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Striga hermonthica (Del.) Benth has Dual Negative Effect on its Host *Sorghum bicolor* (L.) Moench

Tigist Beyene[1,a], Meseret Chimdessa Egigu[2,b,*]

[1]*Department of Biology, Oda Bultum University, P.O. Box: 226, Chiro, Ethiopia*
[2]*School of Biological Sciences and Biotechnology, P.O. Box: 138, Dire Dawa, Ethiopia*
[*]*Corresponding author*

A R T I C L E I N F O	A B S T R A C T
	Striga is a parasitic weed causing remarkable yield lose in *Sorghum bicolor*. In this study, phytotoxicity of leaf and flower extracts of *Striga hermonthica* were investigated on three sorghum varieties under laboratory conditions. Before allelopathic bioassay, total phenolics, alkaloids and terpenoids were quantified in the extracts. For allelopathic bioassay, 5 and 10% (w/v) aqueous extracts were prepared by reconstituting dried crude extracts of leaf and flower of *S. hermonthica* in distilled water. Varieties of sorghum named Muyra 1 (M1), Muyra 2 (M2) and Fendishe (Fe) were treated with equal amounts of 5 and 10% (w/v) extracts in Petri dishes lined with Whatman no.1 filter paper. The control treatment received distilled water and treatments were laid out in a completely randomized design with three replications. Germination parameters were monitored for 15 days and General linear model was used to analyze data. Results showed that both leaf and flower of *S. hermonthica* produce phenolics, alkaloids and terpenoids. Values of total phenolics and total alkaloids did not show significant variation, whereas total terpenoid was significantly higher in flower than leaf. Percent germination, shoot and root lengths as well as their dry weights were significantly affected by extract concentration, sorghum variety and their interactions. Compared to control, percent germination and seedling growth were highly reduced at 5% w/v extract concentration and completely inhibited at 10% w/v extract concentration. Varieties were not differentially affected by leaf extracts, but M1 and M2 appeared to be more affected than Fe by flower extract. The result also showed that root growth was more affected by extracts than shoot growth. Overall, this study for the first time revealed that *S. hermonthica* reduces yield of *S. bicolor* not only due to parasitism, but also through its negative allelopathic effect on seed germination and early seedling growth.

[a] *tgb2016m@gmail.com*   https://orcid.org/0000-0001-5437-636x   [b] *marakye311@gmail.com*   https://orcid.org/0000-0001-5474-1515

Introduction

Striga hermonthica (Del.) Benth. (Orobanchaceae) is commonly known as purple witchweed as its English common name. Locally in Ethiopia, it is called by the name *'Akenchira'*. Sorghum, (*Sorghum bicolor* L. Moench [Poaceae]) is one of the world's major cereal crops and the fifth most important crop globally after rice, wheat, maize and barley (Chala, 2018). It is widely cultivated throughout tropical, semi-tropical, arid and semi-arid environments in over 120 countries (USDA, 2016). It is mainly grown as a food crop by small-scale farmers in sub-Saharan Africa (FAO, 2016) and Ethiopia is the fifth sorghum producing country in the world next to America, India, Nigeria and Argentina (Africa Harvest, 2010). Although sorghum can be cultivated in areas where cultivation of other food grains are difficult due to severe moisture and nutrient stresses, its

production is being constrained due to parasitism by *S. hermonthica* (Rispail et al., 2007; Hayelom, 2014). This parasitic weed grows on the roots of its host and withdraws photosynthates, minerals and water from the host through a direct connection to the host's xylem via its organ called haustorium.

Infestations by *S. hermonthica* causes severe sorghum yield reduction in which farmers lose sometimes up to 100 percent grain production (Zerihun, 2015). The percentage yield loss of course depends on factors such as the density of the parasite, sorghum variety, soil nutrient status and rainfall patterns (Atera et al., 2012). The problem caused by *S. hermonthica* infestation is expected to grow worse due to its adaptive ability of parasitizing new crops or varieties and adaptation to new environmental conditions

(Rodenburg and Bastiaan, 2011). Based on the reports of Hadas (2015), *S. hermonthica* is mainly disseminated across farms through floods from nearby farms, farm tools, and via winds.

Most plants produce some type of organic compounds (secondary metabolites) that are of no direct use for their growth and development (Peteros, 2010), but serve as defense chemicals against biotic and abiotic stresses (Thilagavathi et al., 2015). Secondary metabolites may be synthesized and stored in different parts of the plant body including leaves, stems, roots, flowers, fruits, etc. (Solomon et al., 2013). Alkaloids, terpenoids and phenolic compounds are the most important classes of secondary metabolites (Edeoga et al., 2005), that may have allelopathic effects on neighboring/recipient plants upon liberation into the environments through different means (Mali and Kandae, 2014). Allelochemicals may negatively affect seed germination, seedling growth and establishment of the recipient plant. They exert their negative impacts by modifying resource consumption capacity; alteration of cell membrane permeability and enzymatic activity; triggering genetic defects and disturbing photosynthesis of the recipient plants (Majeed et al., 2012). The allelopathic effect of one plant on another depends on the type and concentration of the allelochemical released as well as the recipient species (Asadduzzaman et al., 2010).

The parasitic nature of striga on sorghum and its negative impact on growth and grain yield of sorghum had long been established by different researchers. For example, Bebawi and Farha (1981) reported that the parasitic striga weed causes reduction in sorghum grain yield by 65% when compared with 32% caused by non-parasitic weeds. Parasitism of sorghum by *S. hermonthica* and reduction of its growth and grain yield due to this parasitic weed had also been reported by Showemimo (2006) as well as by Runo and Kuria (2018). There are many reports on the allelopathic nature of sorghum on weeds too (e.g., Alsaadawi and Dayan, 2009; Hozayn et al., 2011) as well as other crops/plants on sorghum (Hassan et al., 2012). Reports show that chemicals released from a given species upon decomposition may be allelopathic to itself too and inhibit germination and seedling growth of its own (Bonanomi et al., 2005). With this premise, we hypothesized that although *S. hermonthica* is a parasitic weed which depends on its host sorghum, it may also be allelopathic to it and negatively affects its host. This study was, therefore, conducted with the objectives of (i) quantifying the major classes of secondary metabolites from extracts of leaf and flower of *S. hermonthica* and (ii) evaluating the allelopathic effects of different concentrations of *S. hermonthica* extracts on three varieties of *S. bicolor*.

Materials and Methods

Plant Materials

Striga hermonthica was collected from aggressively infested sorghum field around Haramaya University (9°24'53.13''N and 42°01'55.69''E) in East Hararghe Zone of the Oromia Regional state, Ethiopia. Seeds of three sorghum varieties: Muyra-1 (M1), Muyra-2 (M2) and Fendishe (Fe) were obtained from Haramaya University sorghum improvement research center.

Preparation of Plant Extracts

After authentication at the Herbarium of Haramaya University, intact *S. hermonthica* plant was separated into its leaf and flower parts, washed under running tap water and air dried at room temperature in the laboratory. The dried plant parts were separately powdered using mortar and pestle. The powder (100g) was macerated separately in 500 ml of distilled water and shaken for 72 hours on a platform shaker at room temperature. Thereafter, the mixture was filtered using Whatman No. 1 filter paper under suction and the filtrates were evaporated under reduced pressure using a rotary evaporator at 55°C to remove the solvent. The dried crude extracts were then stored in labeled sterile conical flask at 5°C in the refrigerator until use for bioassay.

Quantification of Major Secondary Metabolite Classes

Total phenolics, total terpenoids and alkaloids in the dried crude extracts were quantified following standard methods as indicated below.

Determination of Total Phenolics: The total phenolics in dried crude extracts of leaf and flower were determined according to the Folin–Ciocalteu procedure (Singleton and Rossi, 1965). About 40 mg of the dried crude extract was mixed with 5 ml 80% acetone, shaken well in a Vortex-shaker and centrifuged at 2200 × g for 2 min at room temperature. The supernatant was transferred to a 10 ml volumetric bottle with a Pasteur pipette. The remaining residue was extracted twice with 2.5 ml 80% acetone, shaken well in a Vortex-shaker, centrifuged as before after standing for 5 min and the supernatants were transferred to the same 10 ml volumetric bottle with a Pasteur pipette. Aliquots (100 μl) of supernatant from each sample were added to two 10 ml volumetric bottles and mixed with 1.9 ml deionized water. Folin–Ciocalteu–phenol reagent (1 ml) was added and the solution was shaken vigorously and mixed with 5 ml sodium carbonate (20%). After 20 min, the solution was centrifuged at 2200 × g for 2 min at room temperature. Absorbance at 735 nm was measured in a spectrophotometer (Shimadzu UV-2401PC) and the results were expressed as tannic acid equivalents.

Total Alkaloid Determination: Total alkaloid was determined following the method of Harborne (1973). The dried crude extract (3g) was weighed and added into a 50 ml Erlenmeyer flask. Then, 20 ml of 10% acetic acid in ethanol was added into the flask and the solution was covered and allowed to stand for 4 hrs. Then after, the solution was filtered and concentrated ammonium hydroxide was added drop wise to the filtrate until the formation of precipitate stops. The whole solution was allowed for the precipitate to settle. The precipitate was then collected, washed with dilute ammonium hydroxide and filtered. The obtained precipitate was dried and weighed. Alkaloid content was calculated as mg per g of the dried crude extract used.

Total Terpenoid Determination: The dried crude extract (2 gm) was soaked in 50 ml of ethanol for 24 hr. The solution was then filtered and the filtrate was extracted with 6 ml of petroleum ether using separating funnel. Then, the ether extract was concentrated to dryness using rotary evaporator at 40°C. The dried ether extract was then considered as total terpenoid (Malik et al., 2017).

Allelopathic Bioassay of S. hermonthica Extracts

For allelopathic bioassay, the dried extracts of leaf and flower were first reconstituted in distilled water to have 5 and 10% (w/v) concentrations (Samedani et al., 2013). Prior to germination test, seeds of *S. bicolor* varieties were surface-sterilized for 5 min with 5% sodium hypochlorite (NaOCl) to avoid fungal invasion, and then rinsed thoroughly with distilled water (Hamidi et al., 2013). Thereafter, seeds (10) of each of the *S. bicolor* variety were sown in 9 cm diameter Petri dishes lined with double layer of Whatman no.1 filter paper that received 10 ml of the extracts. Distilled water was applied instead of extracts as a control group for comparison. Petri-dishes were incubated in laboratory for days to complete germination. Each experiment was laid out in completely randomized design (CRD) with three replications. Petri dishes were made to get more or less the same amount of light throughout the incubation period and moved around to avoid position effect. Germination was monitored for 15 days with root and shoot lengths, and seedling shoot and root dry weights measured on the 15th day. Percent germination was calculated using the following formula used by Gairola et al. (2011) as follows.

$$\text{Percent Germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Data Analysis

General linear model was used to analyze the main effects and their interactions on dependent variables using SPSS, version 20. Independent T-test was used to analyze the difference between leaf and flower in terms of each major secondary metabolite class. Differences between means were considered statistically significant at *P*<0.05.

Results

Major Secondary Metabolite Classes of the Leaf and Flower Extracts of Striga hermonthica

In this study; total phenolics, terpenoids and alkaloids were detected both in the leaves and flowers of *S. hermonthica*. The amounts of total phenolics and alkaloids showed no significant (P>0.05, Independent T-test) difference between leaves and flowers, whereas total terpenoid content of the flower was significantly (P<0.05, Independent T-test) higher than that of leaf (Figure 1).

Effects of S. hermonthica Extracts on Germination Percentage of Sorghum bicolor Seeds

Both concentration of aqueous extracts and sorghum varieties as main effects, and their interaction significantly (P<0.01) affected percent seed germination. Under control treatment, germination of seeds of all varieties was 100%, but significantly reduced at 5% (w/v) extract concentration and completely inhibited at 10% (w/v) extract concentration (Figure 2). However, percent germination was unaffected by OT (organ type extracted, i.e., leaf vs. flower) (P=0.198); OT and variety interaction (P=0.493); OT and extract concentration (P=0.194); and OT, variety and extract concentration interaction (P=0.583).

Effects of S. hermonthica Extracts on Shoot and Root Lengths

Inhibition of shoot and root lengths of the three sorghum varieties was significantly (P<0.01) affected by extract concentration, sorghum variety and their interaction (Figure 3 A and B). Shoot and root growth inhibitions increased with increasing extract concentrations with actually complete inhibition at 10% (w/v) as seeds fail to germinate at this concentration. However, shoot was unaffected by OT (P=0.628); interaction of OT and sorghum variety (P=0.333); OT and extract concentration interaction (P=0.904); interaction of sorghum variety, OT and extract concentration (P=0.563). Likewise root length was unaffected by OT (P=0.05), interaction of OT and sorghum variety (P=0.300); interaction of sorghum variety, OT and extract concentration (P=0.557) and OT and extract concentration interaction (P=0.052).
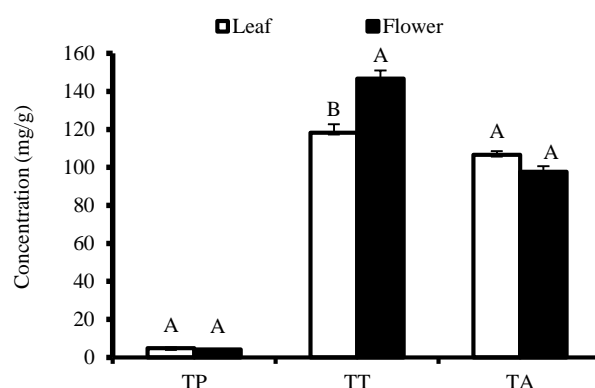

Figure 1. Major Secondary metabolites quantified in Leaf and Flower of *S. hermonthica*.
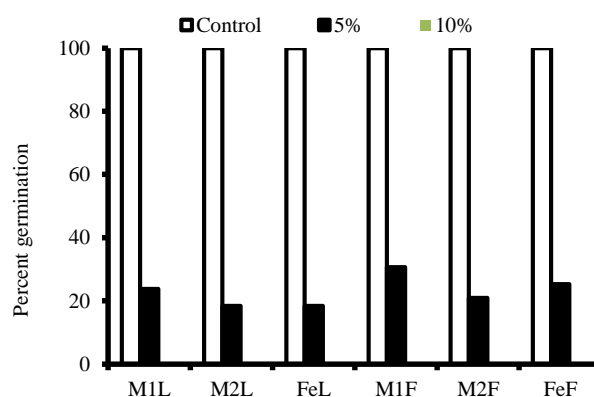Values are Mean ± S.E, n=3. *Note*: TP= Total Phenolics, TT= Total Terpenoids and TA= Total Alkaloids

Figure 2. Germination Percentage of Seeds of Three Varieties of *S. bicolor* treated with different concentrations of Leaf and Flower Aqueous Extracts of *S. hermonthica*.
Values are Mean ± S.E, n=3. *Note*: Bar graph for 10% w/v extract concentration did not appear as values are zero. M1L= Muyra-1 treated with leaf extract, M2L= Muyra-2 treated with leaf extract, FeL= Fendishe treated with leaf extract, M1F= Muyra-1 treated with flower extract, M2F= Muyra-2 treated with flower extract, FeF= Fendishe treated with flower extract
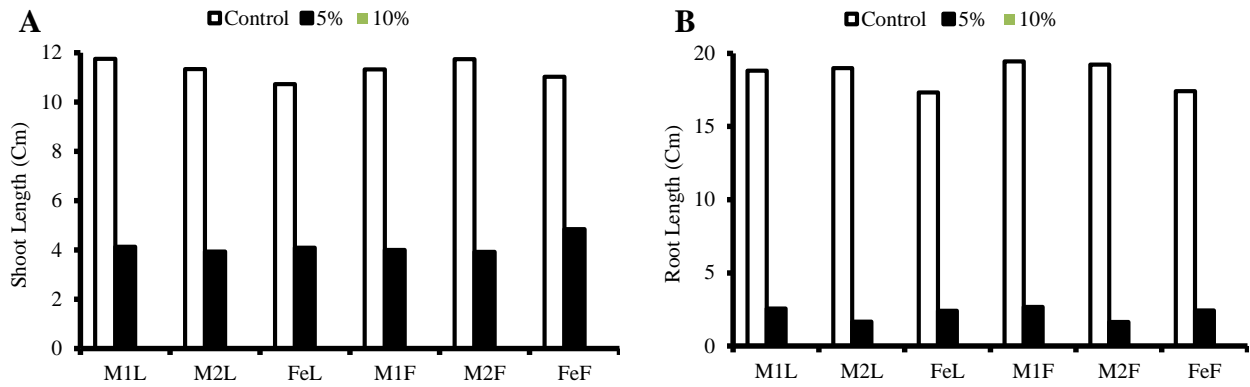
Figure 3. Shoot and Root Lengths of the Three Varieties of *S. bicolor* treated with different concentrations of Leaf and Flower Aqueous Extracts of *S. hermonthica*.

Values are Mean ± S.E, n=3. *Note*: Bar graph for 10%w/v extract concentration did not appear as values are zero. M1L= Muyra-1 treated with leaf extract, M2L= Muyra-2 treated with leaf extract, FeL= Fendishe treated with leaf extract, M1F= Muyra-1 treated with flower extract, M2F= Muyra-2 treated with flower extract, FeF= Fendishe treated with flower extract
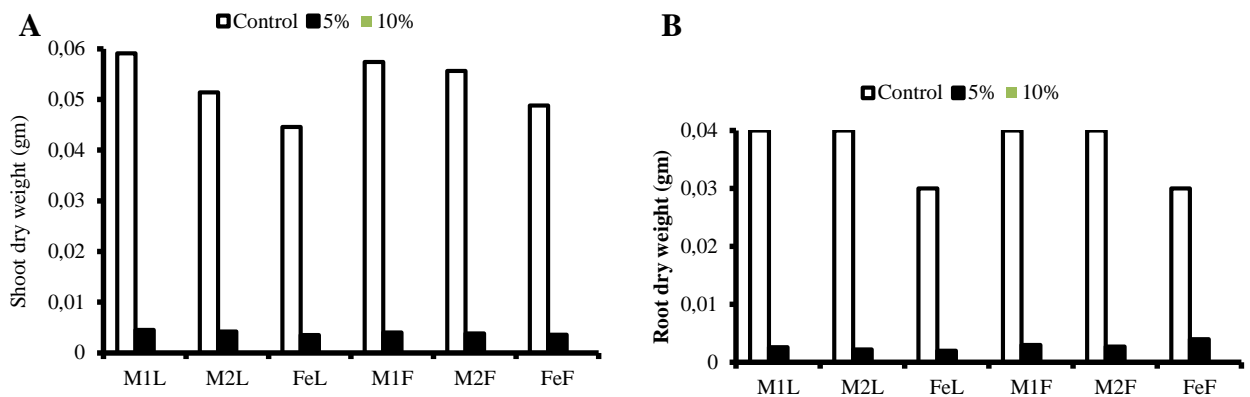

Figure 4. Shoot and Root Dry Weights of the Three Varieties of *S. bicolor* treated with different concentrations of Leaf and Flower Aqueous Extracts of *S. hermonthica*.

Values are Mean ± S.E, n=3. *Note*: Bar graph for 10%w/v extract concentration did not appear as values are zero. M1L= Muyra-1 treated with leaf extract, M2L= Muyra-2 treated with leaf extract, FeL= Fendishe treated with leaf extract, M1F= Muyra-1 treated with flower extract, M2F= Muyra-2 treated with flower extract, FeF= Fendishe treated with flower extract

Effects of S. hermonthica Extracts on Shoot and Root Dry Weights

Shoot dry weight of the three sorghum varieties was significantly ($P<0.001$) affected by extract concentration; sorghum variety and their interaction (Fig. 4A). Although OT did not affect shoot dry weight, the interaction of OT and extract concentration ($P=0.018$); and interactions of OT, sorghum variety and extract concentration ($P=0.046$) significantly affected shoot dry weight (Fig. 4A). Values of shoot dry weight showed highly significant reduction from that of the control treatment at 5% (w/v) extract concentration and no measurement was taken at 10% (w/v) extract concentration because of complete inhibition of seed germination at this concentration (Fig. 4A). Root dry weight of the three sorghum varieties was also significantly ($P<0.01$) affected by extract concentration, but not affected by OT ($P=0.310$); variety ($P=0.388$); the interactions of OT and variety ($P=0.309$); OT and extract concentration ($P=0.351$); variety and concentration ($P=0.423$); and variety, OT and extract concentration ($P=0.332$) (Fig. 4B). Values of root dry weight showed highly significant reduction from that of the control treatment at 5% (w/v) extract concentration and no measurement was taken at 10% (w/v) extract concentration because of complete inhibition of seed germination at this concentration (Figure 4B).

Discussion

Major Secondary Metabolites

Autoinhibitory effect of plants by chemicals released from same plant species upon decomposition was well documented (Bonanomi et al., 2005; Bonanomi et al., 2007). With this point in mind, we hypothesized that a parasitic plant may also have double negative effect on its host through production of allelochemicals apart from parasitism. The main objective of this study was, therefore, to evaluate *S. hermonthica* extracts for its negative allelopathic effects on its host, sorghum. Prior to allelopathic bioassay, we detected the presence of some allelochemicals in leaves and flowers of S. *hermonthica*. Here our interest was not to unravel the complete chemical profiles and specific phytotoxic compound(s) (which should be considered in the future), but gross detection of major secondary compound classes including terpenoids, phenolics and alkaloids that often are reported to have allelopathic effects (Macias et al., 1992) in aqueous crude leaf and flower extracts for net allelopathic effect. In our second phase of this study, we evaluated the allelopathic effect of aqueous crude extracts of leaf and flower of *S. hermonthica* on its host, sorghum. Overall, results of this study showed that *S. hermonthica* which parasitizes sorghum also negatively affects its host through allelochemicals produced in its leaves and flowers.

In this study, results of chemical analysis showed that leaves and flowers of *S. hermonthica* produce phenolics, terpenoids and alkaloids. Okpako and Ajaiyeoba (2004) and Koua et al. (2011), previously detected flavonoids and tannins in aqueous extracts of flowers and other parts of *S. hermonthica*. Koua et al. (2011) showed the presence of trepenes and alkaloids in petroleum ether and ethanol extracts of the entire part of *S. hermonthica* while Baoua et al. (1980) detected alkaloids in *S. hermonthica* tissue extracted using water and ethanol. In this study, the amounts of total phenolics and alkaloids showed no significant difference between leaves and flowers, whereas total terpenoid content of the flower was significantly higher than that of leaf. Ibrahim et al. (2010) and Jiang et al. (2016) reported that floral parts of the plants they investigated produce terpenoids more than leaves. Hansen and Seufert (1999) also reported 7.8-fold higher total emissions of terpenoids from *Citrus sinensis* (L.) branches with flowers than from branches without flowers. Total terpenoids of this study may be array of different components that are produced to give flowers their characteristic scent that may help them attract pollinators (Flamini et al., 2003). Therefore, higher amount of terpenoids in flowers than leaves may be attributed to additional roles they have in flowers. These three classes of secondary metabolites have been reported to have defensive roles against abiotic and biotic stresses (Egigu et al., 2011) and also known for their allelopathic effect (Macias et al., 1992; Mali and Kandae, 2014) upon liberation into the soil mainly through decomposition of the donor plant tissues. Thus, detection of these classes of secondary compounds suggests the potential of *S. hermonthica* to be allelopathic to recipient plants including its host, *S. bicolor*, and the same was confirmed from allelopathic bioassay.

Allelopathic Bioassay

From allelopathic bioassay, we noticed 100% seed germination of the three tested sorghum varieties under control treatment. However, percent germination was significantly reduced at 5% (w/v) extract concentration and completely inhibited at 10% (w/v) extract concentration. Given the same extract concentration, percent germination did not vary with verities tested and organ type extracted. Seed germination may be hindered due to inhibition of water uptake (Tawaha and Turk, 2003). It may also be inhibited due to hampered resource mobilization by allelochemicals during early stages of seed germination (Gniazowska and Bogtek, 2005). It is also possible that allelochemicals such as some phenolic compounds impair the synthesis and/or activity of gibberellic acid (Einhellig, 1996) which regulates the production of amylase (Chandler et al., 1984) so that seed germination is negatively affected.

Growth of shoot and root including their lengths and dry weights of the three tested sorghum varieties was highly reduced by aqueous extracts of both leaf and flower of *S. hermonthica* and growth reduction was more pronounced with increasing extract concentration. Previously, Einhellig (1996) reported that allelochemicals decrease elongation, expansion and division of cells which are growth prerequisites. The inhibition of shoot and root lengths by *S. hermonthica* extracts also suggests the occurrence of some allelochemicals that interfere with important metabolic activities that promote cell division and elongation. Sasikumar et al. (2002) reported the inhibitory effects of phenolic compounds on phosphorylation pathway, Mg activation and ATPase activity. They also mentioned that allelochemicals might decrease synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) hence negatively affect growth. Given the same concentration, OT had no differential effect on the same variety in terms of these parameters. Although leaf extract had no varying effect on the three varieties, flower extract exhibited differential effect on shoot lengths of the three varieties whereby variety Fendishe (Fe) was less affected than Muyra-1 (M1) and Muyra-2 (M2), suggesting that some unique chemicals must have been released from flower extract to more suppress shoot length of M1 and M2 than Fe. In terms of root length also, variety Fe was found to be superior than the others. Sensitivity of crops to allelochemicals varies with species and genotypes within species (Asghari and Tewari, 2007; Baličević et al., 2014). This finding is also in agreement with Hassan et al. (2012) who reported that extract of botanicals have differential negative impacts on shoot dry weight of different sorghum varieties. In contrast to shoot, root length was more suppressed than that of shoot with at least 1.5-fold reduction from that of shoot length at 5%(w/v) extract concentration. This may be attributed to the impairment of endogenous plant growth hormones synthesis and/or activity in the root by allelochemicals.

In conclusion, inhibitory effects of allelochemical at a germination and early seedling growth stage may predispose crops to other abiotic and biotic stresses, hence reduces their productivity (Dekker and Maggitt, 1983). So far, research have shown significant reduction in sorghum yield due to infestation by parasitic *S. hermonthica* for which the mechanism of attack was simply attributed to its parasitism. However, this study for the first time revealed that *S. hermonthica* has dual negative effect on sorghum through production of allelochemicals apart from parasitizing its host.

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