powder from Hamid induced 70.8, 49.4 and 38.4% germination at 30, 40 and 90 DAS, respectively. Root powder from Barac(c) 67, induced 71.3, with a general mean of 21.8%, where powder from seeds provoked 26.8, 22.5 and 54.5 and 42.0%germination at 30, 60 and 90 DAS, respectively. Root powder from Barakat, on the other hand, elicited 77.1, 58.6 and 55.9%germination at 30, 60 and 90 DAS, respectively. The corresponding germination figures for Hamid root powder were 81.5, 72.1and 48.5% germination at 30, 60 and 90 DAS, respectively.

This finding shows a decline in stimulants production with age and clearly indicates that the high GIA displayed during the early stages of cotton growth is expected to contribute significantly to depletion of *Striga* seeds reserves in soil. The late decline in GIA may not be that significant as *Striga* seeds are expected to undergo dormancy on prolonged wetting (Parker, 2009). Within organs GIA was highest for powder from roots followed in descending order by stems, leaves (Table 2). Within cotton cultivars GIA was highest for Hamid followed in descending order by Barakat and Barac(c) 67. The results in conformity with recent literature Koltai and Beveridge, (2013), Daffalla et al., (2014) and Hassan et al., (2011) showed that the *Striga* germination stimulants, probably strigolactones, are present in all cultivars and in all plant parts. It worth mentioning that

Table 1. GIA of 2 mg cotton as influenced by cultivars and plant part on *Striga* germination

Plant part	Striga germination%			Means
	Cotton cultivars			
	Barac(c) 67	Barakat	Hamid	
Leaves	28.2 AB	36.1 B	43.7 A	36.0 AB
Stems	30.8 AB	43.2 A	45.3 A	39.8 A
Roots	31.9 A	46.1 A	48.9 A	42.3 A
Flowers	21.3 BC	31.9 B	29.6 B	27.6 C
Bolls	16.5 C	22.38 C	26.5 B	21.8 D
Seeds	26.8 AB	22.5 C	41.2 A	30.2 BC

Means within a column having the same letter(s) are not significantly different according to the Duncan's Multiple Range Test (DMRT), $P \leq 0.05$

Table 2. Effects of time and cultivar on GIA of 2 mg cotton plants parts powder

Treatment	Striga germination %			
	Plant parts			
Cotton Cultivars	DAS	Leaf	Stem	Root
Barac(c) 67	30	43.7 ^{BCD}	61.1 ^A	71.3 ^{AB}
	60	35.9 ^{DEF}	40.3 ^B	54.5 ^{CD}
	90	31.2 ^F	41.7 ^B	42.9 ^D
Barakat	30	49.6 ^B	65.0 ^A	77.1 ^A
	60	39.9 ^{CDE}	48.2 ^B	58.6 ^{BC}
	90	28.5 ^F	48.0 ^B	55.9 ^C
Hamid	30	58.0 ^A	70.8 ^A	81.5 ^A
	60	47.0 ^{BC}	49.4 ^B	72.1 ^{AB}
	90	35.4 ^{EF}	38.4 ^B	48.8 ^{CD}

Means within a column having the same letter(s) are not significantly different according to the DMRT, $P \leq 0.05$

germination figures obtained in this study were higher than those reported by Botanga et al. (2003) who screened 40 cotton genotypes and recorded GIA of 13.3-50% for *Striga*. However, the results are in line with those of Traore et al. (2011) who reported GIA of more than 75% for the varieties tested. The different results reported by the different researchers could be due to cultivar differences and/or to variability in soil fertility (Yoneyama et al., 2012).

3.3 Activity in Soil of *Striga* Germination Stimulants from Cotton cv HAMID Roots Powder

GIA of cotton cv Hamid root powder in soil, measured 24 h after application, varied with the amount of powder employed (Fig. 1). Germination which was 20% at the lowest powder amount (0.3125 mg/10g soil) increased in a weight dependent manner, reached a maximum 67.5% at 1.25 g/10g soil and then declined to 65.7 and 47.3% at 2.5 and 5 mg/10g soil, respectively. Such performance of germination stimulants were declined suggests involvement of both germination stimulatory and inhibitory substances, possibly phenolics in the

powder as reported by Anicia, et al, (2012) may account for the observed decrease. However, involvement of SLs at high concentration, in the notable inhibition of germination cannot be ruled out. SLs were reported to reduce *Striga* germination at supra-optimum concentrations (Yoneyama et al., 2012).

3.4 Time Course of Persistence of *Striga* Germination Stimulants in Cotton Roots Powder

Germination inducing activity of powder from cotton roots varied with the amount of powder employed and the time after application (Fig. 2). Cotton root powder at 1.25 mg induced 35.4, 63.4, 38.8, 23.7 and 5.7% germination when *Striga* seeds were placed on the soil surface 0, 6, 12, 18 and 24 h after powder application. On increasing powder amount to 5 mg the corresponding germination figures were 41.4, 43.6, 60.6, 26.9 and 30.3%, respectively.

The results showed that the stimulant(s), probably strigolactones (s) displayed considerable activity in soil. Further the notable differential increase in GIA with time displayed

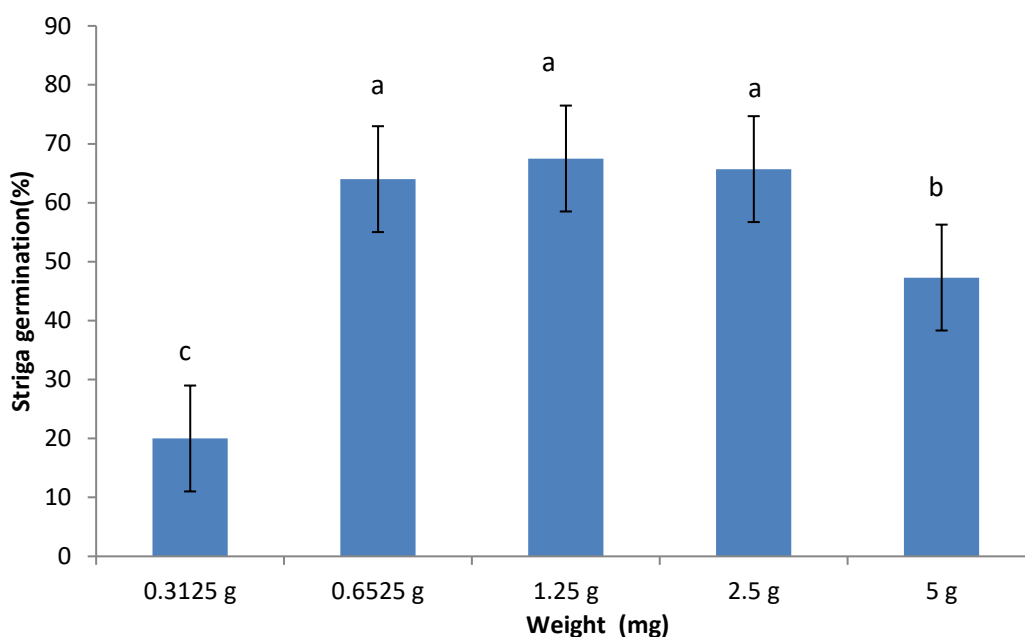


Fig. 1. GIA of *Striga* germination stimulants from cotton cv Hamid root powder in 10 g soil. Bars are represent means \pm standard error (n=5). Bars with the same letter are not significantly different according to the DMRT, (P \leq 0.05)

by both the low and high powder levels corroborates the suggested presence of inhibitory substance(s) in the cotton root powder and that the concentration of the inhibitor(s) also declined with time. The rapid decline of GIA is consistent with reports on persistence of both synthetic and natural SLs in soils particularly at alkaline pH (Babiker & Hamdoun, 1983). It worth mentioning that the GIA of SLs resides, mainly, on the intact C and D rings of the molecule (Zwanenburg & Posipisil, 2013). The rapid loss of activity entails that cotton powder *per se* cannot be used for induction of *Striga* seed germination. Soil incorporation of the powder and/or development of slow release formulation as suggested for synthetic SLs may improve performance under practical field conditions (Babiker & Hamdoun, 1983).

3.5 *Striga* Germination Stimulants in Cotton cv Hamid Root Exudates as Influenced by Time After Sowing

GIA of cotton root exudates varied with time and volume, (Fig. 3). At 5 μ l root exudates collected at 5 and 15 DAS induced 18.5 and 19.5% respectively. Root exudates collected at 30 DAS resulted in significantly higher germination (24.1%). However, root exudates collected at 45 and 60 DAS induced little to negligible

germination (0.0 to 5.3%, respectively. At 15 μ L root exudates collected at 5 DAS induced 18.9%. Increasing the collection time to 15 DAS increased germination significantly to 38.45% (Fig. 3). Increasing collection time to 30, 45 and 60 DAS decreased germination to 32.9, 16.5 and 13.2%, respectively. Root exudates at 25 μ L showed increased GIA with sampling period, which reached a peak 30 days AIOE (33.6% germination) and subsequently declined to 21.6 and 12.7% on extension of the sampling period to 45 and 60 days, respectively (Fig. 3). The decrease of GIA with exudates volume may also suggest involvement of germination inhibitors.

3.6 GIA of cotton (cv Hamid) Root Exudates as Influenced by Plant Population Density and Time After Sowing

Root exudates germination inducing activity increased with increasing number of plants per pot (Table 3). At 5 μ L root exudates collected from 3, 6, and 9 plants per pot induced 39.6, 46, and 50.3% germination, respectively. Root exudates at 15 μ l induced 51.5, 53.4, and 54.7 % germination, respectively when collected from 3, 6, and 9 plants per pot. The corresponding germination figures at higher exudates volume

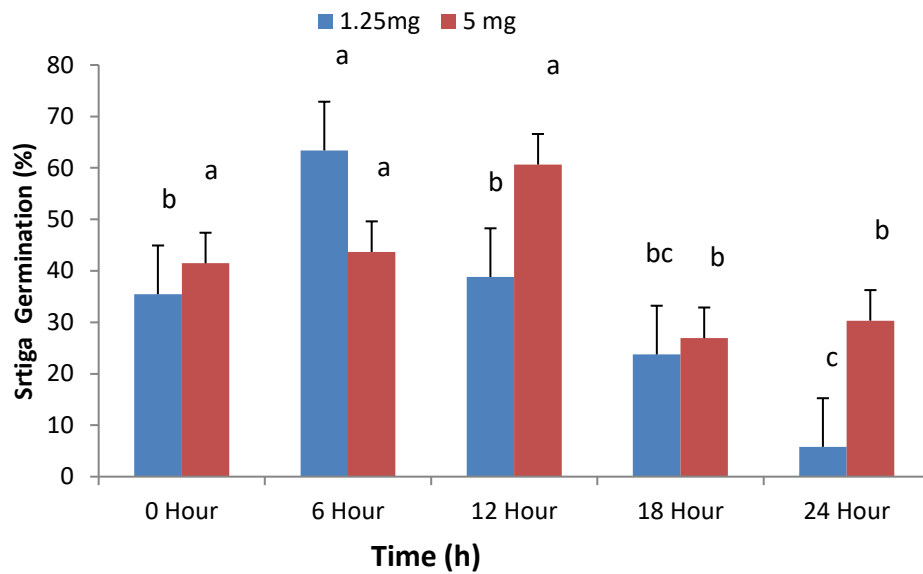


Fig. 2. Persistence in soil of *Striga* germination stimulants in cottoncv Hamid root powder are represent means \pm standard error (n=5). Bars with the same letter are not significantly different according to the DMRT, ($P \leq 0.05$)

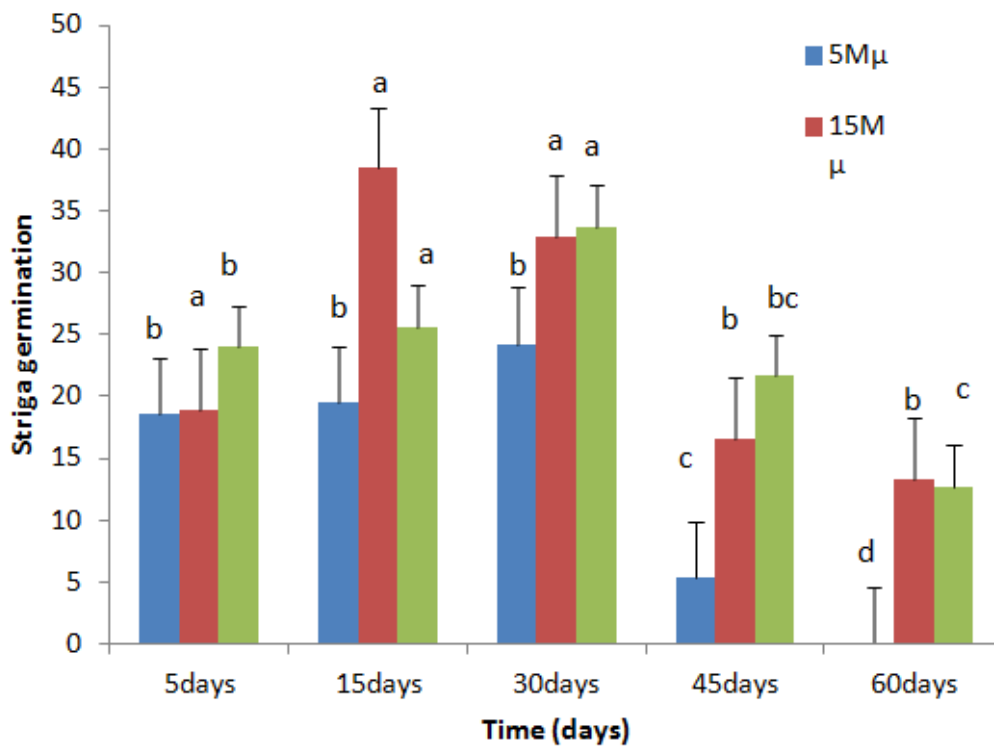


Fig. 3. GIA of cotton cv Hamid root exudates as influenced by time after crop emergence. Bars are represent means \pm standard error (n=5). Bars with the same letter are not significantly different according to the DMRT, $P \leq 0.05$

Table 3. Effect of cotton cv. Hamid population density on *Striga* germination through soil

Number of plant	Volume (μL)		
	5	15	30
0	15.3 ^D	19.3 ^D	22.9 ^D
3	39.6 ^C	51.5 ^{AB}	47.9 ^{ABC}
6	46.0 ^{BC}	53.4 ^{AB}	48.5 ^{AB}
9	50.3 ^{AB}	54.7 ^A	52.0 ^{AB}

Means within a column having the same letter(s) are not significantly different according to the DMRT, $P \leq 0.05$

(30 μl) collected from 3, 6, and 9 plants per pot were 52, 48.5 % and 39.6, respectively. It worth mentioning that exudates collected from bare soil and applied at 5, 15 and 30 μL induced 15.3, 19.3 and 22.9% germination, respectively. GIA of root exudates invariably, albeit not often significantly, increased with the number of plants per pot and was maximal at 15 μL (Table 3). The tendency of GIA to increase with the number of plants per pot may indicate increased stimulant production merely due to increased number of plants involved in the exudation process. However, the possibility of involvement of increased competition for nutrients between plants thus leading to increased production of *Striga* germination stimulants cannot be ruled out. Strigolactones production is known to be enhanced by nutrients deficiency (Yoneyama et al., 2012). The finding that GIA decreased with increased exudates volume further supports production of germination inhibitor(s) as concluded in the experiments (Fig. 3). The notable and consistent GIA of extract from bare soil (Table 3) suggests the plausibility of involvement of microbes in production of *Striga* germination stimulants as previously reported by Berner et al., (1996) for suppressive soils. However, the decrease in stimulant production could also be, among other factors, due to rhizospheric microbes particularly arbuscular mycorrhizal Fungi (AMF). Mycorrhizal plants have been reported to produce less strigolactones than their non-mycorrhizal congeners (Aroca et al., 2013).

4. CONCLUSIONS

- Cotton shoots and roots powder and roots exudates effectively induced germination of *Striga* seeds
- Among cotton cultivar, cotton cv. Hamid shoots and roots powder and roots exudates the most effective to induced germination of *Striga* seeds.
- However, a mechanism other than induction of suicidal germination, through which cotton suppresses *Striga* infection in

subsequent sorghum has to be ascertained.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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