PLANT SCIENCES | RESEARCH ARTICLE

Allelopathic sorghum aqueous extracts reduce biomass of hairy beggarticks

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ABSTRACT: Leachates from the landrace sorghum IS9456 were tested on Bidens pilosa emergence and growth at Henderson Research Station in 2018. The trial had six treatments replicated four times. Stover was put in perforated plastic pots suspended on a frame. Watering released leachates, which were received by clay pots under the suspended pots. Atrazine significantly inhibited percentage emergence (P < 0.05) and leaf area (P < 0.001) of B. pilosa compared to leachates from all sorghum plant parts and the negative control. There was no significant effect (P > 0.05) of sorghum plant part as source of aqueous extract, and atrazine on height of B. pilosa. Sorghum stalks, leaves and sorghum stalks combined with leaves significantly (P < 0.05) reduced dry weight of B. pilosa compared to treatments with heads, atrazine and no sorghum herbage. Heads, stalks, leaves and leaves combined with stalks from mature IS9456 have limited allelopathic effect on the emergence of Bidens pilosa compared to atrazine. However, stalks, leaves and leaves combined with stalks can suppress biomass of Bidens pilosa possibly due to high concentrations of water soluble allelopathic compounds. Atrazine can provide early suppression B. pilosa, while allelopathic leachates from sorghum leaves, stalks and leaves combined with stalks can suppress B. pilosa in later growth stages by reducing weed biomass.

Subjects: Agriculture & Environmental Sciences; Botany; Plant & Animal Ecology; General Science

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PUBLIC INTEREST STATEMENT

Sorghum aqueous extracts have the potential to inhibit weed germination, emergence and growth due to water soluble allelopathic compounds. This can reduce farmers’ reliance on herbicides for weed control. This study investigated the potential of sorghum aqueous extracts prepared from different sorghum plant parts to inhibit emergence and growth of B. pilosa, a weed that has developed tolerance to a number of herbicides globally. Aqueous extracts prepared from heads had little allelopathic effect on B. pilosa emergence and growth compared to those prepared from leaves, stalks and a combination of leaves and stalks, suggesting low concentrations of allelochemicals in sorghum heads. Atrazine did not significantly reduce B. pilosa biomass, suggesting that the weed has some tolerance to the herbicide.
Keywords: Sorghum allelopathy; sustainable weed management; Bidens pilosa

1. Introduction
Weeds can reduce crop productivity (Hartzler, 2009; Leghari et al., 2015; Nandula, 2019; Siddiqui et al., 2010) by competing with crops for resources such as nutrients, light and moisture (Swanton et al., 2015; Trognitz et al., 2016). Weeds directly and indirectly reduce the economic yield of crops (De Matos et al., 2019) through increased production costs, and reduced quality and quantity of produce (Nandula, 2019). Conventional weed management methods are dominated by the use of herbicides in commercial agriculture (Beckie et al., 2019; Dayan, 2019; Harker & O'Donovan, 2013; Westwood et al., 2018), and largely hand weeding in the smallholder farms (Chivinge, 1996; Lee & Thierfelder, 2017; Sims et al., 2018). Heavy reliance on herbicides and their indiscriminate use has raised human health and ecological concerns (Beckie et al., 2019; BMJ, 2019; Davidson et al., 2019; Otorkpa, 2017; Tsai, 2019; Van Bruggen et al., 2018), and herbicide resistance (Baucom & Busi, 2019; Duke et al., 2018). A scary reality concerning reliance on herbicides is that in the last 30 years no herbicides with truly new molecular targets have been introduced (Dayan, 2019) and weeds have evolved resistance to 23 out of the 26 known herbicide sites of action and to 167 different herbicides, with 505 unique cases comprising 259 weed species (Heap, 2019) clearly suggests that continued reliance on herbicides for weed control is not sustainable. Hand weeding is associated with a number of challenges that include high inefficiency (Mashingaidze, 2004), and being labour intensive (Sims et al., 2018). Removing weeds by hand causes drudgery (Krupnik et al., 2016) and this can drive the youthful labour force from the farms to towns in pursuit of better opportunities (Sims et al., 2018). The method is slow and inefficient (Chivinge, 1996), requiring 200–400 man hours ha⁻¹ (Gill, 1982). In sub-Saharan Africa, 50–70% of the labour used in growing crops is dedicated to weeding (Chikoye et al., 2007). Crop fields are seldom adequately weeded (FAO, 2019; Gianessi, 2013). New weed management tools are therefore necessary (Dayan, 2019; Weisberger et al., 2019). One promising tool with potential for inclusion in integrated weed management is the use of allelopathy (Duke, 2010). Harnessing allelopathy for weed control may reduce the use of herbicides (Anaya, 2006; Duke 2007).

Allelopathy refers to the adverse effects of a plant on another plant through production of allelochemicals (Duke, 2015). Many plants, including several weeds and crops have been reported to be allelopathic. These crops release allelopathic compounds in the soil (Anaya, 2006; Blum et al., 1997; Inderjit and Keating, 1999). In Zimbabwe, sorghum, which is primarily cultivated in drought-prone areas in the agroecological that are classified as unsuitable for intensive cropping (Tibugari et al., 2020), can be utilised for allelopathic weed control.

Allelopathic compounds are present in tissues that include leaves, flowers, fruits, buds, seeds, stems and roots, mostly as non-toxic substances, in double or triple bonds separated by a single bond in almost all plants (Putnam, 1988; Xuan et al., 2005). Exposure to stress or death triggers release of the toxic moiety (Putnam, 1988). When residues from sorghum are soaked in water for a period of 24 hours, they can be sprayed as an herbicide (Cheema & Khaliq, 2000). Residues can also be applied as mulch (Abbas et al., 2016; Alsaaadawi et al., 2017; Cheema & Khaliq, 2000; Cheema et al., 2004). Allelopathy can be utilised to manage weeds under different agroecosystems (Chon & Nelson, 2010). Allelopathic activity in the field situations can be caused by the joint action of mixtures of allelochemicals (Einheilig, 1995; Inderjit, 2003, 2005; Lydon et al., 1997; Weston & Duke, 2003). Production of allelochemicals is governed by soil factors (Kobayashi, 2004; Macias et al., 2019) and other external stresses (Macias et al., 2019; Wu et al., 2010) such as presence of other plants (Dayan, 2006; Kong et al., 2004) and nutrient limitation (Weidenhamer, 2006). At subtoxic doses, some allelochemicals may cause stimulatory effects (Belt & Cedergreen, 2010; Belt & Duke, 2014). However, inhibitory effects caused by allelochemicals often outweigh the stimulatory effects (Dayan & Duke, 2009). Multiple modes of action in allelochemical compounds such as sorgoleone help to delay evolution of resistance (Dayan & Duke, 2009; Duke, 2010).
When farmers harvest sorghum grain, the usual practice is to leave sorghum stalks standing in the field for livestock to feed (Bean, 2017; Kristjanson & Zerbini, 1999; Meinders, 1961). The dry sorghum leaves and stalks are an important source of roughage for livestock (Etuk et al., 2012). Other farmers collect the sorghum stalks and stock them for livestock feeding during the winter season when pastures have little grass, while others burn the stalks (Roberts, 2015). Apart from serving as a source of nutritious livestock feed (Etuk et al., 2012; Kristjanson & Zerbini, 1999), mature sorghum herbage contains water-soluble allelopathic compounds. This implies that sorghum leaves, stalks and roots remaining in the field after sorghum is harvested can suppress weeds through allelopathy. Presence of these residues can also physically suppress weeds (Moore et al., 1994). Sorghum residues can also suppress weeds and other crops through release of allelopathic compounds (Kruidhof et al., 2009). Late removal of sorghum residues just before the planting rains in summer can subject the next crop after sorghum to allelochemicals that leach from sorghum residues. The allelopathic residues can also suppress weeds. It is believed that leaves are a consistent source of allelochemicals, while stalks and roots contain less (Miranda et al., 2011; Weston & Mathesius, 2013) the most. Water-soluble compounds that are released by residues prior to decomposition as well as insoluble compounds that are released during decomposition constitute allelopathic potential of a donor plant (Harper & Lynch, 1982). However, for sufficient release of allelochemicals from sorghum herbage to occur, the residues must undergo decomposition. Hairy beggarticks or blackjack (Bidens pilosa L.) is a problem weed in the small-holder farming areas of Zimbabwe (Chivinge, 1988). It has widespread distribution in Zimbabwe (Holm et al., 1979). The weed has developed resistance to herbicides (De la Cruz et al., 2019), making it difficult to manage. In studies that quantified sorgoleone, which is the major allelopathic compound produced by sorghum seedling roots (Dayan & Duke, 2009; Netzly & Butler, 1986; Pan et al., 2018), Tibugari et al. (2019) established that some landrace and wild sorghums produced very high quantities of the lipophilic compound. The South African landrace sorghum IS9456, which produced the highest amount of sorgoleone (584.7 μg mg⁻¹ of root fresh weight) (Tibugari et al., 2019) could also potentially be a good source of water soluble allelopathic compounds. We also hypothesised that allelochemicals leached out of sorghum leaves, stalks and roots are insufficient when these tissues are not decomposed, and therefore sorghum allelopathy does not control the first weeds that germinate and emerge soon after the planting rains in summer. The objective of this study was therefore to examine the potential allelopathic effect of fresh leachates prepared from herbage of mature sorghum on emergence and early growth of B. pilosa.

2. Materials and methods

2.1. Study site, experimental design and procedure

The study was conducted at the Weed Research Unit, Henderson Research Station (17°34’ and 30° 58’E) in Mazowe, Zimbabwe. The experiment was designed as a Completely Randomised Design. Matched experimental units were used and each block contained the same number of pots in four replications. Stover obtained from the South African landrace sorghum IS9456, which produced the highest amount of sorgoleone (584.7 μg mg⁻¹ of root fresh weight) in a previous study (Tibugari et al., 2019) was used. Herbage was obtained from mature sorghum that was at the late dough stage of growth. The sorghum had been planted on a medium grained sandy clay loam soil at Panmure Experiment Station (31°47’E and 17°35’S) in Shamva, Zimbabwe. The soil type at Panmure is classified as Chromic Luvisols (Zimbabwean classification) or Rhodoxeralf Alfisols (USDA classification) (Nyamapfene, 1991). Prior to growing the landrace sorghum crop, the land had previously been fallow. A basal fertiliser, Compound D (7.14.7 NPK), was applied at the rate of 150 kg ha⁻¹ at planting. A seeding rate of 7 kg ha⁻¹ was used. Inter-row distance was 75 cm with planting stations 30 cm apart. The crop was grown under supplementary irrigation and top dressed with Ammonium Nitrate (34.5% N) at a rate of 150 kg ha⁻¹ at four weeks after crop emergence. The crop was harvested at the hard dough stage, air dried, chopped with a sharp chopping knife into 2–3 cm pieces (Cheema et al., 2004; Hussain, 2015) (Figure 1).
The chaffed material was weighed using an American Weigh LB-3000 Compact Bowl scale. The chaffed stover in each pot weighed 35 g. Prior to the experiment, the pots were perforated using a 3-mm-thick hot iron bar. Each pot had three perforations. The small size of perforations and low number of perforations per pot ensured slow release of leachates into the receiving clay pots, and disallowed washing away of pieces of stover from the suspended pots into the receiving pots. The pots containing chopped sorghum stover were suspended on a frame directly above pots containing the weed seeds in such a way that the sorghum leachates directly dropped into pots containing the weed *Bidens pilosa* (Figure 2).

Figure 1. Chopping of sorghum herbage into 2–3 cm pieces using knives.

Figure 2. Metal frame holding perforated plastic pots with chopped sorghum stover (A) and clay pots with emerging *B. pilosa* (B).
Fresh *B. pilosa* weed seeds, that were obtained from the Weed Research Unit, Henderson Research Station in Mazowe, were sown by dibbling in pots containing oven-sterilised sandy loam soil at a rate of 10 seeds per pot. The seeds were sown at a depth of 1 cm. Prior to the experiment, the soil had been analysed for pH and nutrients at the Department of Soil Science and Agricultural Engineering, University of Zimbabwe, Harare. ZFC Cereal Blend + Zn (14:28:14), a basal fertiliser which is commonly applied in cereals such as maize (*Zea mays* L) was applied just below the weed seed during planting at 5 g per pot. This was done so as to expose the weed to normal field conditions. Each plastic pot containing the chaffed sorghum stover was filled up with water. The rate of water flow from the plastic pots was observed for the first 30 minutes and recorded. The leaves let out 450 ml, stalks 400 ml, heads 350 ml, entire plant 403 ml and the blank allowed 500 ml. The average temperature in the glasshouse was 28°C. Watering intervals afterwards were dependant on the need as some of the plant parts drained faster than the others. The herbicide atrazine herbicide was applied as the positive control, and the suspended pots above the atrazine-treated pots had no sorghum stover. The atrazine herbicide containing 50.00 atrazine technical, and 50.00 other ingredients was supplied locally by CureChem Overseas (Pvt) Ltd., Msasa, Harare, Zimbabwe. The herbicide was purchased from Ranchlate Agricultural Supplies and Hardware in Westgate, Harare. It was applied at a normal field application rate of 2 litres per hectare using a 16 Litre Jacto HD-400 Industrial knapsack sprayer fitted with a flat fan nozzle.

### 3. Data collection and analysis

Data on percent weed emergence was calculated by dividing the number of emerged seedlings by the total number of seeds that were planted. Height was measured using a measuring tape. Leaf area was measured using the grid counting method (Igathiathane et al., 2006). A total of four fresh leaves were collected from each pot and they were traced onto a graph paper in order to obtain the leaf area. The numbers of boxes contained within the traced leaves were used to determine the leaf area in cm². The experiment was terminated 28 days after weed emergence. To measure dry weight, five randomly selected plants from each pot were cut at the ground level and placed in brown paper bags and oven dried at 80°C for 72 hours. Dry weight was measured using a digital American Weigh Scale (AWS) LB-3000. Data were checked to determine normality of distribution using the Shapiro–Wilk test. Data were subjected to analysis of variance using GenStat Release 14.1. The treatment standard error of differences were used to separate treatment means at the 5% level of significance.

### 4. Results and discussion

Results (Table 1) show effect of sorghum plant part as source of aqueous extract, and atrazine on % emergence, leaf area, height and dry weight of *B. pilosa*. Atrazine significantly inhibited percentage emergence (*P* < 0.05) and leaf area (*P* < 0.001) of *B. pilosa* compared to leachates from all

| Sorghum plant part | % emergence | Leaf area | Height (cm) | DW (g plant⁻¹) |
|--------------------|-------------|-----------|-------------|----------------|
| Heads              | 92.5⁰       | 27.0⁰     | 6.79        | 0.90⁰          |
| Stalks             | 95.0⁰       | 24.0⁰     | 6.06        | 0.65⁰          |
| Leaves             | 97.5⁰       | 26.0⁰     | 5.45        | 0.65⁰          |
| Stalks + leaves    | 87.5⁰       | 40.2⁰     | 5.83        | 0.60⁰          |
| No sorghum herbage (negative control) | 100.0⁰ | 66.0⁰ | 5.93 | 0.90⁰ |
| Atrazine (positive control) | 55.0⁰ | 14.0 ⁰ | 5.33 | 2.0 ¹ |
| P value            | *P* < 0.05  | *P* < 0.001 | *P* > 0.05  | *P* < 0.05 |
| ± s.e.d.           | 5.03        | 10.39     | 0.718       | 0.1155         |

Sorghum stalks, leaves and sorghum stalks combined with leaves significantly (*P* < 0.05) reduced dry weight of *B. pilosa* compared to treatments with heads, atrazine and no sorghum herbage.
sorghum plant parts and the negative control. There was no significant effect (P > 0.05) of sorghum plant part as source of aqueous extract, and atrazine on height of B. pilosa.

The result that aqueous extracts from sorghum herbage did not inhibit percentage emergence of B. pilosa could suggest that the allelopathic compounds in the herbage have limited effect on this growth parameter of the weed. The result that atrazine significantly reduced emergence and leaf area of B. pilosa compared to sorghum herbage was not surprising. The herbicide controls broadleaf and grass weeds (Williams et al., 2010). However, there was no total suppression of emergence of B. pilosa by atrazine, possibly suggesting that the weed is tolerant to the herbicide. The low level of suppression of B. pilosa emergence and leaf area by sorghum leachates could suggest that there was little leaching of water soluble allelopathic compounds from the herbage. Inderjit (2001, 2005) notes that microorganisms influence the biological activity, or influence allelopathic expression of allelochemicals. It could therefore be possible that lack of allelopathic suppression of emergence, leaf area and height of B. pilosa was due to absence of activation of the allelopathic compounds by microorganisms.

The result that sorghum stalks, leaves and sorghum stalks combined with leaves significantly reduced dry weight of B. pilosa compared to treatments with heads, atrazine and no sorghum herbage may suggest that there is high concentration of water soluble allelopathic compounds in sorghum leaves and stalks compared to the heads. Weston et al. (2013) reported that most water soluble allelopathic compounds produced by sorghum are found in leaves. Results of the current study seem to concur with findings by Ben-Hammouda et al. (1995) who tested the phytotoxicity of extracts from sorghum plant components on wheat, and established that stems, leaves and roots had greater inhibitory effect compared to whole grains and glumes. The leaves, stalks and leaves combined with stalks as sources of aqueous extract significantly reduced dry weight of B. pilosa compared to the heads, suggesting that these sorghum plant parts produced higher quantities of water soluble allelopathic compounds compared to the heads. The leaves, stalks and leaves combined with stalks did not differ significantly in suppressing dry weight of B. pilosapossibly because the water soluble allelopathic compounds were more or less uniformly distributed in stalks and leaves. Atrazine is a photosystem II inhibitor, and it causes irreversible damage to plant cells. Under normal circumstances, it is supposed to inhibit photosynthesis by binding to the D1 proteins of the photosystem II complex in chloroplast thylakoid membranes. Binding at the D1 proteins causes fixation of CO₂ and production of energy required for plant growth to stop (Al-Khatib, 2020). Atrazine can cause oxidative degradation of lipids, pigment and protein degradation in susceptible plants (Wang et al., 2018). By inhibiting photosynthesis, atrazine should reduce photoassimilates required for plant growth and development. In a study that examined the ecological responses of periphyton dry mass and epithecal diatom community structure for different atrazine and temperature scenarios, Chakandinakira et al. (2019) attributed low dry mass to photosynthetic inhibition caused by atrazine treatments. The result that in the current study, atrazine did not reduce dry weight of B. pilosa can suggest that the weed has tolerance to the herbicide. Such tolerance could have been caused by repeated exposure of B. pilosa populations to atrazine resulting in selection for resistance (Singh et al., 2018). Tolerance of B. pilosa to atrazine has been reported in other studies (Takano et al., 2016).

The result that percent emergence, leaf area and height of B. pilosa were measured independently, and were not significantly suppressed by leachates from sorghum herbage, may not suggest that there was totally no allelopathic inhibition of the weed. It is possible that there could have been suppression occurring in small magnitudes, which cumulatively contributed to dry weight suppression. As argued by Ribeiro (2011), analysing parameters used to infer allelopathic activity such as percent and average time of seed germination individually, neglects the effect of cumulative effects of the tested agent. Ribeiro proposes that small differences in parameters used to infer allelopathic effect may pass undetected if they are analysed separately where the number of replicates is small. Dry weight suppression that was
observed in the current study might have been caused by the cumulative influence of the minute allelopathic effects on emergence, leaf area and height.

In the current study, the combination of leachates from sorghum stalks and leaves was more effective in suppressing emergence, height and dry weight of B. pilosa though the effectiveness was not significant. Although sorghum leaf and stem tissues produce the allelochemical dhurrin (β-D-glucopyranosloxy-(S)-p-hydroxymandelontrile) (Pushpa et al., 2019), it has been established that during sorghum growth, stalks produce higher levels of the allelochemical dhurrin compared to leaves because the site of dhurrin synthesis shifts from leaves to stem during sorghum development (Busk & Møller, 2002). In contrast, the content of phenolic acids tends to decrease in shoots as the sorghum plant grows. In a study that evaluated phenolic compounds in sorghum leaf extracts and their effects on weed control, Won et al. (2013) established that amounts of three phenolic acids identified in extracts of sorghum leaves (p-hydroxybenzoic acid, p-coumaric acid and trans-cinnamic acid) varied significantly with growth stage and decreased in shoot with increasing age of sorghum plants. The water soluble allelochemicals in the leachates were possibly phenolic acids contributed largely by leaves, and dhurrin leached from the stalks. The enhanced allelopathic effect from the leaves and stalks that was observed in the current study might have been caused by the joint action of mixtures of dhurrin and simple phenolic acids. Joint action of mixtures of allelochemicals has been reported to cause allelopathic activity, especially under field conditions, by other scientists (Einheilig, 1995; Inderjit, 2003, 2005; Lydon et al., 1997; Weston & Duke, 2003).

5. Conclusions
The study sought to evaluate potential allelopathic effects of Sorghum bicolor leachates on Bidens pilosa emergence and growth. From the study, it is concluded that heads, stalks, leaves and leaves combined with stalks from mature IS9456 have limited allelopathic effect on the emergence of Bidens pilosa compared to atrazine. However, stalks, leaves and leaves combined with stalks can suppress biomass of Bidens pilosa possibly due to high concentrations of water soluble allelopathic compounds. Atrazine can provide early suppression B. pilosa, while allelopathic leachates from sorghum leaves, stalks and leaves combined with stalks can suppress B. pilosa in later growth stages by reducing weed biomass.

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