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Phytotoxicity of *Argemone ochroleuca* L. on Germination and Seedling Growth of *Sorghum bicolor* L. Varieties under *In vitro* Condition

[1]Meseret Muche, [2]Eyayu Molla and [1]Habtamu Teshome

[1]Department of Biology, Woldia University, Woldia, Ethiopia
[2]Department of Natural Resource Management, Bahir Dar University, Ethiopia

Abstract: *Argemone ochroleuca* L. is invasive weed with biological suppression on the neighboring crops by a phenomenon of allelopathy. This study was aimed to examine the potential allelopathic effect of *A. ochroleuca* root, stem and leaf extracts on the widely cultivated *Sorghum bicolor* varieties (*Melkam*, *Gerana* 1 and *Farmers Cultivar*). Complete randomized design was employed. The root, stem and leaf extracts were prepared at concentrations of 5%, 10 % and 15 % and Multivariate Analysis (Two way - ANOVA) was used to analyze relevant data. Extracts caused marked reduction in the seedling growth rates with increasing extraction concentrations and the greatest inhibition of radical length (0.5±0.2cm), plumule length (0.83±0.1cm), germination number (0.33±0.5), germination percent (2.2±0.4), mean germination time (1.7±2.9), vigor index (0.02±0.0) and germination energy (0.1±0.1) produced by root extracts in 15 % concentration in the farmers' cultivar followed by stem and leaf extracts. The overall results indicated the strong phytotoxic effect present in all varieties. However, comparably higher resistance to the allopathic effect of *A. ochroleuca* was observed in *Melkam S. bicolor* variety. The results suggest that integrated management option lunched to improve productivity and sustainability.

Key words: Allelopathy · Aqueous Extract · *A. ochroleuca* · Invasive Weed · *S. bicolor*

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is one of the most important cereal crops in the world next to wheat, maize, rice and barely with estimated average grain yield of 63.18 million metric tons [1]. It is grown in a wide range of agro-ecologies most importantly in the areas of intermittent moisture stresses [2, 3] and a chief cereal grain used for both human consumption and animal feed in dry areas of the semi-arid tropics of Asia and Africa [3]. The average annual national grain yield of *S. bicolor* in Ethiopia is estimated to be 3, 600, 000 metric tons [1, 4]. However, the average yield of sorghum in the north eastern of Ethiopia is even below the national yield average [4]. The major factors that account for this low yield are the abiotic and biotic factors. Among the biotic factors which contribute for the reduction of agricultural production, invasive weeds are the most serious problems with a noticeable yield-deteriorating factor in Ethiopia [5, 6]. It is agreed that in the areas where the invasive weed species occur, the productivity of the crops and forage products are declined by 40 % and 90 % respectively [7, 8], owning to the introduction of phytotoxins or allelopathic chemicals [9].

Allelopathy is a biological phenomenon in which some plants produced subsets of secondary metabolites that inhibited or benefited another plant species by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems [10-13]. Among others, *Aregemone ochroleuca* L (Papeveraceae) is an aggressive alien weed species native to Mexico [10, 14 , 15] and now widely distributed throughout the temperate and subtropical climates [16 , 17]. The plant is an annual, hermaphroditic shrub, mostly pollinated by insects, with terminal lemon yellow to whitish, narrowly elliptical flowers [16 , 18]. The *Aregemon spp* has various phytochemicals like cinnamic, benzoic acid, berberine, protopine and coptisine

Corresponding Author: Meseret Muche, Department of Biology, Woldia University, Woldia, Ethiopia.

[14, 19, 20] and phenolic compounds (*p*-hydroxy benzoic acid, salicylic acid and vanillic acid) [21]. These chemicals are principally inhibits the germination and seedling vigor of various kinds of crops and many vegetables [22]; Lentil (*Lens culinaris*) [23]; mustard, fenugreek and cucumber [24] and Rapeseed and Wheat [25] and the complex alkaloid mixture found in Aregemone spp have nematicidal and bactericidal effects [14]. Westhuizen and Mpedi [26] further noted that the *A. ochroleuca* can compete and potentially displace the wildlife in areas where it has gained dominance. Yet, it is expanding both in scope and magnitude on cultivated and grazing lands in Ethiopia mainly in Northeastern areas of the country. Although it is among the twelve noxious weeds identified in the region which severely limiting productivity; little information is available that revealed to its allelophatic effects and mechanisms to control the alien weed in the Northeastern Ethiopia.

Therefore, the present study was initiated to determine the phytotoxic effect of *A. ochroleuca* on seed germination and seedling growth of three sorghum varieties (*Melkam*, *Gerana 1* and *Farmers Cultivar*) that are widely cultivated in Northeast Ethiopia.

MATERIALS AND METHODS

Preparation of Plant Materials and Extracts: The *Argemone ochroleuca* weed was collected in Febraury 2017 from the farm lands of Gubalafto District (11°50'N and 39°36 E), Northeastern Ethiopia. The intact weed plant wrapped in polyethylene bags and kept in ice upon transfer to the laboratory. Accordingly, the intact parts of the plant were separated into leaf, stem and root and washed thoroughly with distilled water and air dried for seven days at room temperature (25°C -30°C) in the laboratory. Each of the dried plant parts (leaf, stem and root) were separately crushed in an electric blender and sieved through a 40 mm mesh screen and stored in plastic bottles at room temperature. Afterwards, 100 g of each of the pulverized parts of the weed were soaked separately in the beaker containing 1000 mL distilled water and kept in a mechanical shaker for 24 h and filtered by using Whatman No. 1 filter paper. The crude extracts formed was concentrated using a vacuum evaporator at 45°C under low pressure which used to make stock solution using distilled water and hence different concentrations were prepared (5%, 10% and 15%) in a method of dilution [27].

Experimental Design and Assayed Parameters: Complete Randomized Design (CRD) with three replications, three *Sorghum bicolor* varieties (*Melkam*, *Gerana* and *Farmers Cultivars*) × four (extract concentrations namely 0 %, 5%, 10% and 15 %) were employed in a factorial combination. The selected sorghum seeds procured from Serinka Agricultural Research Institute, Northeastern Ethiopia were first washed with distilled water to remove impurities, then sterilized with 2 % sodium hypochlorite for 2 minutes and finally rinsed with distilled water. Accordingly, fifteen randomly selected viable seeds of each variety were placed in 12 cm sterilized plastic Petri dishes lined up with double layer of whatman No. 1 filter paper and with 5%, 10% and 15% *A. ochroleuca* extract concentrations, in addition seeds treated with distilled water were used as a control. Then, the treated groups were designated and tagged as Treatment 0 (control), Treatment 1 (5%), Treatment 2 (10 %) and Treatment 3 (15 %). The Petri dishes were covered and kept inside the laboratory at room temperature. The experiment was carried out for two successive weeks and the seedling performances of the sorghum seeds were observed in every day. The parameters that were assayed are counting of the numbers of germinated seeds every day, measuring radicle and plumule length in centimeter (cm). Based on these total germination percentage (TG %), mean germination time (MGT), germination energy (GE) and vigor index (VI) were computed following the methods of [28 - 30].

$$TG (\%) = \frac{NT}{N} * 100$$

where NT, proportion of germinated seeds in each treatment for the final measurement and N: Number of seeds used in bioassay.

$$GE = \frac{X_1 +}{Y_1} \frac{X_2 - X_1 + ...}{Y_2} \frac{X_n - X_{n-1}}{Y_n}$$

where X_n is the number of germinants on the n^{th} counting date and Y_n, the number of days from sowing to the n^{th} count.

$$MGT \; \frac{\sum_{i=1}^{K} n_i t_i}{\sum_{i=1}^{k} n_i}$$

where t_i, the time from the start of the experiment to i^{th} observation and n_i is the number of seeds germinated in i^{th} time and k, the last time of germination.

$$VI = S * \sum \frac{Gt}{Dt}$$

where S is the seedling height of the seventh day, Gt is the number of germinated seeds in the t^{th} day and Dt, is the number of days from the first day the t^{th} day.

Data Analysis: Statistical difference in the growth assayed parameters of the *Sorghum bicolor* varieties by extracted concentrations were analyzed by General Linear Model of Multivariate Analysis (Two-way- ANOVA) at $p < 0.01$ and 0.05 significant level using R-software (v 3.2.3). Significance tests between the seedling growths of the varieties were performed using univariate analysis of variance (Post hoc) with least significant difference (LSD) at the 0.05 probability level [31].

RESULTS AND DISCUSSION

Effect of Aqueous Extract of *A. ochroleuca* Root on Growth of *Sorghum bicolor* L. Varieties: Root extracted concentrations of *A. ochroleuca* significantly affected the radicle length ($F2,6 = 10.6$, $P < 0.01$, $R^2 = 0.96$) plumule length ($F2,6 = 10.8$, $P < 0.01$, $R^2 = 0.95$) and germination number ($F2,6 = 4.9$, $P < 0.05$, $R^2 = 0.95$) of the selected *Sorghum bicolor* varieties. The seedling parameters were substantially reduced with increasing the extracted concentrations. Thus, among the *S. bicolor* varieties the greater inhibition of radicle length (0.5 ±0.2 cm), plumule length (0.83 ± 0.1 cm) and germination number (0.33 ± 0.5) were observed in the farmers' cultivar in 15 % leaf extract concentration than the control (Table 1). The result showed that even at lower extract concentration the root of *A. ochroleuca* has strong inhibitory effect on the seedling growth of all the sorghum varieties, which could be ascribed to the presence of phytochemicals that suppressed the plant growth hormones. Similar studies reported on other weed plant (*Trianthem aportulacastrum)* showed the inhibitory potential of root extract which was associated with the synthesis of phytochemicals in the root and their subsequent translocation into the stem and leaf [23, 32]. Westhuizen and Mpedi [26] also notified that Argemone spp have allelochemicals mainly the aromatic compounds potentially inhibits germination. In other allelopathic studies of *A. mexicana* depicted that when aqueous extracts of the plant increased there had higher degree of germination and radicle length inhibition in rapeseed and wheat varieties increased [25].

Multivariate analysis revealed a considerable reductions in percent of germination ($F2,6 = 4.9$, $P < 0.05$, $R^2 = 0.90$), MGT ($F2,6 = 4.5$, $P < 0.05$, $R^2 = 0.98$) VI ($F2,6 = 52.2$, $P < 0.01$, $R^2 = 0.90$) and GE ($F2,6 = 12.1$, $P < 0.01$, $R^2 = 0.96$) of the selected *S. bicolor* varieties within the root extract concentrations. This showed that the degree of inhibition of the growth parameters were increased within increase in the extract concentrations. Thus the most suppressed seedling growth parameters in percent of germination (2.2±0.4), mean germination time (1.7±2.9), vigor index (0.02 ±0.00) and germination energy (0.1±0.1) were observed in the farmers cultivar (Fig. 1). Conversely, comparably greater resistance to the phytotoxcity effect of *A. ochroleuca* root extract was noticed in the *Melkam* sorghum variety percent of germination (28.9±3.8), vigor index (2.3±0.6) and germination energy (1.0±0.2) and in *Gerana 1* in mean germination time (13.5±3.4) in the 15 % extract concentration (Fig. 1). This shows percent of germination reduced by 61.86 %, 62.95 % and 96.72 % in *Gerana 1 Melkam* and Farmers cultivar varieties respectively. Therefore, the inhibition effect in sorghum was found to increase with increasing concentrations of different aqueous extracts as reported elsewhere by different studies [33-35]. This could be attributed to suppressive effects of allelochemicals that modify the physiological functions of the crop [19, 36].

Effect of Aqueous Extract of *A. ochroleuca* Stem on Growth of *Sorghum bicolor* L. Varieties: Stem extract of *A. ochroleuca* affected the growth parameters of the selected *Sorghum bicolor* varieties at various extract concentrations. The result showed that there were significant effects in the seedling radicle length ($F2,6 = 4.5$, $P < 0.05$, $R^2 = 0.94$) and germination number ($F2,6 = 2.6$, $P < 0.05$, $R^2 = 0.93$) of the sorghum varieties by stem extract concentrations. The overall inhibitory effects of the radicle length (2.7±0.4cm) and germination numbers (3.3±0.3) were higher in the farmers' cultivar whilst minimal in the *Melkam* variety in 15 % extract concentration (Table 2). However, there was no significant difference in the plumule length ($F2,6 = 2.9$, $P = 0.045$, $R^2 = 0.97$) among all the three varieties and different stem extract concentrations. Regardless of the significant difference, numerically there was a decrease in plumule length of the varieties with increasing extract concentrations and the higher inhibitory effect of the extract was observed in the farmers' cultivar (2.2±0.3cm) of 15% extracts in contrast to the control (6.2±0.5cm) (Table 2). This finding is consistent with the works of [23] that indicated growth regulatory effects of stem extracts of *A. mexicana* than its phytotoxic effect like the root extracts. However, other alien weeds studies showed that aqueous stem extracts of *M. indicus, A. aspera* and *P. hysterophorus* inhibit germination and seedling length of wheat [37]; *S. bicolor* [38] and Peanut and Soybean respectively [39].

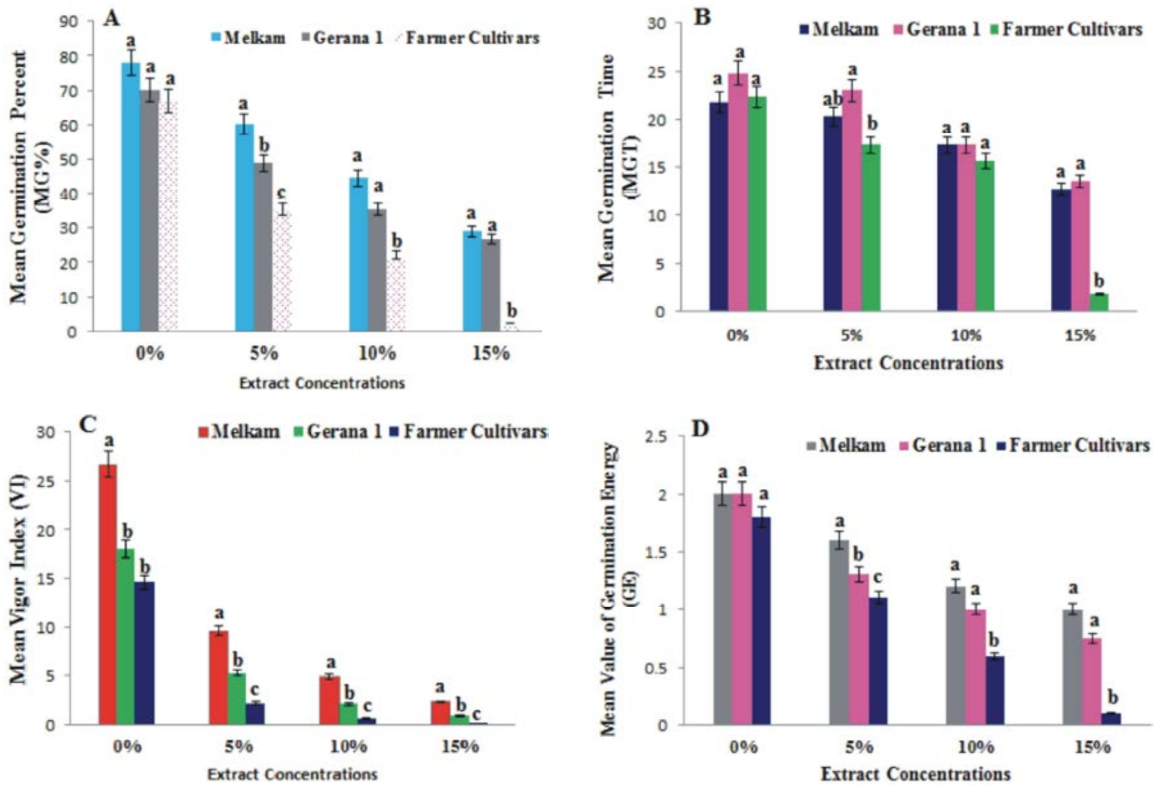

Fig. 1: The effects of *Argemone ochroleuca* L aqueous root extract on A (germination percent), B (mean germination time), C (vigor index) and D (germination energy) of the selected sorghum varieties. The same letters indicate not significant difference ($P \leq 0.05$) at each concentration among the selected varieties in Least Significant Difference (LSD)

Table 1: Seedling growth of *Sorghum bicolor* L. varieties influenced by aqueous extract of *A. ochroleuca* root (Mean±SD)

	Radicle Length (RL in cm)				Pulumule Length (PL in cm)				Germination Number (GN)			
S. bicolor Varieties	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%
Mel	9.8±0.8	8.0±0.5	6.0±0.2	3.3±0.6	8.5±0.5	5.9±0.3	3.9±0.3	2.3±0.4	12.3±0.6	9.0±1.0	6.7±0.6	4.3±0.6
G1	7.3±0.2	4.8±0.2	3.2±0.2	1.2±0.3	5.9±0.4	4.0±0.3	2.0±0.1	1.2±0.3	9.0±0.0	7.3±0.6	5.3±0.6	4.0±0.0
FC	4.2±0.2	2.2±0.3	1.2±0.3	0.5±0.2	3.3±0.6	2.0±0.1	1.0±0.01	0.83±0.1	6.7±0.6	5.3±0.5	3.3±0.5	0.33±0.5
LSD	2.1*				1.65*				1.67**			

* Significant at P<0.001; ** Significant at P<0.005; LSD; Least Significant Difference; SD, Standard Deviation; Mel, *Melkam* sorghum variety; G1, *Gerana* 1 sorghum variety; FC, Farmers cultivar; T(0, 1, 2 &3) are treatments in different extract concentrations

Table 2: Seedling growth of *Sorghum bicolor* L. varieties treated by aqueous extract of *A. ochroleuca* stem (Mean±SD)

	Radicle Length (RL in cm)				Pulumule Length (PL in cm)				Germination Number (GN)			
S. bicolor Varieties	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%
Mel	11.7±1.3	9.5±0.5	8.9±0.4	5.3±1.5	10.5±0.5	7.4±0.4	6.6±0.4	4.9±0.1	13.8±0.6	10.5±1.0	8.7±0.6	6.3±0.6
G1	8.8±0.2	6.2±0.2	5.2±0.3	3.2±0.3	7.9±0.5	5.6±0.4	4.2±0.3	3.2±0.3	10.5±0.0	8.8±0.6	6.8±0.3	6.0±0.0
FC	5.8±0.2	4.5±0.5	3.3±0.3	2.7±0.4	6.2±0.3	4.5±0.5	3.0±0.1	2.2±0.3	7.8±0.6	6.3±0.3	4.8±0.3	3.3±0.3
LSD	1.76*				1.23*				1.78**			

* Significant at P<0.001; SD, Standard Deviation; Mel, *Melkam* sorghum variety; G1, *Gerana* 1 sorghum variety; FC, Farmers cultivar;T(0, 1, 2 &3) are treatments in different extract concentrations

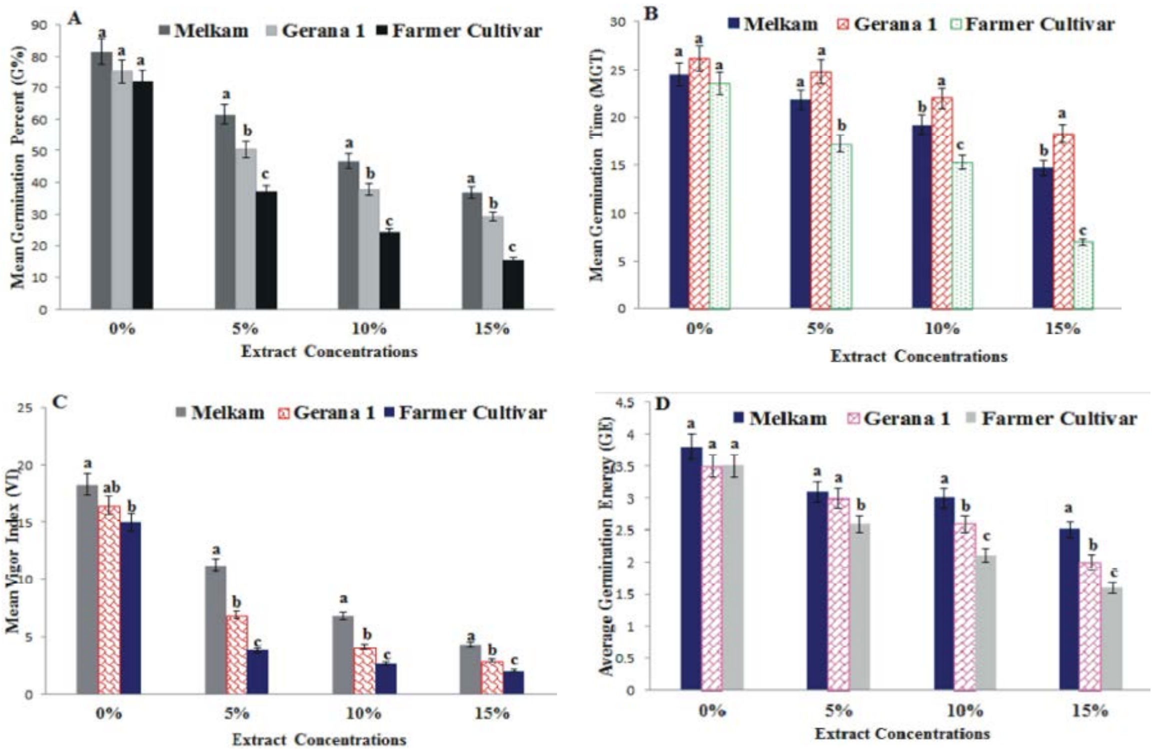

Fig. 2: The effects of *A. ochroleuca*L.aqueous stem extract on A (germination percent), B (mean germination time), C (vigor index) and D (germination energy) of the selected sorghum varieties. The same lettersindicate not significant difference ($P \leq 0.05$) at each concentration among the selected varieties in Least Significant Difference (LSD)

The germination parameters percent of germination ($F2, 6 = 3.9, P < 0.07, R^2 = 0.96$) and MGT ($F2, 6 = 3.6, P = 0.05 < R^2 = 0.8$) didn't show significant difference among the varieties of *Sorghum bicolor* when treated with A. ochroleuca stem extracts. Nonetheless, numerically there was a trend of growth parameters reduction with increasing concentrations of the stem extracts. This inhibitory effect in percent of germination (15.7±2.5) and mean germination time (7.0±1.0) was observed in the farmers' cultivar at 15 % stem extract over the control (Fig. 2). As a result the percent of germination of the varieties were reduced by 54.97 %, 61.07 % and 78.2 % in '*Melkam*', '*Gerana 1*' and 'farmers' cultivar respectively as compared to the control. This result well aligned with the study of [40] that reported insignificant changes in the germination percentage and time of germination of sesame (*Sesamum indicum* L.) seeds at different concentrations of stem extracts of *Artistolochia esperanzae*. However, the present result showed significant difference in VI ($F2,6 = 50.01 < P = 0.01, R^2 = 0.97$) and GE ($F2,6 = 12.08, P = 0.01, R = 0.93$) among the varieties with different extract concentrations. Thus, the higher average value of VI (4.3±0.6) and GE (2.5±0.2) were observed in the *Melkam* variety and the lower VI (2.0±0.06) and GE (1.6±0.1) in the farmers' cultivar at 15 % stem extract concentration (Fig. 2). These results suggest increasing in phytotoxicity with increasing stem extract concentrations and however the stem was a bet lesser to suppress the germination parameters than the root extracts. A similar inhibitor effect for *Melitotus indicus* was reported by [37]. They found that germination rate of *Triticum aestivum* decreased significantly by increasing in concentration of *M. indicus* stem extracts owning to the phenomenon of allelopathy.

Influence of Aqueous Extract of *A. ochroleuca* Leaf on Seedling Growth of Sorghum Varieties: The Allelopathic effect of A. ochroleuca leaf extracts under different concentrations was evaluated against seed germination and seedling growth of the selected *S. bicolor* varieties. The multivariate analysis indicated significant differences in plumule length ($F2,6 = 4.8, P < 0.005, R^2 = 0.95$), germination number ($F2,6 = 12.1, P < 0.001, R^2 = 0.97$), percent of germination ($F2,6 = 8.2, P < 0.01, R^2 = 0.95$) and VI ($F2,6 = 6.9, P < 0.01, R^2 = 0.96$) among varieties by

Table 3: Seedling growth of *Sorghum bicolor* L. varieties treated by aqueous extract of *A. ochroleuca* leaf (Mean±SD)

	Melkam Sorghum variety				Gerana 1 Sorghum Variety				Farmers Cultivar			
Seedling Parameters	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%	T0 0%	T1 5%	T2 10%	T3 15%
RL	13.7±1.3	11.5±0.5	11.5±0.5	8.3±1.5	10.8±0.2	9.2±0.3	8.2±0.3	6.8±0.7	7.8±0.2	6.5±0.5	6.3±0.3	5.7±0.4
PL	12.4±0.4	10.2±0.2	8.5±0.4	7.4±0.4	9.9±0.5	8.0±0.1	7.2±0.3	6.3±0.3	9.2±0.3	8.3±0.3	6.8±0.3	5.4±0.5
GN	14.2±0.3	12.6±0.2	9.8±0.2	8.2±0.3	12.2±0.3	11.0±0.5	9.5±0.5	8.0±0.2	10.7±0.3	9.3±0.2	7.8±0.2	7.3±0.3
G%	85.2±1.6	66.4±1.8	56.1±1.8	49.2±2.9	71.7±0.3	65.1±3.5	54.3±2.0	49.4±0.7	69.3±1.9	63.4±1.7	54.2±3.9	45.7±2.5
MGT	22.0±0.9	23.8±2.9	21.3±2.3	19.0±0.5	24.5±1.0	24.0±0.5	21.3±2.3	23.0±1.7	21.7±1.7	17.8±0.6	16.7±0.3	13.8±0.3
VI	29.0±2.4	19.2±0.7	14.0±0.5	11.7±1.1	24.8±0.8	15.5±2.2	11.1±1.1	8.1±0.91	8.7±0.7	14.2±0.8	10.9±0.6	8.0±0.1

RL, Radicle Legth; PL, Plumule Length; GN, Germination Number; G%, Germination Percent; MGT, Mean Germination Time; VI, Vigor Index; GE, Germination Energy; SD, Standard Deviation

extracted concentrations. However, no significant difference was found in the radicle length between the crop varieties under different extract concentrations ($F2,6 = 3.7$, $P = 0.1$, $R^2 = 0.92$), MGT ($F2,6 = 3.2$, $P = 0.09$, $R^2 = 0.80$) and GE ($F2,6 = 3.9$, $P = 0.07$, $R^2 = 0.94$). Regardless of significant difference, numerically the higher growth rates were recorded in the *Melkam* variety under all concentrations except the MGT value where it was higher in *Gerana 1 S. bicolo*r variety (Table 3). This higher value in *Melkam* variety could be attributed to the greater osmotic potential and strong resistance of the variety to the phytochemicals than the other varieties. The result revealed that the A. ochroleuca leaf extracts found to have less phytotoxic effect to the selected varieties comparatively the stem extract and the more phytotoxic root extracts. However, the overall inhibitory trend is therefore increase with the increasing extract concentrations, might be due to the presence of secondary metabolites. Similar studies indicated the plant growth suppressor effect of *A. mexicana* by Namkeleja, Mokiti and Patrick [41] who reported that the phenolic compounds in particular the cinnamic acid and vanillic acid are potential inhibitory effect. Ehsan *et al.* [42] also elucidated that following the treatment of Maize and Brassica with aqueous leaf extract from *Papaver pavoninum* invariably affected the germination percent, plumule and radicle growth, number of seminal roots and fresh and dry weights.

CONCLUSION

This study indicated that the aqueous extract of *A. ochroleuca* weed plant has phytotoxic effect on seed germination and seedling growth of *Sorghum bicolor varieties* especially on the farmer cultivar where it showed minimal resistance to the phytotoxic effect of the weed. The results obtained in this study showed that substantial reductions in the seedling growth parameters of the sorghum with increasing extract concentrations. *A. ochroleuca* root extract showed strong phytotoxic effect followed by the stem and comparatively the least inhibitory effect was observed in the leaf extract. Appropriate weed management strategies should be prime importance and further identification of allelochemicals which suppress the plant growth and development may help the breeder to develop the desire gene that resist the phytotoxicity of the weed. Despite its phytoxicity effect further study is also needed to isolate and identify novel phytochemicals that could have medicinal values and environmental friendly phytochemicals.

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