

# Original Article: Assessment of *Sorghum bicolor* (L.) Moench. varietal resistance and the effect of aqueous extracts on *Striga hermonthica* (Del.) Benth. seed germination



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## ABSTRACT

*Striga hermonthica* is widespread in several regions of Burkina Faso. It attacks crops of agronomic interest, causing losses ranging from 20 to 100% of grain yield in sorghum varieties. This study carried out at the weed science laboratory from October 2022 to February 2023 aims to identify sorghum varieties resistant to *Striga hermonthica* and local plants that inhibit or stimulate its germination. Fifteen varieties of sorghum has been evaluated for their germination stimulant capacity production using the agar gel method. The roots of two seedlings of each variety were buried in the opposite position in the agar medium containing *Striga* seeds. The effect of nine local plants on *Striga hermonthica* seeds germination has been evaluated by preparing 10% aqueous extract for each plant by macerating a mass of 10 g of powder in 100 ml of sterile distilled water. The inhibitory effect was estimated by conditioning the *Striga hermonthica* seeds during 14 days in each of the plant aqueous extracts and the stimulatory effect by the application of aqueous extracts on the *Striga hermonthica* seeds. The results revealed Rsoe 38 (MGD=0.74cm), Icsv 1049 (MGD = 0.74cm), Ouédzouré (MGD = 0.81cm), Grinkan (MGD = 0.85cm), Rsoe 15(MGD = 0.85cm) and B 35 (MGD = 0.97cm) as resistant to *Striga hermonthica* germination. *Parkia biglobosa* and *Tephrosia purpurea* have proved effective in inhibition while *Andira inermis* and *Albizia zygia* stimulate it. The promotion of resistant sorghum varieties and plants with inhibitory or stimulatory effects could be an important asset for the biological control of *Striga hermonthica* in the most infested areas.

## Introduction

*Striga* genus plants constitute one of the main constraints to the production of cereal crops in West Africa. They develop at the expense of their host by attacking their roots by forming a sucker which eventually establishes a connection between the conductive vessels of the xylem of the crop and the parasitic plant (Rathnayaka *et al.*, 2022). These plants

produce small seeds that only germinate in the presence of strigolactone elicitors. Parasitic plants affect the growth of crops of agronomic interest such as maize, rice, sorghum, and millet, thus causing significant yield losses. In West Africa, around 50 million hectares are infested by *Striga* (Gressel *et al.*, 2004). Grain yield losses due to this infestation range from 20 to 100%, depending on agroclimatic conditions (Diyanat *et al.*, 2020). In Burkina Faso, *Striga hermonthica*,

*Striga gesneroides*, and *Striga aspera* cause considerable losses to cereal crops (Boussim *et al.*, 2011). The economic impact of each species varies according to local agro-ecological conditions and the type of crop grown. The extent of damage caused to sorghum cultivation has made *S. hermonthica* the most feared plant pest by farmers. Heavily infested fields are sometimes abandoned in favour of new clearings (Dembelé & Sidibe, 2009). Until now, crop protection against *Striga* remains dominated by the use of synthetic chemical herbicides (Koichi *et al.*, 2010). This chemical control, in addition to its toxicity for the environment, has a high cost. It also leads to the proliferation and resistance of other weeds (Rathnayaka *et al.*, 2022). It is therefore urgent to develop new sustainable solutions, respectful of the environment, to fight against *Striga*, which causes significant losses to agricultural crops. The selection of cereal varieties resistant to *Striga* remains one of the best control options (Koichi *et al.*, 2010). In addition, the use of plants inhibiting plants (Sourabié *et al.*, 2022) or stimulating plants in the absence of the crop (Dallali *et al.*, 2017) represents an alternative solution. As *S. hermonthica* is a hemiparasite, this stimulation in the absence of the crop could lead to the reduction of seed reserves in the soil. The objective of this study is to identify sorghum varieties resistant to *Striga hermonthica* and local plants that inhibit or stimulate its germination.

## Materials and methods

### Experimental location

The experiment was conducted at the Weed Science Laboratory of the Institute for the Environment and Agronomic Research (INERA/Kamboinsé) from October 2022 to February 2023. Kamboinsé whose geographical coordinates are: 12°28 North latitude, 1°32 of the West longitude is located in the North-Sudanian climatic zone, semi-arid with two seasons during the year: a long dry season of 6 to 7 months which extends from October-November to April-May and a wet season from May-June to September-October. Average temperatures vary between 26 °C and 31 °C during the wet season and between 25.3 °C and 32.5 °C in the dry season.

### Plant Materials

Herbicide plants, *Striga hermonthica* seeds, and sorghum seeds were used in this study. Nine herbicide plants (Table 1) were selected for their potential inhibitory or inducing effect on the germination of *Striga hermonthica* seeds. The choice of these plants follows the work of (Sourabié *et al.*, 2020) in the Cascades region. *Striga hermonthica* seeds were collected from sorghum fields in Comoé Province during the 2020-2021 agricultural seasons. They were stored in the weed science laboratory in plastic bags until use. Fifteen improved sorghum varieties of diverse origin were evaluated. These were Gnonfing, Gnossiconi, Kapelga, Ouedzouré, Pse 144, Rsoe 15, Rsoe 38, Sarioso 07, Icsv 1049, Samurai 1, Samurai 2, Sarioso 14, Grinkan, B 35, and Sepon 82. These sorghum cultivars are from the Burkinabé, Nigerian and Malian varietal assortment.

**Table 1** Plant organs used for the preparation of aqueous extracts for herbicidal effects

Species	Families	Organ used
<i>Albizia zygia</i> (DC.) J.F.Macbr.	Fabaceae-mimosoideae	Leaves (L)
<i>Andira inermis</i> (W.Wright) DC.	Fabaceae-faboideae	Leaves (L)
<i>Azadirachta indica</i> A. Juss.	Meliaceae	Bark and leaves (B+L)
<i>Balanites aegyptiaca</i> (L.) Delile	Zygophyllaceae	Bark (B)
<i>Khaya senegalensis</i> (Desr.) A. Juss.	Meliaceae	Bark (B)
<i>Ficus polita</i> Vahl.	Moraceae	Bark and leaves (B+L)
<i>Eucalyptus camaldulensis</i> Dehnh. [cult.]	Myrtaceae	Leaves (L)
<i>Parkia biglobosa</i> (Jacq.) R.Br.ex G.Don.	Fabaceae-mimosoideae	Pod (P)
<i>Tephrosia purpurea</i> (L.) Pers.	Fabaceae-faboideae	Stem and leaves (St-L)

### Methods

*Striga* seeds and sorghum were initially disinfected, and then the aqueous extracts of each plant were prepared. The resistance of each variety of sorghum to the germination of *Striga* seeds and the effect of stem extracts on the germination of parasite were finally evaluated.

#### *Disinfection of seeds and production of sorghum seedlings*

The seeds of *Striga hermonthica* were successively immersed in the solution of ethanol 70 % and sodium hypochlorite (NaOCl) 1 %. In this last solution, two drops of tween 80 were added, and then the seeds were rinsed 3 times with sterile distilled water. They were preconditioned for two weeks at 28 °C (Rathnayaka *et al.*, 2022). Sorghum seeds were disinfected by immersion in a 1% sodium hypochlorite solution for about 30 minutes. Thereafter, they were rinsed three times with sterile distilled water. About 15 sterilized sorghum seeds were germinated in Petri dishes containing moistened filter paper, and then covered with aluminum foil. The whole set was kept at room temperature in the laboratory for 24 hours, after which the vigorous sorghum seedlings were selected for testing.

#### *Preparation of aqueous extracts*

The sample of each plant was completely dried at the room temperature of the laboratory for three weeks and then reduced to a fine powder with a traditional mortar. For each herbicidal plant species, the 10% concentration was prepared by macerating 10 grams of powder in 100 ml of sterile distilled water. A New Brunswick Scientific apparatus was used to agitate the mixture for 24 hours at a constant temperature of 28 °C after which the decoctions were filtered to give the aqueous extracts.

#### *Evaluation of sorghum varietal resistance*

Preconditioned *Striga hermonthica* seeds were diluted in sterile distilled water. One milliliter of this dilution was pipetted and placed in the middle of a 9 cm diameter Petri dish. Autoclaved agar medium (0.70% Agar), then cooled to about 36

°C, was poured over this dilution to obtain an even distribution of seeds at the bottom of the petri dish. The rootlets of two sorghum seedlings were buried in the solidified agar medium in opposite positions at the edges of the Petri dish. Petri dishes containing *Striga hermonthica* seeds and sorghum seedlings were covered with aluminum foil and incubated in the dark at 28 °C for 72 hours, after which each Petri dish was observed under a binocular loupe.

#### *Evaluation of the inhibitory effect of aqueous extracts on *Striga hermonthica* seeds germination*

Approximately forty to fifty seeds of *Striga hermonthica* were placed on filter paper discs that overlay the Whatman paper on the bottom of the petri dish. Three milliliters of aqueous extract was used to condition the seeds in each Petri dish. The Petri dishes were incubated in an incubator at 28 °C for 14 days after which the seed-bearing discs were transferred to new petri dishes. In these dishes, twenty-five microliters of GR24 were applied to the seeds to stimulate their germination. The Petri dishes were placed in an incubator at 28 °C for 48 hours.

#### *Evaluation of the germination stimulating effect of *Striga* seeds by aqueous extracts*

Approximately forty to fifty seeds of *Striga hermonthica* were deposited on filter paper discs that overlay the Whatman paper on the bottom of the Petri dish. Three milliliters of sterile distilled water was used to condition the seeds in each Petri dish. The Petri dishes were placed in an incubator at 28 °C for 14 days after which the seed-bearing discs were transferred to new Petri dishes. In each Petri dish, 25 µl of aqueous extract was applied to the seeds to stimulate their germination. The dishes were incubated at 28 °C for 48 hours.

#### *Data analysis and processing*

Sorghum varietal resistance was assessed by estimating the Maximum Germination Distance (MGD). The MGD represents the greatest distance measured in centimeters between the radicle of the sorghum seedling and the furthest germinating *Striga hermonthica* seed. Sorghum seedlings are classified as low germination

stimulant producers if this measured MGD is less than or equal to 1 cm. The test is repeated in three trials under the same experimental conditions and in each trial, three Petri dishes are used per variety. To estimate the herbicidal effect (inhibitor or stimulator) induced by aqueous extracts, germinated seeds were counted with a binocular magnifying glass. Germination rate was expressed as follows (Rathnayaka *et al.*, 2022):

$$\text{Germination rate (\%)} = \frac{N_g \times 100}{N_t}$$

Where,  $N_g$  indicates the number of germinated seeds and  $N_t$  is the total number of seeds. The means of the different tests were subjected to statistical analysis using R version 4.0.2 software. One-way analysis of variance (ANOVA) was performed to assess the herbicidal effect of aqueous extracts and varietal resistance. Tukey test was also used to compare means. The significance threshold was set at 5% ( $p < 0.05$ ).

## Results and Discussion

### *The maximum germination distances of Striga hermonthica seeds in relation to sorghum radicles*

The means of the maximum germination distances of *S. hermonthica* seeds recorded by individual test are presented in [Table 2](#). The maximum germination distance less than or equal to 1 cm was recorded with four varieties Grinkan, Icsv1049, Ouédzouré and Rsoe 38 in the test 1, and with five varieties B35, Icsv1049, Rsoe15, Rsoe38, and Sepon82 in test 2. On the other hand in test 3, eight varieties such as Gnonfing, Grinkan, Icsv1049, Kapelga, Ouédzouré, Rsoe15, Rsoe38, and Sariasso14 recorded low maximum germination distances ( $\leq 1$ cm). Indeed, Gnessiconi and Kapelga were developed by seed producers, while Icsv 1049 and Rsoe38 come from the varietal breeding units of ICRISAT and INERA. This difference in origin could therefore justify the sensitivity of Gnessiconi and Kapelga to *S. hermonthica*. Our results are similar to the study conducted by Oliver *et al.* (1992) who reported the sensitivity of Gnessiconi and Kapelga to the *S. hermonthica* seed germination.

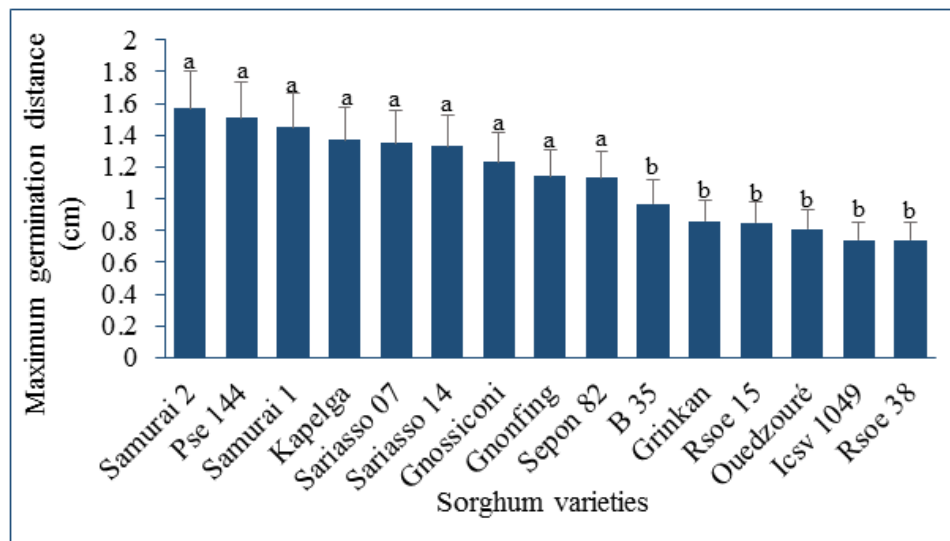
**Table 2** Variation in the maximum germination distances of *Striga hermonthica* seeds from sorghum radicle

Sorghum varieties	Test 1	Test 2	Test 3
B35	1.07 <sup>b</sup>	0.53 <sup>c</sup>	1.63 <sup>a</sup>
Gnonfing	1.40 <sup>b</sup>	1.30 <sup>b</sup>	0.73 <sup>b</sup>
Gnessiconi	1.27 <sup>b</sup>	1.37 <sup>b</sup>	1.55 <sup>a</sup>
Grinkan	0.75 <sup>c</sup>	1.02 <sup>b</sup>	0.81 <sup>b</sup>
Icsv1049	0.15 <sup>c</sup>	0.63 <sup>c</sup>	0.77 <sup>b</sup>
Kapelga	2.17 <sup>a</sup>	1.33 <sup>b</sup>	0.63 <sup>b</sup>
Ouedzouré	0.55 <sup>c</sup>	1.33 <sup>b</sup>	0.55 <sup>b</sup>
Pse144	1.70 <sup>b</sup>	1.37 <sup>b</sup>	1.87 <sup>a</sup>
Rsoe15	1.07 <sup>b</sup>	0.93 <sup>c</sup>	0.57 <sup>b</sup>
Rsoe 38	0.60 <sup>c</sup>	0.73 <sup>c</sup>	0.90 <sup>b</sup>
Samurai1	1.47 <sup>b</sup>	2.20 <sup>a</sup>	2.70 <sup>a</sup>
Samurai2	1.83 <sup>b</sup>	1.43 <sup>b</sup>	1.77 <sup>a</sup>
Sariasso07	1.43 <sup>b</sup>	1.60 <sup>b</sup>	1.93 <sup>a</sup>
Sariasso14	1.60 <sup>b</sup>	1.63 <sup>b</sup>	0.77 <sup>b</sup>
Sepon82	1.43 <sup>b</sup>	0.82 <sup>c</sup>	2.97 <sup>a</sup>
Mean	1.23	1.21	1.34
P value	<0.001	<0.001	<0.001
C.V. (%)	46.03	37.93	35.86

\*In the same column, means followed by the same letter are not significantly different at the 5 % level. CV, coefficient of variation; P, probability.

In the same column, means followed by the same letter are not significantly different at 5% level. CV: Coefficient of variation and P: Probability. The means maximum germination distances derived from the three tests (Figure 1) show that the lowest maximum germination distances ( $\leq 1$  cm) are obtained with six varieties Icsv1049 (0.7 cm), Rsoe38 (0.7 cm), Ouedzouré (0.8 cm), Grinkan (0.9 cm), Rsoe15 (0.9 cm), and GnoSSiconi (1.0 cm). Thus, on the basis of the

evaluated parameters, the results obtained with the cultivars Icsv1049, Ouédzouré, Grinkan, Rsoe15, GnoSSiconi, and Rsoe38 are promising and could constitute a database in sorghum improvement programs in Burkina Faso (Oliver *et al.*, 1992). However, the analysis of variance (ANOVA) of each test revealed the existence of a significant difference ( $p < 0.001$ ) between the maximum germination distances induced by the sorghum varieties.

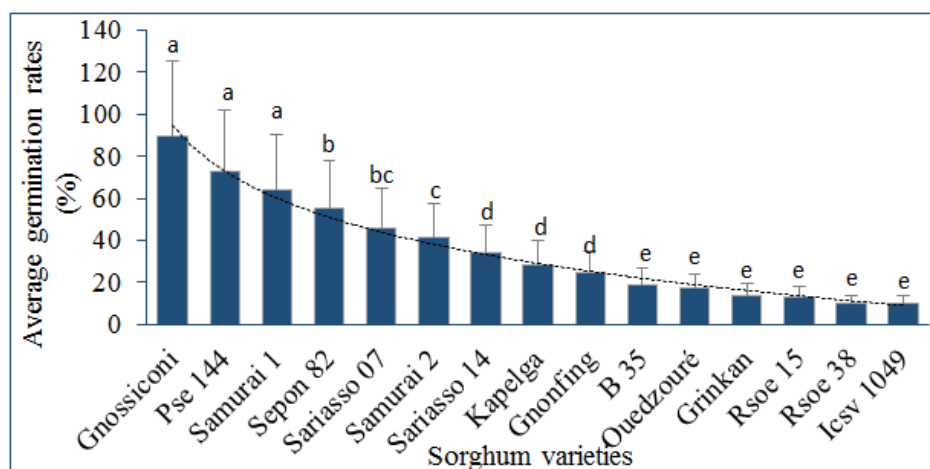


**Figure 1** Average maximum germination distances of *Striga hermonthica* seeds from the three tests. Each bar represents the standard error of the mean

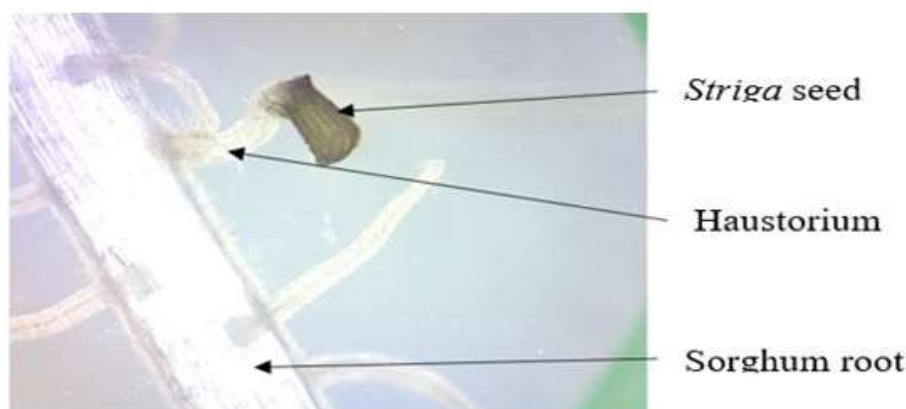
#### Average germination rates of *Striga hermonthica* seeds in relation to sorghum varieties

The mean *S. hermonthica* seed germination rates induced by each sorghum variety are shown in Figure 2. The lowest germination rates ( $\geq 20\%$ ) of *S. hermonthica* seeds were recorded with six varieties Icsv1049 (9.9 %), Rsoe38 (10.1%), Ouedzouré (13.1%), Grinkan (13.8%), Rsoe15 (17.3%), and GnoSSiconi (19.1%). The highest germination rates ( $\geq 50\%$ ) were induced by B35 (89.3%), Samurái2 (73.0%) Pse144 (64.4%), and Samurái1 (55.5%). With these same varieties, the case of fixation of the haustorium of the seeds of *S. hermonthica* (Figure 3) was observed.

Sariasso07 and Kapelga, although they induced strong germination of *S. hermonthica* seeds, proved resistant to haustorium attachment. This resistance could be explained by the structure of radicles which is thought to resist haustorium penetration (Dembelé & Sidibe, 2009). A similar result was recently reported in the Democratic Republic of Congo by Jadika *et al.* (2018) with three maize varieties Tze-y-dtc4 str c5, Tze-w-dtc4 str c4, and Katoki wa lukasa. These researchers showed that the resistance to attachment of the haustorium of *S. hermonthica* could depend on the covering of the walls of the radicle.



**Figure 2** Germination rates of *Striga hermonthica* seeds from the three tests. Each bar represents the standard error of the mean



**Figure 3** Attachment of the haustorium in the radicle of sorghum variety Psc144

#### *Inhibitory effect of aqueous extracts on Striga hermonthica seeds germination*

The results reported in [Table 3](#) show that the extracts inhibited *S. hermonthica* seeds germination. The manifestation of inhibition phenomena on *S. hermonthica* seeds germination could be explained by the phytochemical compounds. The involvement of biological compounds with an inhibitory effect on the germination of weeds has already been reported in the work of Ben Kaab *et al.* (2020). These authors reported the inhibitory effect of naringenin and myricitrin from *Cynara cardunculus* Crude on the germination of seeds of *Trifolium incarnatum* L. Among the factors likely to explain the effect of phytochemical compounds on *S. hermonthica* seed germination

are the number of seeds present and the volume of aqueous extract applied (Othman *et al.*, 2020). The mean germination rates of the seeds conditioned in the aqueous extracts of each of the plants ranged from 0 to 52.8% in test 1; 0 to 40.2% in test 2 and 0 to 49.2% in test 3. The highest germination rates of *S. hermonthica* seeds in each test were recorded with the aqueous extracts of *A. zygia* and *A. indica*. The same observations result as for the means derived from the 3 tests ([Figure 4](#)). The *S. hermonthica* seed was completely inhibited by aqueous extracts of *P. biglobosa* pods. This result corroborates the work of (Kambou *et al.*, 2000) who reported the inhibitory effect of *P. biglobosa* pods. The percentages of seed germination inhibition induced by the aqueous extracts of *P. biglobosa*

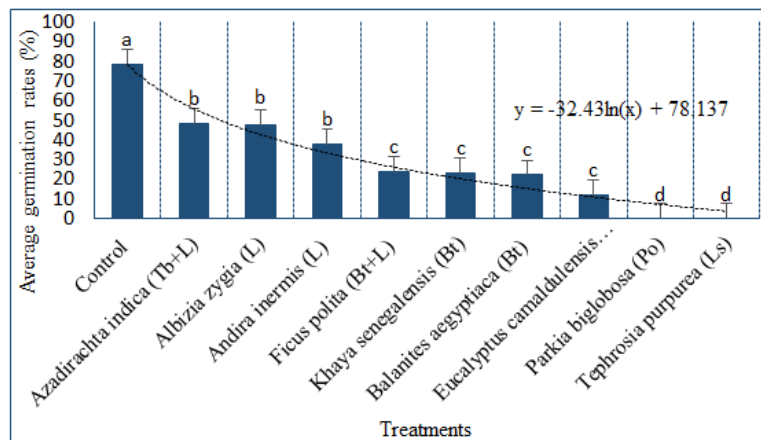
and *T. purpurea* are not significantly different. This does not explain an equitable concentration of phytochemical compounds with an inhibitory effect in both plants. It shows that each plant contains the biological compounds with an inhibitory effect which allows to produce the effect. The fact that the aqueous extracts of *P. biglobosa* pods are more active on *S. hermonthica*

seeds germination could be due to their high concentration in bioactive compounds. This justifies the observation of Evert *et al.* (2017) which shows that phytochemical compounds with an inhibitory effect are produced at the level of the leaves, stored in the envelopes and released into the environment by roots exudation; with the stems acting essentially as transitional organs.

**Table 3** Variations in germination rates of *Striga hermonthica* seeds conditioned with 10% aqueous plant extracts

Treatments	Germination rate (%)		
	Test 1	Test 2	Test 3
Control (GR24)	87.25 <sup>a</sup>	76.51 <sup>a</sup>	70.88 <sup>a</sup>
<i>Albizia zygia</i> (L)	52.84 <sup>b</sup>	40.21 <sup>b</sup>	49.24 <sup>b</sup>
<i>Andira inermis</i> (L)	27.05 <sup>c</sup>	28.12 <sup>c</sup>	36.37 <sup>c</sup>
<i>Azadirachta indica</i> (B+L)	42.23 <sup>b</sup>	50.41 <sup>b</sup>	52.21 <sup>b</sup>
<i>Balanites aegyptiaca</i> (B)	16.50 <sup>cd</sup>	27.21 <sup>c</sup>	22.02 <sup>d</sup>
<i>Khaya senegalensis</i> (B)	17.59 <sup>cd</sup>	29.01 <sup>c</sup>	21.63 <sup>d</sup>
<i>Ficus polita</i> (B+L)	25.35 <sup>c</sup>	27.54 <sup>c</sup>	18.33 <sup>d</sup>
<i>Eucalyptus camaldulensis</i> (L)	12.59 <sup>d</sup>	17.89 <sup>d</sup>	19.63 <sup>d</sup>
<i>Parkia biglobosa</i> (P)	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
<i>Tephrosia purpurea</i> (St-L)	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>
Mean	26.88	29.69	29.03
P value	0.003	0.017	0.020
C.V. (%)	13.01	22.18	15.90

\*Means followed by the same letter in the same column are not significantly different ( $P > 0.05$ ). L, leaf; B, bark; P, pods; St-L, stem and leaves



**Figure 4** Average germination rates of *Striga hermonthica* seeds from the three tests. Each bar represents the standard error of the mean

#### Stimulating effect of aqueous extracts on *Striga hermonthica* seeds germination

The recorded germination rates (Table 4) ranged from 3.3 to 79.9% in test 1; 2.4 to 80.3% in test 2, and 5.7 to 75.3% in test 3. The effect induced by the aqueous extracts on the germination of *S. hermonthica* seeds varied from one plant to another (Figure 5). Our results revealed that the

aqueous extracts of *A. inermis* and *A. zygia* stimulated more the germination of *S. hermonthica* seed. This result is corroborated by the work of Rathnayaka *et al.* (2022) where burying the green leaves of *A. inermis* and *A. zygia* as a green manure in the soil stimulated seed germination of *S. hermonthica*. The germination rates caused by the aqueous extracts

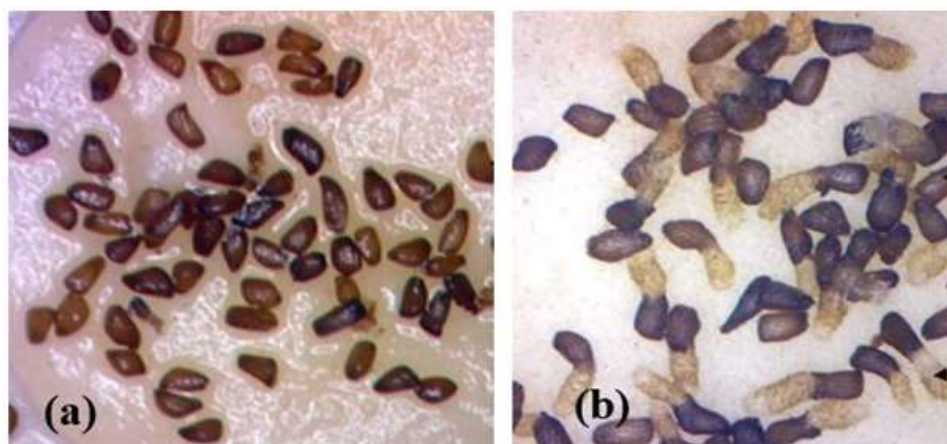
of these two plants were statistically identical to those induced by GR24 with which they form a homogeneous group. The same observations result as for the means derived from the 3 tests (Figure 6). This result suggests that *A. inermis* and *A. zygia* can be used in biological control

since they cause the suicidal germination of *S. hermonthica* seeds. However, this suggestion remains a matter of agronomic interest given that bioassays do not allow extrapolation of results to field conditions (Othman *et al.*, 2020).

**Table 4** Variations in germination rates of *Striga hermonthica* seeds after stimulation with aqueous plant extracts

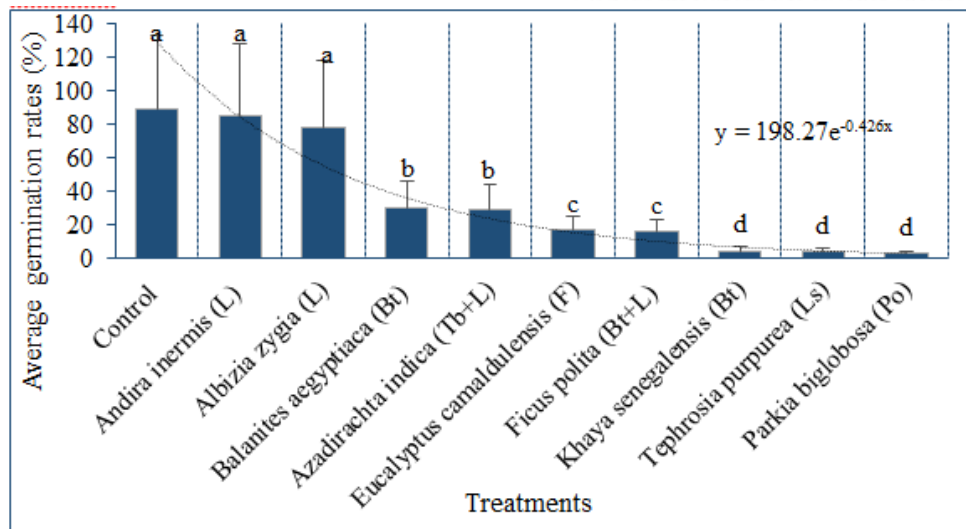
Treatments	Germination rate (%)		
	Test 1	Test 2	Test 3
Control (GR 24)	89.39 <sup>a</sup>	96.27 <sup>a</sup>	80.38 <sup>a</sup>
<i>Albizia zygia</i> (L)	79.89 <sup>a</sup>	80.37 <sup>a</sup>	75.29 <sup>a</sup>
<i>Andira inermis</i> (L)	87.11 <sup>a</sup>	92.42 <sup>a</sup>	76.77 <sup>a</sup>
<i>Azadirachta indica</i> (B+L)	22.24 <sup>b</sup>	38.40 <sup>b</sup>	27.91 <sup>b</sup>
<i>Balanites aegyptiaca</i> (B)	36.52 <sup>b</sup>	30.29 <sup>b</sup>	24.39 <sup>b</sup>
<i>Khaya senegalensis</i> (B)	4.09 <sup>d</sup>	6.00 <sup>d</sup>	3.11 <sup>c</sup>
<i>Ficus polita</i> (B+L)	15.35 <sup>c</sup>	7.51 <sup>d</sup>	18.00 <sup>b</sup>
<i>Eucalyptus camaldulensis</i> (L)	12.59 <sup>c</sup>	17.89 <sup>c</sup>	19.63 <sup>b</sup>
<i>Parkia biglobosa</i> (P)	2.10 <sup>d</sup>	1.93 <sup>d</sup>	3.95 <sup>c</sup>
<i>Tephrosia purpurea</i> (St-L)	3.32 <sup>d</sup>	2.40 <sup>d</sup>	5.70 <sup>c</sup>
Mean	35.55	37.64	33.51
P value	0.001	0.013	0.007
C.V. (%)	9.47	14.83	10.35

\*Means followed by the same letter in the same column are not significantly different ( $P > 0.05$ ). L, leaf; B, bark; P, pods; St-L, stem and leaves



**Figure 5** Condition of *Striga hermonthica* seeds treated with the aqueous extracts (a) aqueous extracts of the stem and leaves of *T. purpurea* and (b) aqueous extracts of *A. inermis* leaves





**Figure 6** Average germination rates of *Striga hermonthica* seeds stimulated by extracts. Each bar represents the standard error of the mean

### Conclusion

In this study six varieties of sorghum were investigated which were resisted to *Striga hermonthica* seeds germination. The low production of germination stimulants by these varieties is at the origin of their resistance. Regarding the effect of aqueous extracts, *P. biglobosa* and *T. purpurea* inhibit *Striga hermonthica* germination while *A. inermis* and *A. zygia* stimulate it. It is important for the continuation of the work leading to the biological control of *S. hermonthica*, to carry out an additional study in the greenhouse with the varieties of sorghum, the plants with an inhibitory effect, and those with a stimulatory effect.

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### Conflict of interest

The authors declared that there is no conflicts of interest in this article.

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