

Allelopathic Effects of Sodom Apple (*Calotropis procera* L.) on Seed Germination and Seedling Growth of Some Poaceous Crops

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Abstract

Laboratory and greenhouse experiments were conducted at the Faculty of Agricultural Sciences, University of Gezira, Sudan in 2014/15 season to study the allelopathic effect of aqueous extract and powder of sodom apple (*Calotropis procera* L.) leaves on seed germination and seedling growth of sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetum glaucum* [L.] R. Br.), maize (*Zea mays* L.) and wheat (*Triticum vulgare* L.). Treatments were arranged in completely randomized design with four replicates. Data were subjected to analysis of variance ($P \leq 0.05$) and means were separated using LSD test. The results showed that the leaves aqueous extract of sodom apple significantly reduced seed germination and there was direct relationship between concentration and reduction in germination. Also, the results showed that incorporating the leaves powder into the soil at the rate of 1 - 5%, significantly, reduced plant height, number of leaves, and root length of crop seedlings as well as plant fresh and dry weight. In addition, the reduction in seedling growth was increased as the leaves powder increased in the soil. This study confirmed that the sodom apple had allelopathic effects on seed germination and seedling growth of the poaceous crops. Therefore, isolation and identification of allelochemical compounds from this plant could provide means to minimize their negative effects over the cultivated plants and potentially could provide structural models for the development of bio-herbicides.

Keywords: Allelopathic; *Calotropis*; Sorghum; Millet; Maize; Wheat.

Introduction:

Sodom apple (*Calotropis procera* L.), a member of family Apocynaceae, and known as milk weed, is an evergreen poisonous shrub or small tree (Parihar *et al.*, 2011). It is distributed throughout the tropical and sub-tropical regions in world. It is able to thrive in warm and hot climate areas having dry, sandy and alkaline soils (Hassan *et al.*, 2015). It is mostly noted in waste and fallow lands along roadsides, streets, residential colony parks, sand dunes as well as in crop fields as weed (Parsons and Cuthbertson, 2001). Sodom apple has a perennial growth habit with tall and erect stem having large number of branches thus assuming the shape of shrub or sometimes small trees which can grow up to 2-3 m height. Leaves are arranged in opposite pairs along the stems and are sessile, glaucous, ovate to obovate, 5–20

cm long and 4–10 cm wide (D'Souzaa *et al.*, 2010). It reproduces mainly through the seed which are transported by wind, water and animals, while vegetative propagation occurs through suckers that produced from the roots (Parsons and Cuthbertson, 1992). Sodom apple has widespread and persistent occurrence near sorghum, maize, cotton and sugarcane crop fields, which suggests some adverse effects on these crops through allelopathic interactions. So, there is always a threat that it may become a major weed of cropping systems (Yasin *et al.*, 2012). The plant was suggested to have some adverse effects on other plants through allelopathic interactions (Yasin *et al.*, 2012).

Allelopathy phenomenon has been defined as direct or indirect suppression of growth of one plant (donor) species by another (receptor) through release of phytotoxic substances into the surrounding environment (Bhadoria, 2011). These substances are known as allelochemicals (Singh and Chaundhary, 2011). Allelochemicals, secondary metabolites, are toxic to other plants at early growth stages and slow down seed germination and inhibit root and shoot growth of the neighboring plants. (Farooq *et al.*, 2008; Jabran *et al.*, 2010). Many weed and crop species have been reported to have allelochemicals which are present in various plant parts, including roots, rhizomes, stems, buds, leaves, flowers, inflorescences, fruits and seeds (Yasin *et al.*, 2012; Gulzar and Siddiqui, 2017). Allelochemicals are released to the environment through litter decomposition, root exudation or direct volatilization (Bhadoria, 2011). In sustainable agriculture, allelochemicals are potential for the development of future herbicides and proposed for weed management (Kohli *et al.*, 1998; Dragoeva *et al.*, 2015).

Therefore, this study was carried out to investigate the allelopathic effect sodom apple (*Calotropis procera* L.) leaves powder of on seed Germination and seedling growth of some poaceous crops, in particular sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetum glaucum* [L.] R. Br.), maize (*Zea mays* L.) and wheat (*Triticum vulgare* L.).

Materials and Methods:

Experimental site:

Two experiments were conducted at the Faculty of Agricultural Sciences (FAS), University of Gezira, Sudan. The first was germination test, which was conducted in the biology laboratory having an average temperature of $26\pm 3^{\circ}$ C and relative humidity of 65 ± 5 %. The second was pot experiment, which was conducted in a greenhouse of horticulture nursery under field conditions. The experimental site was located at $14^{\circ} 24' N$, $33^{\circ} 29' E$ and 407m asl. The climate of the region is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12° C in January and 42° C in May, respectively. The soil of the area is characterized by heavy soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous (Elbasher, 2016).

Materials collection

Leaves of mature plants of sodom apple were collected from Experimental Farm of FAS in season 2015/16. The leaves were transferred to the biology laboratory of FAS. Then, the leaves were washed with sterilized distill water, air dried on bench for 15 days at room temperature and in a dark room to avoid the direct sun light that might cause undesired reactions. The dried leaves were then crushed into powder and kept in brown bottles till used. Certified commercial seeds of sorghum (cv. *Tabat*), millet (cv. *Baladi*), maize (cv. *Hudeiba* I) and wheat (cv. *Imam*), that have a germination percentage of 95-100% and purity of 100%, were obtained from the central market of Wed Medani city, Gezira state,

Sudan. The seeds were surface sterilized by sodium hypochlorite, (NaOCl) 1% (v/v), solution, for 3 min continuously agitated to reduce fungal infection. Subsequently the seeds were washed with sterilized distill water for several times and stored at room temperature till used.

Laboratory experiments:

These experiments were conducted in the biology laboratory to study the allelopathic effects of leaves aqueous extract of apple of Sodom on seed germination of sorghum, millet, maize and wheat. Fifty grams of leave powder of Apple of Sodom were placed in a conical flask, sterilized distilled water was added to give a volume of 1000 ml and then flasks were shaken for 24 hr at room temperature ($27\pm 3^{\circ}\text{C}$) by an orbital shaker (160 rpm). The extracts were strained through 2 layers of cheese cloth and then through 2 layers of Whatman no-2 filter paper to remove solid material. The filtrate was centrifuged at 3000 rpm for 20 min. The supernatant was collected and filtered through a $0.22\ \mu\text{m}$ membrane filter paper. The stock solution was stored at 4°C until further use. Six concentrations (0, 20, 40, 60, 80 and 100%) of the aqueous extract were prepared from the stock solution (50 g/l). Seeds of sorghum, millet, maize and wheat (100 seeds each) were sprinkled on Glass Fiber Filter Paper (GFFP) (Whatman GF/C) placed in a glass Petri-dish (GPD), 9 cm internal diameter (i.d). Each GPD moistened with 25 ml of leaves aqueous extract of apple of Sodom, sealed with Parafilm, covered with black polyethylene bag, were incubated at 30°C in the dark. Treatments, for each crop, were arranged in completely randomized design (CRD) with four replicates. The seeds were examined for germination at two days after initial germination.

Greenhouse experiments:

These experiments were conducted at the greenhouse of horticulture nursery to study the allelopathic effects of leaves powder of apple of Sodom on seedling growth of sorghum, millet, maize and wheat. Plastic pots, 10 cm internal diameter and 18 cm high with drainage holes at the bottom, were filled with Gezira soil and river silt that mixed in the ratio 1:1, oven dried at $120\ \text{C}$ for 48 h and screened to pass a 2-mm sieve. Leaves powder of apple of Sodom was incorporated into the soil at rate of 0, 1, 2, 3, 4 and 5% on w/w biases. Seeds of sorghum, millet, maize and wheat were sown in pots, five seeds per pot for each crop. The pots were kept free from weeds, irrigated and the seedlings were thinned to 3 plants per pot, 7 days after emergence. Treatments, for each crop, were laid out in completely randomized design (CRD) with four replicates. At 30 days after sowing the experiments were terminated and plant height (cm), number of leaves and root length (cm) of crop seedlings were measured as well as plant fresh and dry weight (g).

Statistical analysis:

Collected data were subjected to analysis of variance (ANOVA) procedure. Significant means were separated for significance using LSD test at $p \leq 0.05$. The statistical analysis was done using the Statistical Analysis System software v.9.0 (SAS, 2004).

Results:

Allelopathic effects of the leaves aqueous extract of sodom apple on seed germination of some poaceous crops:

The results showed that the leaves aqueous extract of sodom apple significantly ($P \leq 0.05$) decreased seed germination of the tested poaceous crops in comparison to the controls (Table 1). The reduction in seed germination increased with further increase in concentration of leaves aqueous extract. The highest seed germination was recorded in the control treatments. On the other hand, the greatest concentration (100%) attained the lowest seed germination which was 20.5, 24.3, 31.0 and 15.5% in sorghum, millet,

maize and wheat, respectively. There was direct negative relationship between concentration and seed germination.

Allelopathic effects of the leaves aqueous extract of sodom apple on seedling growth of some poaceous crops:

The results of the greenhouse experiments showed that incorporating leaves powder of sodom apple into the soil at rate of 1, 2, 3, 4 and 5% significantly hindered seedling development of the tested crops in comparison to controls (Table 2, 4, 5 and 6).

Table 1. Allelopathic effects of the leaves aqueous extract of sodom apple on seed germination of some poaceous crops

Concentration (w/v)	Germination (%)			
	Sorghum	Millet	Maize	Wheat
0%	96.3 a	96.3 a	96.3 a	98.5 a
20%	82.5 b	80.3 b	83.5 b	85.5 b
40%	72.3 c	72.5 c	71.8 c	59.5 c
60%	63.0 d	32.8 d	62.3 d	40.0 d
80%	29.8 e	26.5 e	52.5 e	16.5 e
100%	20.5 f	24.3 f	31.0 f	15.5 e
SE _±	0.96	0.72	0.88	1.02
CV%	3.2	2.6	2.7	3.9

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

Allelopathic effects of leaves powder of sodom apple on plant height of some poaceous crops:

At 30 days after sowing, the greatest plant height of crop seedlings were recorded in the control treatments (Table 2). The plant height of sorghum, millet, maize and wheat in the control treatments were 40.1, 44.3, 35.5 and 27.0 cm, respectively. However, increasing the concentration of leaves powder of sodom apple into the soil exhibited lowest plant height in all tested crops. The leaves powder when incorporated into the soil at rate of 1 to 5% reduced the plant height of poaceous crops in comparison to control treatments (Table 2). Moreover, the reduction in the plant height was increased as leaves powder increased in the soil. The greatest reduction in plant height was observed when leaves powder was incorporated into the soil at the rate of 5%. At high concentration leaves powder, the plant heights were significantly reduced to 25.0 cm in sorghum, 28.8 cm in millet, 18.5 cm maize and 6.3 cm in wheat seedlings.

Table 2. Allelopathic effects of the leaves aqueous extract of sodom apple on plant height of some poaceous crops

Concentration (w/w)	Plant height (cm)			
	Sorghum	Millet	Maize	Wheat
0 %	40.5 a	44.3 a	35.5 a	27.0 a
1 %	34.8 b	40.0 b	30.5 b	17.5 b
2 %	32.5 bc	38.5 b	27.3 c	14.8 bc
3 %	30.8 bc	33.8 c	24.5 d	13.8 c
4 %	28.5cd	30.8 cd	22.3 d	10.0 d
5 %	25.0 c	28.8 d	18.5 e	6.3 e
SE _±	1.33	1.29	0.80	0.94
CV%	8.4	7.2	6.0	12.6

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

Allelopathic effects of leaves powder of sodom apple on number of leaves of some poaceous crops:

At 30 days after sowing, the results showed that the incorporated leaves powder of sodom apple into the soil at a rate of 1, 2, 3, 4 and 5% negatively affected the leaves number of seedlings of all tested poaceous crops in comparison to the control treatments (Table 3). The highest leaves numbers of crop seedlings were obtained in the control treatments. The leaves number of sorghum, millet, maize and wheat in the control treatments was 7.3, 7.8, 7.0 and 8.8, respectively (Table 3). Incorporating leaves powder into the soil at the rate of 1% and more significantly ($P \leq 0.05$) reduced leaves number of seedlings of sorghum, millet and maize in comparison to the control treatments. While, significant reduction in leaves number of seedlings of wheat was obtained as leaves powder incorporated into soil at the rate of 3% or more.

Table 3. Allelopathic effects of the leaves aqueous extract of sodom apple on number of leaves of some poaceous crops

Concentration (w/w)	Number of leaves			
	Sorghum	Millet	Maize	Wheat
0 %	7.3 a	7.8 a	7.0 a	5.8 a
1 %	5.8 b	6.8 b	5.8 bc	5.3 ab
2 %	5.5 b	6.3 b	5.3 bc	4.8 abc
3 %	5.0 bc	5.3 c	5.0 bc	4.3 bc
4 %	4.8 bc	4.8 c	4.8 bc	4.0 c
5 %	4.3 c	4.5 c	4.5 c	3.8 c
SE _±	0.33	0.31	0.36	0.33
CV%	12.3	10.4	13.3	14.2

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

Allelopathic effects of leaves powder of sodom apple on root length of some poaceous crops:

Incorporation of leaves powder of sodom apple into the soil significantly inhibited root length of tested poaceous crops (Table 4). The reduction in root lengths was increased with leaves powder concentration in the soil. At 30 days after sowing, the longest root lengths of crop seedlings were displayed by the control treatments and amounted to 19.5, 24.5, 19.3 and 14.5 cm in sorghum, millet, maize and wheat, respectively. The root length was reduced to 9.3 cm in sorghum, 14.0 cm in millet, 13.8 cm maize and 7.0 cm in wheat seedlings as the leaves powder was incorporated into the soil at concentration of 5%.

Allelopathic effects of leaves powder of sodom apple on fresh weight of some poaceous crops:

The greatest fresh weights of crop seedlings, at 30 days after sowing, were recorded in control treatments (Table 5). The incorporation of leaves powder of sodom apple into soil significantly reduced fresh weight of the tested crops in comparison to control treatments. The greatest fresh weight of crop seedlings were observed in the control treatments and amounted to 12.5, 10.1, 14.3 and 8.0 g in sorghum, millet, maize and wheat, respectively. Moreover, the reduction in the fresh weight was increased as leaves powder increased in the soil. The incorporation of leaves powder into the soil at rate of 5% resulted in seedling fresh weights amounted to 5.7, 4.7, 8.5 and 3.7g in sorghum, millet, maize and wheat, respectively.

Table 4. Allelopathic effects of the leaves aqueous extract of sodom apple on root length of some poaceous crops

Concentration (w/w)	Root length (cm)			
	Sorghum	Millet	Maize	Wheat
0 %	19.5 a	24.5 a	19.3 a	14.5 a
1 %	15.0 b	19.8 b	17.8 b	12.8 b
2 %	14.0 bc	17.8 c	16.0 c	12.3 bc
3 %	12.5 cd	16.0 cd	15.0 cd	11.0 c
4 %	10.8 de	15.8 cd	14.5 d	9.0 d
5 %	9.3 e	14.0 d	13.8 d	7.0 e
SE _±	0.65	0.66	0.49	0.45
CV%	9.5	7.3	6.1	8.1

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

Table 5. Allelopathic effects of the leaves aqueous extract of sodom apple on fresh weight of some poaceous crops

Concentration (w/w)	Fresh weight (g)			
	Sorghum	Millet	Maize	Wheat
0 %	12.5 a	10.1 a	14.3 a	8.0 a
1 %	10.9 b	8.9 b	12.5 b	7.3 a
2 %	10.0 c	7.7 bc	11.3 bc	5.6 b
3 %	8.0 d	7.4 c	10.8 c	5.4 bc
4 %	6.8 e	5.0 d	10.3 c	4.5 cd
5 %	5.7 f	4.7 d	8.5 d	3.7 d
SE _±	0.28	0.39	0.45	0.36
CV%	6.2	10.7	8.1	12.6

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

Allelopathic effects of leaves powder of sodom apple on dry weight of some poaceous crops:

The results of incorporated leaves powder of sodom apple into the soil at rate of 1, 2, 3, 4 and 5% on seedling dry weight had same trend as seedlings fresh weight (Table 6). Incorporating the leaves powder into the soil at rate of > 1% significantly reduced fresh weight of sorghum, millet and wheat in comparison to the control treatments. While, significant reduction in dry weight of maize seedlings were obtained when seed powder incorporated into the soil at rate of 2% or more compared to the control treatments. The incorporation of leaves powder into to the soil at concentration of 5% (w/w) decreased the seedling dry weight to 1.1 g in sorghum, 0.9 g in millet, 1.8 g maize and 0.9 g in wheat seedlings.

Discussions:

The results of this study revealed that the leaves aqueous extract of sodom apple significantly reduced seed germination of the tested poaceous crops and there was a direct relationship between concentration and reduction in germination. Sodom apple has been reported to possess water-soluble organic compounds that hindered the growth of plants by inhibiting the reduction of gibberlins and indole acetic acid (Yasin *et al.*, 2012). The presence of water soluble allelochemicals in sodom apple leaf extracts can significantly delay seed germination and other growth attributes of crops (Samreen *et al.*, 2009). Similar results were found by Akhtar *et al.*, (2013) who pointed out that the leaf extract of sodom apple imposed allelopathic effects on seed germination and this inhibition of germination increased with an increase in the concentration of the leaf extract.

Table 6. Allelopathic effects of the leaves aqueous extract of apple of Sodom on dry weight of some poaceous crops

Concentration (w/w)	Dry weight (g)			
	Sorghum	Millet	Maize	Wheat
0 %	3.8 a	2.2 a	3.1 a	1.8 a
1 %	3.3 b	2.0 b	2.9 ab	1.4 b
2 %	2.8 c	1.7 c	2.8 b	1.3 b
3 %	1.6 d	1.3 d	2.6 c	1.1 c
4 %	1.3 de	1.1 e	2.1 d	1.0 cd
5 %	1.1 e	0.9 f	1.8 e	0.9 d
SE _±	0.01	0.04	0.06	0.05
CV%	8.5	5.7	4.5	8.4

* Means in the same column followed by the same letter(s) are not significantly different according to LSD test ($P \leq 0.05$).

The results were further supported by Gulzar and Siddiqui (2017) who studied the effect of aqueous extract of sodom apple on the seed germination of some crops. They concluded that the inhibition in seed germination after treatment by the aqueous extracts, because the extracts might damage the membrane system of the seeds. The various concentrations of the leaves aqueous extracts of sodom apple had varying degrees of inhibition on the seed germination. The inhibition of seed germination was concentration dependent. Inhibition of the seed germination as a result of allelochemical stress might be attributed to inhibition of water uptake, gibberellic acid activity, cell division and elongation during germination process (Tawaha and Turk, 2003).

Also, this study indicated that incorporating leaves powder of sodom apple into the soil at rate of 1, 2, 3, 4 and 5% (w/w) significantly decreased plant height, number of leaves per seedling, root length of crop seedlings as well as plant fresh and dry weight. In addition, the reduction in seedling growth was increased as leaves powder increased in the soil. These results are in lined with Umar *et al.* (2014) who carried out a pot experiment to investigate the allelopathic effect leaf aqueous extract of sodom apple at different concentrations (0,5,10,15 nd 20%) on growth of sorghum and maize. They concluded that the leaf aqueous extracts have phytotoxic effects and inhibition in the growth of leaf area, plant height, plant fresh and dry weight of sorghum and maize (Umar *et al.*, 2014). Al-Zahrani and Al-Robai, (2007) confirmed that sodom apple has strong phytotoxic effects which can affect the normal growth of agricultural crops.

This result is consistent with that of Shah *et al.*, (2017) who studied the allelopathic effects of aqueous extracts of leaves, stems and root of sodom apple on wheat crop at of various concentrations. They concluded the sodom apple must be removed near the wheat fields, because it releases some allelopathic compounds that cause serious losses to the crop. This result is in agreement with that of Aslam *et al.*, (2016) who designed an experiment to find out phytotoxic effects of sodom apple on seed germination and seedling length of wheat. It is found that the leaves aqueous extracts of sodom apple have phytotoxic effect and inhibited seed germination and seedling length of wheat. Also, it is found that the inhibitory effect increases with the increase of extracts concentration. It is thus concluded that this inhibition is due to the presence of different compounds in the leaves aqueous extracts.

The phytochemical analysis on the aerial parts of sodom apple showed that the leaves contains tannins, flavonoids, glycosides, steroids, saponins and cardiac glycosides that might contribute its allelopathic potential (Sharma *et al.*, 2011; Umar and Mustapha, 2014). Inhibition (%) in seed germination of the

tested poaceous crops as a result of allelochemical stress may be due to inhibition of water uptake, cell division, elongation and alteration in the activity of gibberellic acid (Chandler *et al.*, 1984; Tawaha and Turk, 2003) which is known to regulate de novo amylase production during germination process. The inhibition of seed germination was found to be concentration-dependent (Oudhia, 1999).

Conclusion:

This study confirmed that the leaves powder of sodom apple had allelopathic effects on seed germination and seedling growth of sorghum, millet, maize and wheat crops. Therefore, isolation and identification of allelochemical compounds from this plant could provide means to minimize their negative effects over the cultivated plants and potentially could provide structural models for the development of bio-herbicides.

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تأثير التضاد الحيوي لمسحوق أوراق نبات العشر (*Calotropis procera* L.) في إنبات البذرة ونمو البادرة في بعض المحاصيل النجيلية

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الملخص

أُجريت تجارب في المعمل والبيت المحمي بكلية العلوم الزراعية، جامعة الجزيرة، السودان في الموسم 15/2014 لدراسة تأثير التضاد الحيوي للمستخلص المائي ولمسحوق أوراق نبات العشر (*Calotropis procera* L.) على إنبات البذور ونمو بادرة الذرة الرفيعة (*Sorghum bicolor* [L.] Moench)، والدخن (*Pennisetum glaucum* [L.] R. Br.)، والذرة الشامية (*Zea mays* L.)، والقمح (*Triticum vulgare* L.). وُضعت المعاملات في تصميم كامل العشوائية بأربعة مكررات. أُخضعت البيانات لتحليل التباين (ANOVA)، وتمت مقارنة المتوسطات باستخدام اختبار أقل فرق معنوي (LSD) باحتمال $0.05 \geq$. أوضحت النتائج أن المستخلص المائي للأوراق خفض معنوياً إنبات البذرة، وكانت هنالك علاقة مباشرة بين التركيز والانخفاض بنسبة الإنبات. كما أوضحت النتائج أن خلط مسحوق الأوراق في التربة بمعدل 1-5% قد خفض طول النبات، وعدد الأوراق، وطول الجذر، كما خفض الوزن الرطب والجاف للنبات. إضافة إلى ذلك انخفض نمو البادرات بزيادة تركيز مسحوق الأوراق في التربة. تؤكد هذه الدراسة أن مسحوق أوراق العشر له تأثير تضادي على إنبات البذرة ونمو بادرة المحاصيل النجيلية. لذلك، فإن عزل وتعريف المركبات الاليلوكيميائية من هذا النبات يمكن أن يوفر وسائل للتقليل من آثارها السلبية على النباتات المزروعة ويمكن أن يوفر نماذج هيكلية لتطوير مبيدات الأعشاب البيولوجية.

الكلمات المفتاحية: نبات العشر، التضاد الحيوي، الذرة الرفيعة، الدخن، الذرة الشامية، القمح.