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REDUCED ATRAZINE DOSES COMBINED WITH SORGHUM AQUEOUS EXTRACTS INHIBIT EMERGENCE AND GROWTH OF WEEDS

Tibugari H^{1*}, Chiduza C¹, Mashingaidze AB² and S Mabasa³



Handsen Tibugari

*Corresponding author email: htibugari@yahoo.com.au

¹Dr Handsen Tibugari (PhD Crop Science, University of Fort Hare), Department of Agronomy, Faculty of Science and Agriculture, University of Fort Hare, Alice 5700, South Africa

¹Professor Cornelius Chiduza (PhD Crop Science, University of Zimbabwe), Chairperson & Lecturer, Department of Agronomy, Faculty of Science and Agriculture, University of Fort Hare, Alice 5700, South Africa

²Professor Arnold Bray Mashingaidze (PhD Crop Weed Ecology, Wageningen University, The Netherlands), Chairperson & Lecturer, Department of Crop Science and Post-Harvest Technology, School of Agricultural Sciences and Technology, Chinhoyi University of Technology, Chinhoyi, Zimbabwe

³Dr Stanford Mabasa, PhD Ecological Plant Physiology (University of Sheffield), Lecturer, Department of Crop Science, Faculty of Agriculture, University of Zimbabwe, Harare, Zimbabwe

ABSTRACT

Combining low doses of herbicides with allelopathic plant extracts subject weeds to different mechanisms of action, which reduces herbicide resistance. The effects of reduced atrazine doses combined with sorghum aqueous extracts (*sorgaab*) from sorghum accessions IS9456, IS22320 and Mahube on emergence and growth of *Bidens pilosa* and *Eleusine indica* were evaluated in a greenhouse experiment at the University of Zimbabwe in 2017. Two experiments were set up as a 3 × 5 factorial arrangement in a completely randomized design testing sorghum varieties as sources of *sorgaab*, and five atrazine-*sorgaab* mixtures (100% *sorgaab*, 10% of the label recommended dosage (LRD) of atrazine for maize + 90% *sorgaab*, 30% LRD atrazine + 70% *sorgaab*, 100% LRD atrazine, and untreated check). Percent emergence, height, and total chlorophyll content in leaves significantly decreased ($P < 0.001$) as influenced by sorghum variety in the order IS22320 > Mahube > IS9456, for *B. pilosa* and *E. indica*. Sorghum variety significantly ($P < 0.001$) affected dry weight of *B. pilosa* similarly to other parameters but did not significantly ($P > 0.05$) affect dry weight of *E. indica*. Percent emergence, height, total chlorophyll content and plant dry weight significantly ($P < 0.001$) decreased in the order untreated control > 100% *sorgaab* > 10% LRD atrazine + 90% *sorgaab* > 30% LRD atrazine + 70% *sorgaab* > 100% LRD atrazine. There were significant ($P < 0.05$) effects of sorghum variety as source of *sorgaab* × atrazine-*sorgaab* mixture interactions on *B. pilosa* emergence and height and *E. indica* height. There is potential to exploit sorghum allelopathy using aqueous extracts alone, and in mixture with reduced doses of atrazine in controlling certain weeds. However, the allelopathic efficacy of *sorgaab* was dependent on sorghum variety. The sorghum variety IS9456 possibly produces high amounts of water soluble allelochemicals, making it a suitable candidate for use in integrated weed management.

Key words: Herbicides, reduced dose, sorghum, allelopathy, *Eleusine indica*, *Bidens pilosa*

INTRODUCTION

Herbicides are an integral part of weed management [1]. Their usage is reported to be increasing worldwide, including in Africa owing to rising rural-urban migration, which is causing scarcity of labour for hand weeding [2, 3, 4]. However, the numerous advantages of chemical weed control have resulted in over-reliance on herbicides in field crops [5] causing a number of challenges that include unintentional damage to non-target vegetation, environmental and human health risks [6]. Residual effect and soil persistence of some herbicides [7], such as atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) [8] can reduce crop establishment [9]. Atrazine can be detected in the soil more than two decades after its last application [10]. Due to its ability to persist and contaminate water bodies, it has been banned in some countries [11-14]. Repeated exposure of weed populations to atrazine can lead to resistance [15], threatening the benefits of chemical weed control [16].

Reduced herbicide doses can be combined with allelochemicals for sustainable weed management [17]. Reduced herbicide dosages reduce the risk of carry-over phytotoxicity problems to susceptible crops in a rotation [18] and are less liable to leaching and contamination of underground water sources as compared with full label rates. Reduced herbicide dosages reduce the aggregate cost of the herbicide option and render the use of herbicides more attractive to smallholder farmers [19].

Allelochemicals produced by sorghum can inhibit emergence and seedling growth of susceptible weed species [20]. A study by Cheema *et al.* [21] demonstrated that herbicide dose could be reduced by 50 to 60 percentages but still achieve good results when combined with sorghum (*Sorghum bicolor* L. Moench) allelochemicals in wheat (*Triticum aestivum*) or maize (*Zea mays*).

Bidens pilosa (L.) and *Eleusine indica* (L.) Gaertn. are important weeds of arable lands. *E. indica* features in the list of top 10 worst agronomic weeds globally [22] and is considered a serious weed in at least 42 countries [23]. In Zimbabwe, Chivinge [24] described the two weeds as aggressive, due to their fast and vigorous early growth which makes them highly competitive against crops. The weeds have also developed resistance to herbicides in different parts of the world. For example, Takano *et al.* [25] made the first report of *B. pilosa* multiple resistance to atrazine and imazethapyr. The objective of this study was to test how reduced atrazine doses combined with sorghum aqueous extracts affect emergence and early growth of *E. indica* and *B. pilosa*.

MATERIALS AND METHODS

Study site and preparation of sorghum herbage

The study was conducted in September 2017 in a greenhouse in the Department of Crop Science, Faculty of Agriculture at the University of Zimbabwe (17.7850° S, 31.0546° E). Herbage of three sorghum accessions, namely IS9456 (a South African landrace), Mahube (an open pollinated variety from Botswana) and IS22320 (a landrace sorghum from Botswana) was used to prepare sorghum water extracts. These three sorghum accessions produced the highest amount of sorgoleone (584.7 µg mg⁻¹ of root fresh weight), low sorgoleone (17.38 µg mg⁻¹ of root fresh weight) and no

sorgoleone ($0.0 \mu\text{g mg}^{-1}$ of root fresh weight), respectively in a study that quantified sorgoleone in 353 sorghum accessions from southern Africa [26]. Herbage was obtained from mature sorghum that was at the late dough stage of growth. The sorghum had been planted on 24 February 2017 on a medium grained sandy clay loam soil at Panmure Experiment Station ($31^{\circ}47'E$ and $17^{\circ}35'S$) in Shamva, Zimbabwe. The soil type at the station is classified as Chromic Luvisols (Zimbabwean classification) or Rhodexeralf Alfisols (USDA classification) [27]. 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. The crop was top dressed with Ammonium Nitrate (34.5% N) at a rate of 150 kg ha^{-1} at four weeks after crop emergence. Weeds were controlled through hand hoeing in the second and fifth weeks after crop emergence. The crop was harvested at the hard dough stage. In order to collect the roots, a soaking irrigation was applied. Prior to harvesting, sorghum received a soaking overhead irrigation of 35 mm followed by carefully digging up whole plants in order to retain most of the roots. The harvested plants were taken to the Department of Crop Science, University of Zimbabwe, gently washed with a light shower to remove excess soil from the roots, and left to dry under shade at $25^{\circ}\text{C} \pm 2$, for 14 days. The dried plant material was chopped into 2-3 cm pieces using a chopping knife. To extract, the chopped sorghum herbage was soaked in distilled water for 24 hours at room (23°C) temperature [28]. After 24 hours, the herbage-water mixtures were filtered using a funnel fitted with a Whatman No. 2 filter paper.

Experimental design

Experiments were designed as 3×5 factorials with three sorghum varieties (IS9456, IS22320 and Mahube) obtained from the regional International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Matopos, Bulawayo, Zimbabwe, as sources of sorgaab, and four atrazine-sorgaab mixtures (100% sorgaab, 10% atrazine + 90% sorgaab, 30% atrazine + 70% sorgaab, 100% atrazine, and a check in which only distilled water was applied). The 30% atrazine dose was used by Khan *et al.* [29], in a study which examined the effect of allelopathic plant water extracts combined with $\frac{1}{2}$, $\frac{1}{3}$ and $\frac{1}{4}$ doses of atrazine on *Trianthema portulacastrum* L. in maize. In our study, we aimed to test if reducing the atrazine dose further to 10%, and combining the atrazine with sorghum aqueous extracts could control weeds. Atrazine, was supplied locally by CureChem Overseas (Pvt) Ltd., Msasa, Harare, Zimbabwe and was purchased from Ranchlate Agricultural Supplies and Hardware in Westgate, Harare. The effects of sorgaab from the three sorghum varieties and the atrazine-sorgaab mixture on the emergence and growth of *E. indica* and *B. pilosa* was evaluated. Each weed species was treated as a separate experiment. The pots were arranged in a completely randomized design (CRD) with six replicates for each treatment.

Preparation of planting media and planting

A red fersiallitic clay soil classified as Harare 5E.2 according to the Zimbabwe soil classification, and rhodustalf according to USDA classification [27] was used. The soil was obtained from a fallow field overlooking the greenhouses in the Department of Crop Science. Before the start of the experiment, soil samples were collected and sent to the Department of Research and Specialist Services, Harare, Zimbabwe, for analysis.



The soil was sterilised in an oven at 110 °C for 3 days in order to kill weed seeds. Clay pots measuring 15 cm diameter and 13 cm high were filled with 1.65 kg of the soil. Weed seeds that were obtained from the Weed Research Unit at Henderson Research Station (17°35' S, 30°38' E) in Mazowe were surface sterilised by immersion in 0.6% sodium hypochlorite solution before planting. The label recommended dose of atrazine (4500 ml in 250 l of water/ha) [30] was diluted with distilled water. In the current study, 33.75 ml of atrazine was mixed with 1500 ml of water to make a 100% atrazine solution. The herbicide-sorgaab mixtures were applied pre-emergence, soon after planting of the weed seeds. Spraying was done using a 2-litre hand-held Berthoud sprayer (Taurus Spraying Systems Pvt. Ltd., Amby, Harare, Zimbabwe) fitted with a calibrated jet nozzle at a volume of 250 litres ha⁻¹. After every two days, 150 ml of tap water was applied to the pots. The average greenhouse temperature for the duration of the experiment was 25 ± 2°C.

Data collection and analysis

Data on percentage emergence, plant height, leaf chlorophyll content and dry weight were collected. Weed seedling emergence was measured seven days after sowing (DAS) and after every two days thereafter until Day 14. Weed seedling emergence data at Day 14 were used to calculate percentage emergence. Weed seedling height was measured using a measuring tape. Leaves at the top of the canopy usually contain more chlorophyll than those near the ground [31]. The method used by Peng *et al.* [32] was used to measure leaf chlorophyll concentration with modifications. For each treatment two plants with fully expanded leaves were identified. On each plant, two uppermost fully expanded leaves were then selected. Three Soil Plant Analysis Development (SPAD) readings were taken around the midpoint of each leaf blade on one side of the midrib [32]. The SPAD readings were averaged to represent the mean SPAD value of each treatment. The experiment was terminated 28 DAS. To measure dry weight, plants were cut at the ground level and placed in brown paper bags and oven dried at 80 °C for 72 hours. Data were evaluated to determine whether it was normally distributed using the Shapiro–Wilk test. Data were subjected to analysis of variance using GenStat Release 14.1 (VSN International 2011). The treatment and interaction standard error of differences were used to separate treatment means at the 5% level of significance.

RESULTS AND DISCUSSION

Results (Table 1) show effect of sorghum variety as source of aqueous extract (sorgaab), atrazine-sorgaab mixture and their interaction on % emergence, height, chlorophyll and dry weight of *E. indica*. Sorghum water extracts from IS9456 significantly ($P<0.001$) reduced percent emergence of *E. indica* by 54.7%. Percent emergence was reduced by 50.8% and 47.6% by sorgaab from Mahube and IS22320, respectively. Percent emergence of *E. indica* significantly ($P<0.001$) decreased as atrazine content was increased. There was no sorghum variety × atrazine-sorgaab mixture interaction on percent emergence.

There was a significant ($P<0.05$) sorghum accession × atrazine-sorgaab mixture interaction on height of *E. indica* (Fig. 1).



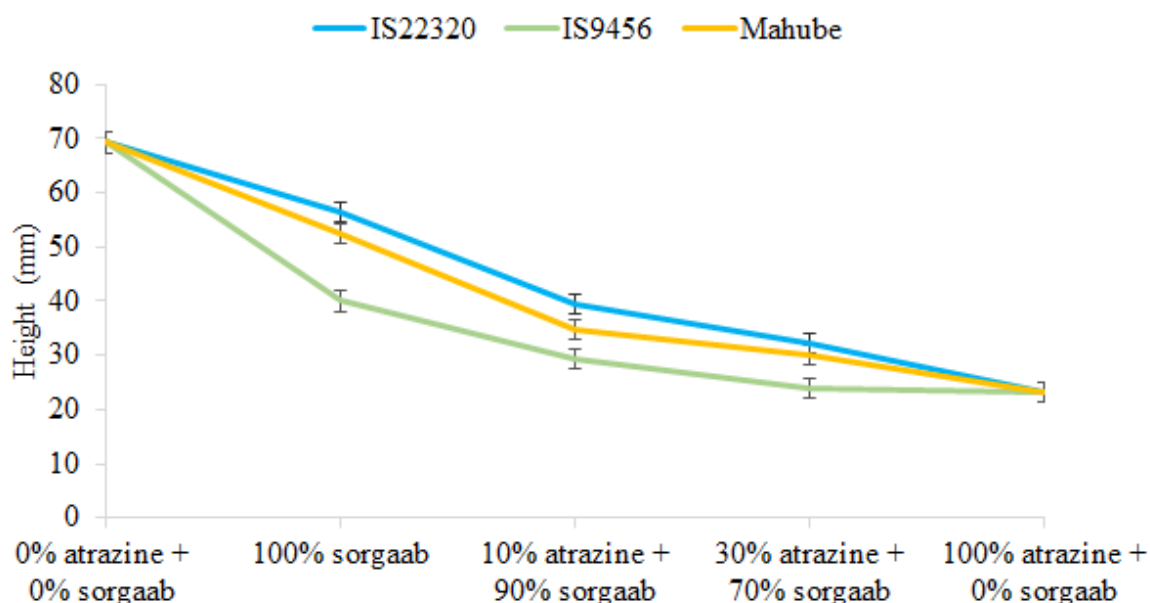


Figure 1: Effect of atrazine-sorgaab mixtures on height of *E. indica*.

Bars indicate standard error

The interaction shows that crude aqueous extracts and sorghum aqueous extracts – atrazine mixtures from the sorghum accession IS9456 were more effective in reducing *E. indica* height than from the sorghum varieties IS22320 and Mahube. The 10% atrazine + 90% sorghum aqueous extracts and 30% atrazine + 70% sorgaab from the sorghum accession IS9456 reduced *E. indica* height similarly to the 100% atrazine. The 10% atrazine + 90% sorghum aqueous extracts and 30% atrazine + 70% mixtures of sorghum aqueous extracts from IS22320 and Mahube were significantly ($P < 0.001$) less effective than 100% atrazine in reducing *E. indica* height. Aqueous extracts from IS9456 significantly ($P < 0.001$) reduced leaf chlorophyll content of *E. indica* compared to extracts from Mahube and IS22320, respectively (Table 1). There was no significant sorghum accession \times atrazine-sorgaab mixture interaction on leaf chlorophyll content ($P > 0.05$) (Table 1). Sorghum accession as source of aqueous extracts did not significantly ($P > 0.05$) reduce dry weight of *E. indica* (Table 1). The 30% atrazine + 70% sorgaab mixture significantly reduced *E. indica* dry weight ($P < 0.001$) compared to 100% sorgaab and 10% atrazine + 90% sorgaab (Table 1). There was no significant ($P > 0.05$) sorghum accession \times atrazine-sorgaab mixture interaction on *E. indica* dry weight (Table 1).

There was a significant ($P < 0.05$) sorghum accession \times atrazine-sorgaab mixture interaction effect on percent emergence of *B. pilosa* (Fig. 2). The interaction shows that crude aqueous extracts and sorghum aqueous extracts + atrazine mixtures from the sorghum variety IS9456 were more effective in reducing *B. pilosa* emergence compared to sorghum varieties IS22320 and Mahube.

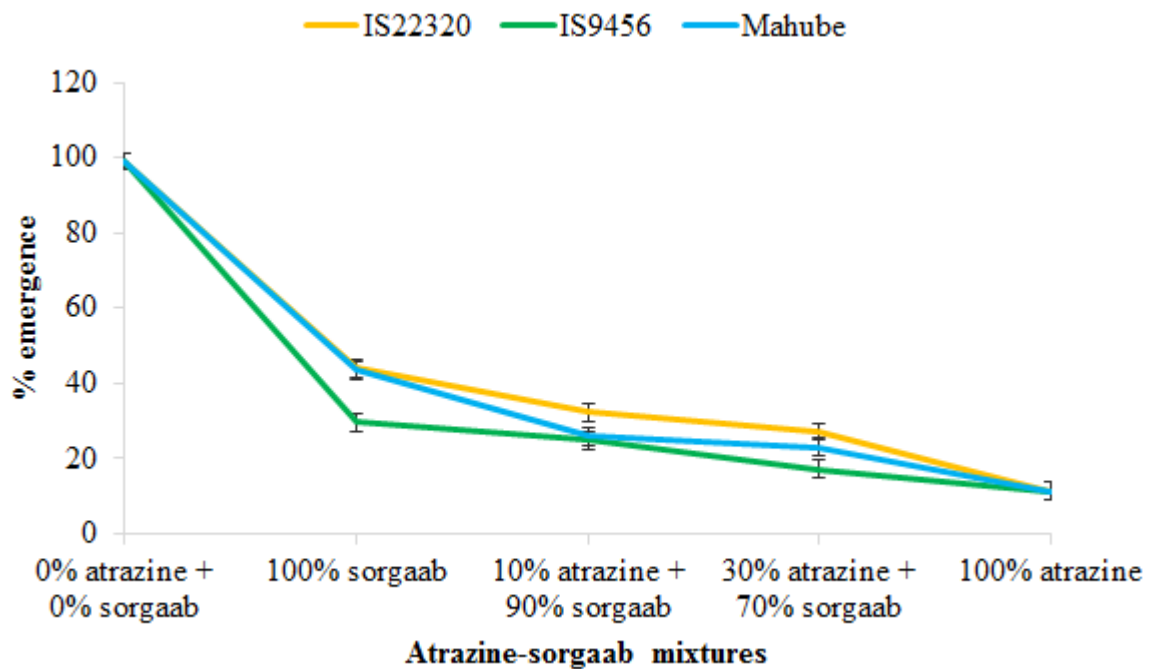


Figure 2: Effect of atrazine-sorgaab mixtures on percent emergence of *B. pilosa*.
Bars indicate standard error

The 10% atrazine + 90% sorghum aqueous extracts from the sorghum variety IS9456 reduced *B. pilosa* emergence similarly to the 100% sorgaab while the same mixture with sorghum aqueous extracts from IS22320 and Mahube were significantly less effective than 100% sorgaab in reducing *B. pilosa* emergence (Fig. 2).

There was a significant ($P < 0.05$) sorghum accession \times atrazine – sorgaab mixture interaction effect on the height of *B. pilosa* (Fig. 3). The interaction shows that crude aqueous extracts and sorghum aqueous extract – atrazine mixtures from the sorghum variety IS9456 were more effective in reducing *B. pilosa* height compared to sorghum varieties IS22320 and Mahube. The 10% atrazine + 90% sorghum aqueous extracts from the sorghum variety IS9456 reduced *B. pilosa* height similarly to the 100% sorgaab while the same mixture with sorghum aqueous extracts from IS22320 and Mahube were significantly less effective than 100% sorgaab in reducing *B. pilosa* height (Fig. 3).

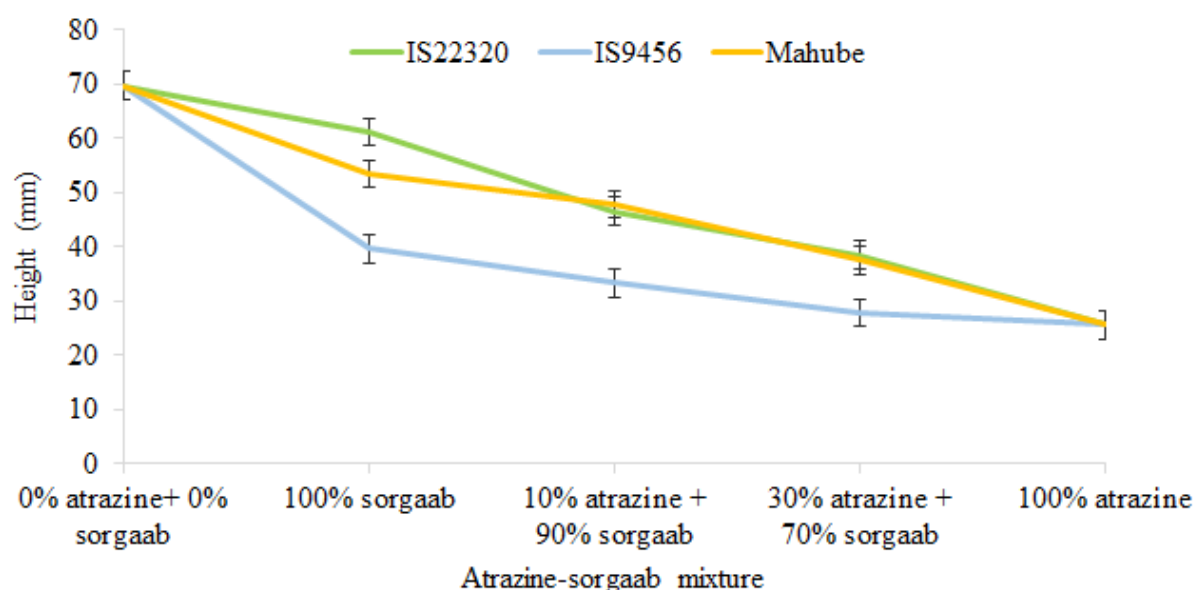


Fig. 3: Effect of atrazine-sorgaab mixtures on height of *B. pilosa*.

Bars indicate standard error

Aqueous extracts from IS9456 significantly ($P < 0.001$) reduced *B. pilosa* leaf chlorophyll content compared to aqueous extracts from IS22320 and Mahube (Table 2). The 30% atrazine + 70% sorgaab mixture significantly reduced *B. pilosa* leaf chlorophyll content compared to the 10% atrazine + 90% sorgaab and the sole application (100%) of sorgaab 100% sorgaab. There was no significant sorghum variety \times atrazine-sorgaab mixture interaction ($P > 0.05$) on *B. pilosa* leaf chlorophyll content. Aqueous extracts from IS9456 significantly ($P < 0.001$) reduced *B. pilosa* dry weight compared to extracts from Mahube and IS22320. The 30% atrazine + 70% sorgaab mixture significantly reduced *B. pilosa* dry weight compared to the sole application (100%) of sorgaab. There was no significant sorghum variety \times atrazine-sorgaab mixture interaction effect ($P > 0.05$) on *B. pilosa* dry weight.

Interactions between sorghum accession \times atrazine-sorgaab mixture on height of *E. indica*, and on percent emergence and height of *B. pilosa* showed that there are differences in the effectiveness of sorgaab extract in suppressing weed emergence and growth, with sorgaab extracts from IS9456 being more efficacious than those from IS22320 and Mahube. The same effect is expressed when sorgaab is mixed with the 10% atrazine + 90% sorgaab and the 30% atrazine + 70% sorgaab equally suppressing weed emergence and growth as 100% atrazine dose. This result suggests that in addition to producing very high sorgoleone [26], IS9456 could also be producing high amounts of water soluble allelochemicals, making the sorghum accession a suitable candidate for use in weed management through allelopathy.

The results show that the 10% atrazine + 90% sorgaab and the 30% atrazine + 70% sorgaab combinations are as effective as the label recommended dosage of atrazine and therefore, there may be potential of reducing atrazine dosages when sorgaab from

IS9456 is combined with atrazine for the control of *E. indica* and *B. pilosa*. Sole applications of sorgaab and atrazine inhibited weed emergence and growth, confirming the potency of atrazine and sorgaab against weeds. When applied alone, sorgaab from IS22320, IS9456 and Mahube significantly reduced emergence and growth of *E. indica* and *B. pilosa* compared to the negative control. Sorgaab alone significantly reduced *E. indica* and *B. pilosa* height compared to 0% atrazine + 0% sorgaab, suggesting that the aqueous extracts from the three sorghum varieties contain growth inhibiting phytotoxic compounds. Combining 30% of the recommended label dose of atrazine with 70% sorgaab reduced *E. indica* and *B. pilosa* height compared to sorgaab alone, suggesting that sorgaab and atrazine had synergistic effects.

The label recommended dosage of atrazine caused the highest inhibition of weed emergence, height, chlorophyll content and dry weight of *E. indica* and *B. pilosa*. This was expected. Atrazine, which is mainly applied for the pre- and post-emergence control of broadleaf weeds [33], blocks the electron flow in photosystem II (PSII) [34], leading to the inhibition of carbon dioxide assimilation [35] and the generation of large amounts of reactive oxygen species [36].

Reduction in chlorophyll content in the 100% label recommended dose of atrazine was perhaps not surprising. It has been reported by other scholars that reactive oxygen species destroy pigment molecules when they are generated after application of atrazine [15]. In the current study, the 100% sorgaab averaged across sorghum varieties also caused reduction in chlorophyll content. It is possible that allelochemicals in sorgaab might have reduced chlorophyll content of *E. indica* and *B. pilosa*. A study by Khaliq *et al.* [37] also demonstrated that chlorophyll content of weeds such as *Cyperus rotundus* L. and *C. eragrostis* Lam. was reduced by sorghum aqueous extracts. As suggested by Dakshini and Dakshini [38], phenolic acids in sorgaab might have caused a reduction in chlorophyll content of *E. indica* and *B. pilosa*. Phenolic acids such as ferulic, *p*-coumaric, *p*-hydroxybenzoic, and vanillic acids, which have been implicated in sorghum allelopathy [39] inhibited chlorophyll content and photosynthesis of test plants in other studies [40].

Compared to *B. pilosa*, *E. indica* generally appeared to tolerate atrazine and sorgaab, a result that shows that atrazine has greater inhibitory effects on broadleaf weeds than grasses. Reports that some grass weeds tolerate atrazine applications have been made by other researchers. Popov and Cornish [41] tested the response of four grasses [*Pennisetum clandestinum* Hochst, *Stipa aristiglumis* F. Muell., *Themeda australis* (R. Br.) Stapf, *Danthonia* spp. (L.)] to photosynthetic inhibition by the herbicide atrazine. Their study established that all the four weed species showed signs of quick recovery within 7 to 21 days to normal photosynthetic activity. A study on the effects of reduced dosages of atrazine and nicosulfuron on weed control in maize by Mashingaidze [19] showed that *E. indica* was one of the four weed species that tolerated atrazine. Combining sorghum aqueous extracts with atrazine may possibly reduce *E. indica* tolerance to the herbicide.

The study showed that reduced doses of atrazine (10% and 30% of atrazine dosage) combined with aqueous extracts from the sorghum variety IS9456 were as effective as

100% atrazine in suppressing weeds. In wheat, Khaliq *et al.* [42] found that the mixture of allelopathic plant water extracts from sorghum, sunflower [*Helianthus annuus* (L.)] and mulberry [*Morus rubra* (L.)], combined with reduced doses of iodo + mesosulfuron gave weed control equal to the recommended dose of the herbicide. The results of the current study imply that atrazine use could be reduced by 70 to 90% by combining the allelopathic aqueous extracts from IS9456 with lower doses of the herbicide. When low doses of atrazine combined with allelopathic aqueous extracts are included in an integrated weed management system, farmers will manage weeds in an ecologically sustainable way. This may reduce the risk of herbicide resistance, shifts in weed populations and environmental pollution. By applying less herbicide, farmers reduce input costs, which may translate to increased profits.

Aqueous root extracts from some sorghum accessions with high inhibitory effects on weeds can be used in integrated weed management exploiting allelopathy and atrazine. However, while integrating sorghum aqueous extracts with reduced doses of herbicide can control certain weed species, some crops may also be negatively affected by the sorghum allelochemicals [43]. For example, a continuous sorghum cropping system can result in the accumulation of phytotoxins, which can cause adverse effects on sorghum itself [17] through autotoxicity [44]. It was established that hot water extracts of decomposed sorghum residues inhibit root growth of wheat that follows sorghum in crop rotation [45]. Additionally, extracts of sorghum residues reduced germination and growth of rice, wheat, and corn [46], suggesting that farmers must take precautions that reduce phytotoxicity of these extracts on food crops in cropping systems in which the extracts are used.

CONCLUSION

The study shows that there is potential to exploit sorghum allelopathy alone and in mixture with reduced doses of atrazine in weed management. However, the allelopathic efficacy of sorgaab was dependent on sorghum variety. The sorghum variety IS9456 possibly produces high amounts of water soluble allelochemicals, making it a suitable candidate for use in integrated weed management. Future studies should focus on extracting, characterizing and testing the allelochemicals produced by allelopathic sorghum varieties for bio-herbicide development.

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Conflicts

The authors declare no conflict of interest.



Table 1: Effect of sorghum variety and sorgaab-atrazine mixture on emergence and growth of *E. indica*

	% emergence	Height (mm)	Chlorophyll (nmol/cm ²)	Dry weight (g plant ⁻¹)
<i>Sorghum variety</i>				
IS22320	52.37 a	42.74 a	32.63 a	8.25
IS9456	45.34 c	31.11 c	29.39 c	7.91
Mahube	49.21 b	39.11 b	31.26 b	8.64
P value	P<0.001	P<0.001	P<0.001	P>0.05
±s.e.d	0.955	1.066	0.446	0.477
<i>Atrazine-sorgaab mixture</i>				
0% atrazine+0% sorgaab	99.33 a	69.17 a	37.15 a	14.43 a
100% atrazine	29.55 e	20.33 e	18.72 e	4.95 e
100% sorgaab	61.78 b	49.67 b	35.09 b	9.98 b
10% atrazine+90% sorgaab	49.51 c	34.46 c	30.72 c	8.67 c
30% atrazine+70% sorgaab	35.63 d	28.83 d	27.48 d	6.15 d
P value	P<0.001	P<0.001	P<0.001	P<0.001
±s.e.d	0.955	1.066	0.446	0.477
<i>Sorghum variety x atrazine-sorgaab mixture interaction</i>				
P value	P>0.05	P<0.05	P>0.05	P>0.05
±s.e.d	1.654	1.846	0.772	0.826

Means followed by the same lower-case letters in the same column are not significantly different

Table 2: Effect of sorghum accession and sorgaab-atrazine mixture on emergence and growth of *B. pilosa*

	% emergence	Height (mm)	Chlorophyll (nmol/cm ²)	Dry weight (g plant ⁻¹)
<i>Sorghum variety</i>				
IS22320	34.40 a	48.72 a	31.55 b	4.827 a
IS9456	23.78 c	33.61 c	30.96 b	3.546 c
Mahube	30.80 b	46.28 b	33.42 a	4.241 b
P value	P<0.001	P<0.001	P<0.001	P<0.001
±s.e.d.	1.348	1.493	0.568	0.2322
<i>Atrazine-sorgaab mixture</i>				
0% atrazine + 0% sorgaab	99.00 a	69.67 a	36.80 a	12.96 a
100% sorgaab	38.97 b	51.44 b	36.61 a	5.824 b
10% atrazine + 90% sorgaab	27.63 c	42.56 c	31.71 b	3.711 c
30% atrazine + 70% sorgaab	22.37 d	34.61 d	27.61 c	3.079 d
100% atrazine	11.25 e	25.70 e	20.43 d	1.800 e
P value	P<0.001	P<0.001	P<0.001	P<0.001
±s.e.d.	1.348	1.493	0.568	0.2322
<i>Sorghum variety x atrazine-sorgaab mixture interaction</i>				
P value	P<0.05	P<0.05	P>0.05	P>0.05
±s.e.d.	2.334	2.586	0.983	0.4022

Means followed by the same lower-case letters in the same column are not significantly different

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