Journal of Agricultural Technology 2012 Vol. 8(4): 1423-1469
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Allelopathic effects of some botanical extracts on germination and seedling growth of *Sorghum bicolor* L.

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The allelopathic potential of the aqueous extracts and powder of Khella (*Ammi majus*), Ghobaish (*Guiera senegalensis*) and Safsaf (*Salix spp*.) on germination and seedling growth of two *Sorghum bicolor* L. cultivars was studied. Petri-dish trial showed that the different extracts level reduced total germination percentage (G %) and mean germination time (MGT). Khella extract sustained the maximum reduction in G % and MGT. Botanical extracts exhibited extra inhibitory effects on radical emergence than on plumule growth. Pot experiment indicted variations in seedlings germination and post-germination growth between the two cultivars in response to different botanical residues. Fatarita seedling emergence improved by Khella and decreased with Ghobaish and Safsaf. While, Hybrid seedling emergence improved with Ghobaish and Safsaf and reduced by Khella compared to control. The higher MGT was recorded in Fatarita in some treatments compared to control. Hybrid exposes a constant MGT in all treatments. Botanical extracts stimulated some growth parameters and reserve others in both cultivars. The results suggested that allelopathic potentials of these plants may entitle them to control specific weeds especially in non-sequential crops by preparing them as natural herbicides.

Key words: allelopathy, botanical extracts, sorghum germination and growth.

Introduction

Allelochemicals emancipated as residues, exudates and leaches by many plants from leaves, stem, roots, fruit and seeds reported to interfere with growth of other plants (Asgharipour and Armin, 2010). These chemicals products mainly affect plants at seed emergence and seedling levels (Alam and Islam,

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2002; Hussain *et al*., 2007; Mohamadi and Rajaie, 2009; Naseem *et al*., 2009). Allelopathy plays an important role in agricultural ecosystems and in a large scale, in the plant covers among the crop-crop, crop-weed and tree-crop covers. These interactions are detrimental and occasionally, are useful and gave attention to allelopathy in natural and agricultural ecosystems. Today, allelopathy is recognized as appropriate potential technology to control weeds using chemicals released from decomposed plant parts of various species (Naseem *et al*., 2009).

Sorghum [*Sorghum bicolor* (L.) Moench] is a very economic important cereal crop and represent major staple food crop for many developing countries. Sorghum was severe affected by weeds invention during the four-five weeks after seeds emergence and seedling growth. As a consequence, severe uncontrolled weed infestations often cause poor crop establishment or complete crop failure (Pannacci *et al*., 2010). Bioherbicides represent solution to heavy use of synthetic herbicides which it causes serious threats to the environment, consumers and increases costs of crop production (Asghari and Tewari, 2007). Unavailability of grass herbicides registered both for pre- and post-emergence applications (Pannacci *et al*., 2010). Moreover, continuous use of herbicides for weeds control causes herbicide resistant (Naseem *et al*., 2009). Many author reported employ plants extracts for controlling weeds with variable success (Hussain *et al*., 2007; Iqbal *et al*., 2009; Naseem *et al*., 2009). However, allelochemicals might affect both crop and weeds when found together. The crop was distress directly or indirectly by the allelochemicals and lead to either stimulation or inhibition of growth (Asgharipour and Armin, 2010). Several works have demonstrated the harmful influence of application of some plant species to sorghum including reduced seeds germination, seedlings emergence and biomass gain. Aqueous extracts of leaves have notably inhibited seed germination of sorghum with application of *Parthenium hysterophorus* (Murthy *et al*., 1995), *Ipomoea cornea* (Jadhav *et al*., 1997) *Commelina bengahalensis* and *Cyperus rotundus* (Channappagoudar *et al*., 2003) and *Eucalyptus camaldulensis* (Mohamadi and Rajaie, 2009). However, the allelochemicals sometimes have positive affects on sorghum growth. For example, *Moringa oleifera* leaf extracts enhanced germination of sorghum by 29% (Phiri, 2010).

The same kind of germination pomotary behaviour was also observed in extract of *Cassia angustifolia* (Hussain *et al*., 2007). Study the allelopathic of plant organs extract at seed germination and seedling growth stages was beneficial for it is difficult to separate the allelochemicals effects from that of competition among crop and allelopathic plants (Asgharipour and Armin, 2010). Understanding the response of crop cultivars to allelopathic plants potential for weed control is very important. Therefore, the objective of this study was to determine the effect of aqueous extracts from some Sudanese medicinal plants on germination and seedling growth of sorghum under lab and greenhouse conditions.

Materials and methods

Botanical material

A total of three plants known for ability to produce allelochemicals were selected for the present study namely Khella, *Ammi majus* (Umbellifereae), Ghobaish, *Guiera senegalensis* (Combretaceae) and Safsaf, S*alix spp.* (Salicaceae). Fully grown healthy leaves collected from these plants were washed thoroughly with distilled water and dried in the open for 14 days. Then the dried samples were separately ground into fine powder and stored dry until used. Aqueous leaf extract was prepared by soaking 10 gram of powdered leaf material in 100 ml distilled water for 24 hours. Then, this extract was filtered using filter paper (Whatman No. 1). The filtered solutions (stock solutions) were held in a refrigerator for a short time until experiment start. Stock solution (10% w/v) was diluted appropriately with distilled water to give the final concentrations of 25, 50 and 75 %. To evaluate the phytotoxicity of allelochemicals produced by the plants, the effects of water soluble compounds on seed germination and powdered leaves on seedling growth was analyzed.

The control treatment, distilled water, was used to estimate potential germination of seeds.

Plant material

Seeds of two local cultivars, *Sorghum bicolor* (L.) Moench, viz. Fatarita and Hybrid were obtained locally. The plant seeds were sterilized with 15:1 water/bleach (commercial NaOCl) solution for 5 minutes and subsequently washed with distilled water.

Seed germination

Five tests corresponding to the treatments mentioned above were performed on cultivar Fatarita in a germination test consisting of seeds incubated on filter paper with; irrigated solely with concentrations of 25, 50, 75 and 100 % along with 0% (control, distilled water). For each treatment, three different replications were tested. Each replicate was composed of 10 seeds placed in a 10-cm diameter plastic Petri dish on paper tissues, kept saturated by regular watering. The Petri dishes were kept in a germinator (25±3° C, 70%

humidity and constantly dark) for 5 days. The Petri dishes were watered once in every 2-3 days with either different concentrations of aqueous solution of leaf extract or distilled water for the control, if necessary.

Germinated seed (considered when radicle emergence $\geq$ 1 mm) were daily counted for 5 days or until the last seed germination. To assess the rate of germination, final germination percentage (G %) and mean germination time (i.e., time from imbibition to radicle emergence) (MGT) were calculated using the formulas:

$G\% = (a/b)\ 100$,

Where, a is the proportion of germinant and b the total number of seeds germinates in control treatment.

Whereas, MGT was calculated according to Ganaie *et al.* (1992) as:
$MGT = \Sigma\ (n \times d)/N$,

Where, n is the number of seeds which germinated after each period in days (d) and N is the total number of seeds germinated at the end of the experiment. Radicle and plumule growth elongation measurements were recorded after the 5^{th} day.

Seedling growth

In order to test the short-term allelopathy of the three plants, effect of botanical residues incorporated with soil was studied. For incorporation treatments, residues of dried botanicals were mixed with a silt soil per 50 cm3 pot. The amount of plant residues incorporated in a soil medium was 0.0, 0.5 and 1.0 g/pot. In each pot 10 seeds of the two cultivars were sown at a depth of 1 cm. Pots were kept under green house condition with daily irrigation. After 2 days of incubation, seeds start to germinate and seedlings emergence was counted each day for a period of 9 days to calculate G% and MGT as above.

The length of shoot (seedling height) was measured. At the end of the experiment, all plants were harvested to determine the dry weight of roots and shoots of seedlings. The experiment was performed in three replicates with 8 seeds in each.

Statistical analyses

Germination and seedling growth bioassays were conducted in a complete randomized design (CRD) with three replications. All experiments repeated twice and the data were subjected to analysis of variance (ANOVA). The significant differences between treatments means were separated using LSD test ($P< 0.05$).

Results and discussion

Results of variance analysis for germination experiment showed that germination, plumule and radicle growth and dry biomass of sorghum plant was significantly affected by different concentrations of botanical extract (allelopathic chemical).

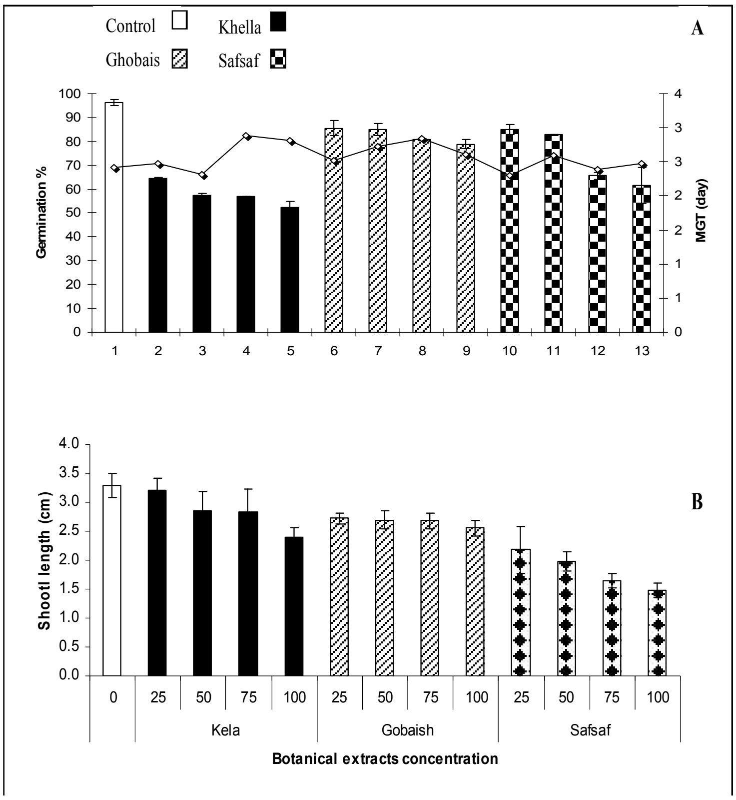
Seed germination

Seed germination is considered to be the most critical stage especially under stress conditions. During germination, biochemical changes take place, which provide the basic framework for subsequent growth and development. Aqueous crude extracts of the three plants leaves appreciably affected germination and growth of the *Sorghum bicolor* cultivar Fatarita (Figure 1).

However, there were considerable variations on parameters measured in responses to different treatments at all concentrations. Different botanical extracts revealed different influence on Fatarita seeds germination (Figure 1 a). Among the botanical extracts, Khella displayed the lowest germination (52.5%) and therefore showed greater inhibition on germination as compared to control (96.3%), Ghobaish (85.5%) and Safsaf (85.0%). No correlation between germination percentage (G %) and time to germination (MGT) was found (Figure 1 a). Results displayed that when concentration of extract increased the G % decreased while MGT increased. This representing that seeds in higher concentrations need more time to germinate (i.e. has lower germination rate). In consequent, control has the lower MGT with 2 days indicating high germination rate. Nevertheless, Ghobaish increase MGT to around 3 days almost in all concentrations. The leaves extract inhibited the shoot and root length sorghum Fatarita seedlings, compared to the related controls. The inhibitions were relatively enhanced with the increasing amount of each extract concentrations. Fatarita shoot length showed less effect with application of Khella at 25% (Figure 1b). Results indicated that root length was relatively more sensitive to allelochemicals compared to the shoot elongation (Figure 1c).

Ghobaish has same effect under all concentrations. Safsaf leaf extracts proved to have more allelopathic effect in comparison to other extracts. Effect of botanical extracts on sorghum seed germination was reported through several experimentations. This study is in agreement with other studies which showed that sorghum seeds germination was significantly reduced when treated with

Eucaliptus camaldulensis (Mohamadi and Rajaie, 2009) and *Spina christi*, *Sesbania sesban* and *Tamarindus indica* (Mubarak *et al*., 2009). In contrast, *Moringa oleifera*, *Khaya senegalensis* and *Albizia lebek* leaf extracts found to have no significant effects on seed germination of sorghum (Mubarak *et al*., 2009; Phiri, 2010). The differences in the germination percentage between the cultivars could be attributed to differences in the selective permeability of the seeds coat of sorghum to inhibitory substances (Zakaria and Razak, 1990). Therefore, effects of allelochemicals on seeds germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage or organelles (Mohamadi and Rajaie, 2009). It can be summarized from the results that extract concentrations of allelochemcial will reduce sorghum seeds germination and ultimately results in reduction in yield. These results are in agreement with those of Singh *et al*. (1992), Nandal *et al*. (1999 a, b) and Patel *et al*. (2002) who all observed reduction in germination percentage with extract/ leachates application to wheat seed.



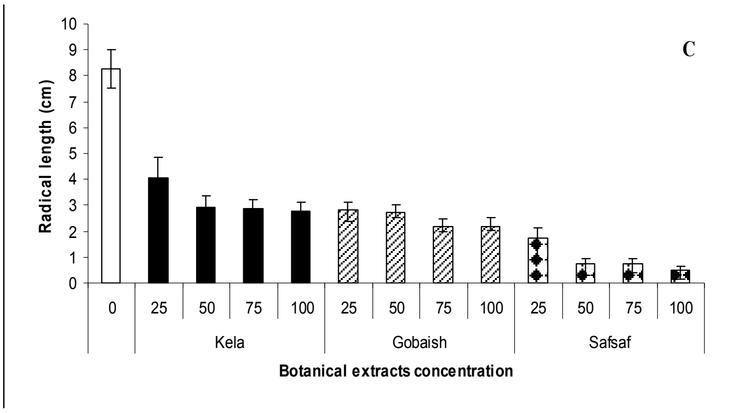


Fig.1. Effect of botanical extracts on Fatarita (*a*) seed G% (column) and MGT (line), (*b*) shoots length and (*c*) radicle length. Bars at the tip of columns indicate ± standard errors of means.

Results showed that the two sorghum cultivars differed in their reaction in seed emergence with botanical residues application (Figure 2). Germination percentage (G %) of Fatarita consistently increased when Khella was added to the substrate, whereas decreased with Ghobaish and Safsaf compared to control. On the other hand, germination of Hybrid was increased in order of Safsaf > Ghobaish > Khella > Water. Germination rate considered when the G% increased. However, Fatarita gave better seeds germination than Hybrid in all treatments excluding Safsaf 1.0g. The differences in the tolerance levels between the two sorghum cultivars Fatarita and Hybrid could possibly be due to the selective permeability of the seed coat to the inhibitory substances (Mubarak *et al.*, 2009). Experiment on effect of *M. oleifera* leaf extracts on sorghum indicated 15.3% reduction in survival seedlings (Phiri, 2010). The leaches of *E. camaldulensis* (Mohamadi and Rajaie, 2009) and many plant extracts (Mubarak *et al.*, 2009) were also reported to reduce seedlings growth of sorghum.

The mean days to germination (MGT) was relatively decreased (i.e. germination rate increased) by botanical application compared to control (Figure 2). The fastest MGT exhibited in Fatarita by the Safsaf with 2.6 days. Fatarita has wide range in MGT between 2.6 to 11.7 days. Also, seeds germinated rapidly (MGT improved) in all of treatments (except Ghobaish 1.0g) than control. The mean time to germination of all treatments on Hybrid was almost same and near to 4 days.

Comparison results in seeds germination percentages between Petri dish and pot experiments, displayed that seeds were germinated better in pot experiment as compared to Petri dishes. Allelochemicals might be inactivated in the soil by different factors such as chelation with ions, complexation with soil colloids, decomposition by micro-organisms or mechanical forces (Mubarak *et al.*, 2009).

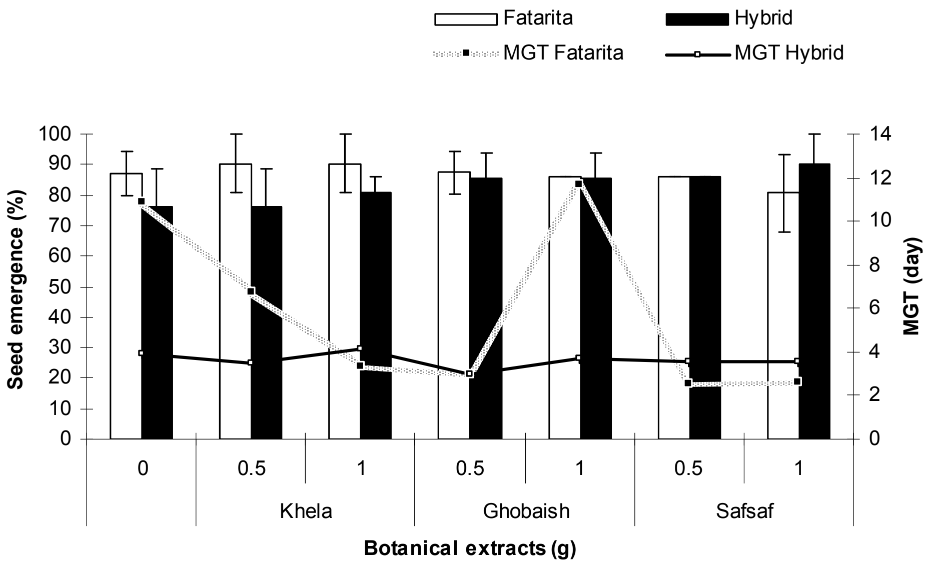


Fig. 2. Effect of botanical extracts on seed emergence and mean germination time of Fatarita and Hybrid. Bars at the tip of columns indicate ± standard errors of means.

Seedling growth

The data on sorghum seedling growth indicated considerable differences due to different botanicals and their concentrations (Table 1). Among the two cultivars the concentrations of the three botanical residues decreases the shoot length of Fatarita as compare to control (distilled water). The control showed 15.9 ± 0.8 cm for shoot length where the highest length produced by botanical was (15.7 ± 0.8) in Ghobaish treatment. Likewise, cultivar Hybrid treated with botanicals leaf powder enhanced shoot growth of Khella at 0.5g (10.6 ± 1.3 cm), Ghobaish at 1.0g (8.5 ± 1.1 cm) and Safsaf at 0.5 (9.1 ± 0.6 cm) over the control (8.3 ± 1.3 cm).

With respect to plant biomass, Khella and Ghobaish reduced shoot and root dry weight of cultivar Fatarita as compared to the control (Table 1). However, Safsaf decreased shoot dry weight of sorghum Fatarita although it enhanced its root dry weight. In among all treatments, sorghum Hybrid treated with botanical extracts displayed the highest shoot dry weight except Ghobaish at 0.5g as compared to the control (0.029 ± 0.00 cm). However, all botanical treatments decreased root dry weight compared to control. From these results it can be stated that (Table 1), the botanical materials reveal negative effects on seedling growth but with variation between the two cultivars. Sorghum treated with Safsaf increased shoot and root dry weight of both Hybrid and Fatarita.

Table 1. Effect of botanical residues on shoot length and dry biomass of Sorghum cultivars after 2 weeks of germination

Botanical Residue (g/pot)	**Shoot length (cm) (Means ± SE)**		**Shoot dry weight (g) (Means ± SE)**		**Root dry weight (g) (Means ± SE)**	
	Fatarita	Hybrid	Fatarita	Hybrid	Fatarita	Hybrid
0.0	15.9 ± 0.8	8.3 ± 1.3	0.066 ± 0.00	0.029 ± 0.00	0.037 ± 0.01	0.046 ± 0.02
Khella						
0.5	14.5 ± 1.0	10.6 ± 1.3	0.054 ± 0.01	0.031 ± 0.01	0.018 ± 0.01	0.031 ± 0.02
1.0	12.4 ± 0.7	8.7 ± 1.3	0.051 ± 0.01	0.030 ± 0.01	0.014 ± 0.00	0.026 ± 0.01
Ghobaish						
0.5	15.7 ± 0.8	7.0 ± 0.9	0.064 ± 0.01	0.027 ± 0.00	0.036 ± 0.004	0.031 ± 0.00
1.0	15.7 ± 0.1	8.5 ± 1.1	0.063 ± 0.00	0.033 ± 0.00	0.026 ± 0.00	0.035 ± 0.02
Safsaf						
0.5	12.8 ± 1.5	9.1 ± 0.6	0.050 ± 0.02	0.031 ± 0.01	0.051 ± 0.01	0.028 ± 0.00
1.0	12.6 ± 0.3	8.2 ± 0.5	0.057 ± 0.00	0.030 ± 0.00	0.040 ± 0.002	0.025 ± 0.01
LSD(p=0.05)						
Cultivar (V)		1.2		0.01		0.01
Botanical (B)		1.4		0.01		0.01
Conc. (C)		1.4		0.01		0.01
V*B*C Interaction		2.5		0.01		0.02

The reduction of biomass was correlated with seedling height growth. This reduction may be due to stunted and reduced seedlings growth (Garcı´a *et al*., 2002). A reduction of 31% in dry matter of sorghum by leaf leaches of *E. camaldulensis* (20%) had been reported (Mohamadi and Rajaie, 2009). Khella, *Ammi majus* is known for its high coumarin content, particularly furanocoumarins, Coumarin, umbelliferone, bergapten, xanthotoxin, isopimpinellin, imperatorin and isoimperatorin (Garcı´a *et al*., 2002). It is inhibitors of seeds germination and seedling growth.

In conclusion, results of this study showed that most leaves aqueous extracts and residues of Khella (*Ammi majus*), Ghobaish (*Guiera senegalensis*) and Safsaf (*Salix spp*.) have phytotoxic effects on seeds germination and seedling growth of sorghum. Allelopathic potentials of these plants which induces identifying and purification of allelopathic substances, may entitle them to control specific weeds especially in non-sequential crops by preparing them as natural herbicides.

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(Published in July 2012)