

## Phytotoxic Effects of *Calotropis procera* (Aiton) W.T. Leaves Aqueous Extract on Seed Germination of some Cereal Crops Using Probit Analysis

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### ABSTRACT

Certain plants suppress development of other plants by releasing many chemical substances into the environment. This study was carried out to investigate the phytotoxic effects of the leaves aqueous extract of apple of Sodom [*Calotropis procera* (Aiton) W.T.] on seed germination of *Sorghum bicolor* (L.) Moench (sorghum), *Pennisetum glaucum* (L.) R. Br. (millet), *Zea mays* L. (corn) and *Triticum aestivum vulgare* L. (wheat) using probit analysis. The experiments were conducted at the University of Gezira in Sudan, in the 2014/15 agricultural season. Ten concentrations (2.11 to 21.05 g/l) were prepared from the stock solution (50 g / l). Sterilized-distilled water was used as control. The experiment for each crop, was laid out in a completely randomized design and the treatments were replicated four times. The crop seeds were checked for germination and its inhibition three days after the onset of germination. Data were subjected to probit analysis procedure ( $P \leq 0.5$ ). The results revealed that apple of Sodom's leaves aqueous extract inhibited seed germination of the tested cereal crops and there was direct positive relationship between concentration (g/l) and inhibition (%). In addition, the result revealed that the  $LC_{50}$  was 17.6, 17.0, 19.2 and 16.7 g/l for sorghum, millet, corn and wheat, respectively. It was concluded that the leaves aqueous extract of apple of Sodom had toxic effect to the seeds of the tested cereal crops.

**Key Words:** Herb, Poaceae, Toxicity, Weeds.

### INTRODUCTION

Certain plants may have the capacity to suppress germination and development of other plants by releasing many chemical substances into the environment (Wang *et al.*, 2017). These chemicals are known as allelochemicals (Singh and Chaudhary, 2011). These allelochemicals are actually secondary metabolites and are phytotoxic (Farooq *et al.*, 2011). Phytotoxic impact of these compounds on other plants is usually dominant at early growth stages. The most cited effects include reduction in seed germination and seedling growth (Farooq *et al.*, 2008; Jabran *et al.*, 2010). Allelochemicals have been defined as compounds synthesized by plants to manage various biological processes (Viter *et al.*, 2015). Allelochemicals are formed in leaves, stems, fruits rhizomes, and seed of plants and move to the soil through degradation, perfusion, washing, and volatilization of plant materials (Zeng *et al.*, 2008). Allelochemicals disrupt numerous biological functions of plants like absorption of water and nutrients, photosynthesis,

respiration and induction of the nucleic acids (Einhelling, 2002; Lebedev *et al.*, 2019). Allelochemicals can lower cell division or inhibit auxins that stimulate the development of plant root and shoot systems (Gholami *et al.*, 2011). Allelochemicals, like phenolic compounds, have the ability to hinder the growth of the roots and shoots (Hussain and Reigosa, 2011). Allelochemicals freed into the ecosystem can prevent root and/or shoot prosperity, nutrient absorption, or may adverse the symbiotic relationship that occur in nature, thus destroy the plant's source of nutrients. Allelochemicals could provide a potential source for natural herbicides and biological control agents (Razzaq *et al.*, 2012).

Apple of Sodom [*Calotropis procera* (Aiton) W.T.], belonging to the family Asclepidaceae, is a long-lived toxic shrub or short tree. This species is distributed in tropical and subtropical regions of the world in Africa and Asia. The plant population flourish in different soil types ranging from fine to rough texture, and from unsaturated to saline soils (Hassan *et al.*, 2015). It survives heavily in

arid and semi-arid zones in the absence of agricultural inputs and without any cultural practice or agricultural operation (Sharma *et al.*, 2012). The plant has deep branching roots that permeate the soil granules. The plant scarcely grow on surface soils over un-cracked rock (D'Souzaa *et al.*, 2010). The plant develops in open environments, particularly in natural grazing areas or poor soils, where the competition decreases with other weeds. The plant is also spread on the sides of streets and roads, in river and canals arid land, in sand dunes, and in coastal marshland (Parsons and Cuthbertson, 2001). Apple of Sodom communities are important in the performance of ecosystems because the plants create a suitable environment for the development of insects such as butterflies, and form a good food source for many arthropods. In addition, the plant is used in several countries in the tropics for medical purposes. The non-traditional uses of this plant include the treatment of soil contaminated by toxic trace elements in addition to using the plant biomass as an alternative source of renewable energy (Hassan *et al.*, 2015). Apple of Sodom is widely spread close to the fields of important crops such as sorghum and corn, which imposes some negative effects on these crops through allelopathic interactions. Thus, there is a constant risk that it may turn into a noxious herb in agricultural systems (Yasin *et al.*, 2012).

Considering the economic importance of cereal crops, this study was carried out to investigate the phytotoxic effects of the leaves aqueous extract of apple of Sodom on germination of the seeds of sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum* (L.) R. Br.), corn (*Zea mays* L.), and wheat (*Triticum aestivum vulgare* L.) using probit analysis.

## MATERIALS AND METHODS

### Experimental site

A series of germination tests were conducted in the biology laboratory at the Faculty of Agricultural Sciences (FAS), University

of Gezira (UofG), Sudan in 2015. The laboratory has an average temperature range between 25 - 30°C and the relative humidity ranging between 60 - 70 %.

### Materials collection

The leaves of Apple of Sodom were separated from fully grown plants in the fields of the Experimental Farm of the FAS in season 2014/15 then transported to the biology laboratory of the FAS. The leaves were cleaned out of dust and impurities, washed with sterilized distilled water, and dried by air on the table for 15 days at room temperature in dark to bypass direct sunlight, which may cause unwanted interactions. The dried leaves were triturated and crushed to powder, which was preserved in brown bottles until used. The seeds of sorghum cultivar *Tabat*, millet cultivar *Baladi*, corn cultivar *Hudeiba* I, and wheat cultivar *Imam* were obtained from the local Market of Wad Medani town in the Gezira State, Sudan. The germination percentage of seeds ranged from 95% to 100% and the purity was 100%. The seeds were sterilized with a solution of sodium hypochlorite at a concentration of 1% (v/v) for three minutes with continuous shaking to reduce the incidence of fungal infections. Immediately, the seeds were washed with sterilized distilled water several times, dried, and kept at room temperature until used.

### Preparation and calculation of the actual concentration of the leaves aqueous extract

Fifty grams, initial weight (IW), of leaves powder of apple of Sodom were placed in a conical flask, sterilized distill water was added to give a volume of 1000 ml and then the flasks were shaken for 24 hours at room temperature (27±3°C) using an orbital shaker (160 rpm). Subsequently, the aqueous extract of the leaves was filtered by muslin cloth and the leachate was dried and the precipitation (cake) weight (PW) was determined by a sensitive balance. The final volume (FV) of the water extract for the Apple of Sodom

leaves was measured by measuring cylinder. The final weight (FW), dissolved powder, was calculated using the following equation:  
 $FW = IW - PW$

The actual concentration (AC) of the aqueous extract of the leaves was calculated using the following equation:

$$AC (G/l) = (FW/FV) 1000$$

**Bioassay procedure**

Ten concentrations (n) of the leaves aqueous extract were prepared by sequential dilution of the stock extract with sterilized-distilled water to give 2.11, 4.21, 6.34, 8.42, 10.53, 12.63, 14.13, 16.84, 18.94, and 21.05 g/l. A control with sterilized-distilled water was included for comparison. Glass fiber filter papers were placed in 9 cm Petri dishes. One hundred seeds, for each tested crop, i.e. sorghum, millet, corn, and wheat, were placed on a glass fiber filter paper. The seeds on the papers were wetted with an aqueous extract of the leaves of Apple of Sodom (30 ml). The Petri dishes were then sealed with adhesive tape, kept in a black polyethylene bag and incubated at 30 ° C in the dark. The experiment, of each crop, was laid out in a completely randomized design with four replicates. Crop seeds were checked for germination and inhibition of germination during three days from the onset of germination. The percentage of the inhibition of seed germination was calculated using the following equation:

$$Inhibition (\%) = (Total\ number\ of\ seeds - number\ of\ germinated\ seeds) / Total\ number\ of\ seeds \times 100$$

The inhibition (%) was corrected using Abbott’s formula. It is given by:

$$Corrected\ Inhibition (\%) = (X - Y) / X \times 100$$

Where:

X is the % survivorship of the control group  
 Y is the % survivorship in the experimental group

**Statistical analysis**

Data were transformed using Abbott’s formula and subjected to probit analysis procedure. Results from probit analysis were reported as a concentration to inhibit a certain rate of the tested seeds (LC<sub>50</sub> and LC<sub>90</sub>). The statistical analysis was carried out using Microsoft Excel and SPSS software v.16.

**RESULTS**

The results showed that the leaves aqueous extract of apple of Sodom inhibited the seed germination of the tested cereal crops (sorghum, millet, corn, and wheat) and there was direct positive relationship between concentration (g/l) and inhibition (%) (Fig. 1, 2, 3 and 4).

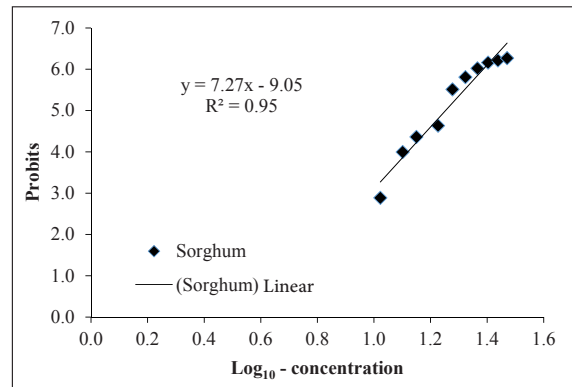


Fig. 1. Relationship between Log<sub>10</sub> of concentration of leaves aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of sorghum

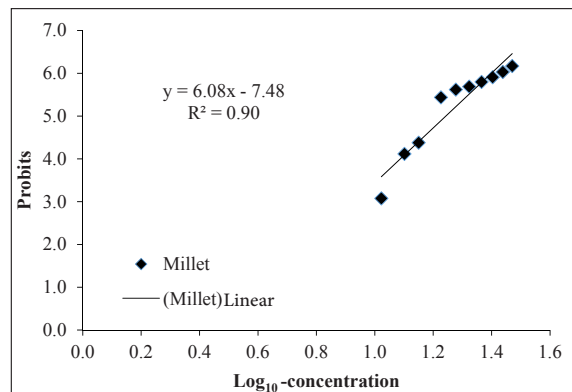


Fig. 2. Relationship between Log<sub>10</sub> of concentration of leaves aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination

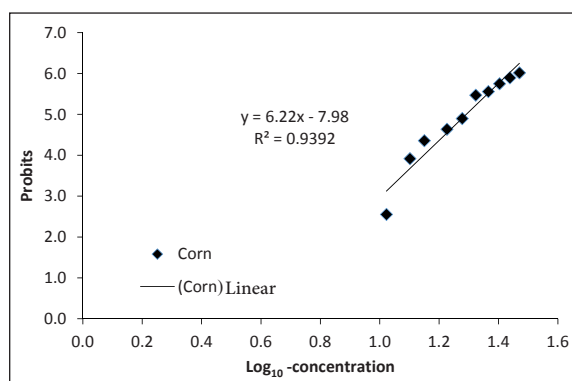


Fig. 3. Relationship between  $\text{Log}_{10}$  of concentration of leaves aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of corn

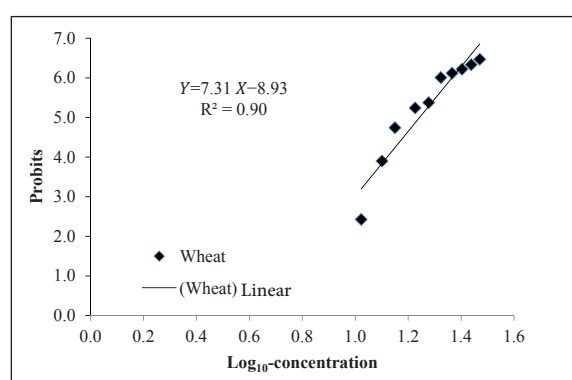


Fig. 4. Relationship between  $\text{Log}_{10}$  of concentration of leaves aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of wheat

### Phytotoxic effect on sorghum

Simple linear regression equation was  $Y=7.27 X-9.05$ . The value of coefficient of simple determination ( $R^2$ ) was 0.95. The  $LC_{50}$  and  $LC_{90}$  were 17.6 and 26.4 g/l, respectively (Table 1).

### Phytotoxic effect on millet

The simple linear regression equation was  $Y=6.08 X-7.48$ . The value of coefficient of simple determination ( $R^2$ ) was 0.90. The  $LC_{50}$  and  $LC_{90}$  were 17.0 and 27.6 g/l, respectively (Table 1).

### Phytotoxic effect on corn

The simple linear regression equation was  $Y=6.22 X-7.98$ . The value of coefficient of simple determination ( $R^2$ ) was 0.94. The  $LC_{50}$  and  $LC_{90}$  were 19.2 and 30.8 g/l, respectively (Table 1).

### Phytotoxic effect on wheat

The simple linear regression equation was  $Y=7.31 X-8.93$ . The value of coefficient of simple determination ( $R^2$ ) was 0.90. The  $LC_{50}$  and  $LC_{90}$  were 16.7 and 124.9 g/l, respectively (Table 1).

Table 1. Phytotoxic effects of the leaves aqueous extracts of apple of Sodom (*C. procera* W.T) on inhibition (%) of seed germination of some cereal crops using probit analysis

Cereal crops	No. of Tested (.seeds (Rep	Inhibition % values (Confidence limits 95%)		Slope $\pm$ SE	$\chi^2$	Df	.Sig
		$LC_{50}$	$LC_{90}$				
Sorghum	(4) 400	17.6 (16.5-16.6)	26.4 (24.8-29.9)	$0.25 \pm 27.7$	83.9	8	<sup>a</sup> 0.000
Millet	(4) 400	17.0 (15.4-18.5)	27.6 (24.7-33.0)	$0.24 \pm 6.08$	137.5	8	<sup>a</sup> 0.000
Corn	(4) 400	19.2 (18.3-20.0)	30.8 (28.6-33.4)	$0.24 \pm 6.22$	41.9	8	<sup>a</sup> 0.000
Wheat	(4) 400	16.7 (15.5-17.8)	124.9 (23.0-29.3)	$0.25 \pm 7.31$	102.9	8	<sup>a</sup> 0.000

a. A heterogeneity factor is implemented in the calculation of the limits of the confidence, because the significance level is less than 0.150.

## DISCUSSIONS

The results showed that apple of Sodom leaves aqueous extract was toxic to seed germination of the tested cereal crops. There was a positive relationship between

concentration (g/l) and inhibition (%). The  $LD_{50}$  was ranged between 16.7 g/l and 19.2 g/l and  $R^2$  ranged between 0.90 and 0.95. The suppression of seed germination in this study could be attributed to allelochemical

compounds presence in the leaves aqueous extracts of apple of Sodom. This result is consistent with those of Yasin *et al.* (2012) who indicated that apple of Sodom possesses organic chemical compounds that have the potential to be dissolved in water and hindered other plant development *via* suppressing the induction of important secondary metabolites such as phytohormones. The presence of water-soluble allelochemical compounds in the water extract of apple leaves have the ability to delay seed germination of the plants as well as inhibit the growth and development of the plants (Samreen *et al.*, 2009). Similar results were found by Akhtar *et al.* (2013) who pointed out that the leaf extract of apple of Sodom imposed adverse allelopathic effects on seed germination and the adverse effects was enhanced with further increment in the concentration in the extract.

The results showed a direct negative relationship between concentration (2.11 – 21.05g/l) and germination that might be as a result of the concentration of the toxic compound in the extract. The results are also supported by Gulzar and Siddiqui (2017). They studied the effect of aqueous extract of apple of Sodom on seed germination of some crops and concluded that the inhibition in seed germination after treatment by the aqueous extracts is due to the damage caused to the seed membrane system. They reported that seed germination inhibition was concentration dependent. Al-Zahrani and Al-Robai (2007) found that the leaves aqueous extract of apple of Sodom (at 5, 10, 20, 40 and 60 %) has phytotoxic effect on seed germination of solanaceous crops and the germination percentage was reduced with increasing concentration of the aqueous extract. The delay in seed germination and the reduction in germination index might be due to the presence of water-soluble inhibitors in apple of Sodom extract (Yasin *et al.*, 2012). Furthermore, apple of Sodom has powerful toxic effect that makes it a potent candidate for bi-rational management of agricultural pests (Samreen *et al.*, 2009). 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 (Olofsson, 1998; Tawaha and Turk, 2003).

The current results showed that leaves aqueous extract of apple of Sodom inhibited seed germination of sorghum, millet, corn, and wheat. These results are in accordance with previous studies reported Umar *et al.* (2014). They carried out a pot experiment to investigate the allelopathic effect leaf aqueous extract of apple of Sodom at different concentrations (0,5,10,15 and 20%) on the growth of sorghum and corn. They concluded that leaf aqueous extract has poisonous effects that can suppress leaf production and plant height as well as fresh and dry weight of sorghum and corn (Umar *et al.*, 2014). In addition, Al-Zahrani and Al-Robai (2007) confirmed that apple of Sodom has potent toxic impact on healthy growth of beneficial plants.

This research results is also consistent with those of Shah *et al.* (2017) who studied the allelopathic effects of aqueous extracts of leaves, stems, and root of apple of Sodom on wheat crop at various concentrations. They concluded that apple of Sodom must be removed near wheat fields, because it releases some allelopathic compounds that cause serious losses to the crop. The current result are in agreement with that of Aslam *et al.* (2016). They designed an experiment to find out phytotoxic effects of apple of Sodom on seed germination and seedling length of wheat. It is found that leaves aqueous extracts of apple of Sodom have a toxic and inhibitory effect for the germination of seeds and seedlings growth of wheat. Also, it is reported 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 plant has allelopathic behavior and has extensively been used for the control of many plants. The phytochemical studies on the aerial parts of the plant showed

that the leaves contain mainly tannins, flavonoids, glycosides, steroids, saponins, and cardiac glycosides that might contribute its allelopathic effect (Sharma *et al.*, 2011; Umar and Mustapha, 2014). The presence of chemical compounds like coumarin, flavonoids, resin, phenol, and alkaloid in different parts of apple of Sodom, regardless of their quantity, has been found to prevent cell division, reduce induction and alter the stability of phytohormones, defect absorption and delivery of water in plant tissues. It is also reported that the aqueous leaf extract of apple of Sodom has allelopathic properties including germination inhibition and yield reduction (Chon *et al.*, 2002; Ghasemi, *et al.*, 2012).

## CONCLUSION

It is concluded that the leaves aqueous extract of apple of Sodom had toxic effect on the seed germination of the tested cereal crops. Therefore, it is necessary to take into account the effective management of apple of Sodom in the fields of cereal crops to prevent its adverse effect the seed germination rate and the density of the crops stand. Subsequent studies are expected to reveal the extent to which the active substances that present in different parts of the plant can be used to produce rational measures to combat weeds.

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## تأثير السمية النباتية للمستخلص المائي لأوراق نبات العشر (*Calotropis procera* (Aiton) W.T) على إنبات بذور بعض محاصيل الحبوب باستخدام تحليل «Probit»

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### الملخص

العديد من النباتات تعوق تطور نباتات أخرى؛ لأنها تطلق العديد من المركبات الكيميائية في البيئة. أجريت هذه الدراسة للتحقق من تأثير السمية النباتية للمستخلص المائي لأوراق نبات العشر [*Calotropis procera* (Aiton) W.T] على تثبيط إنبات بذور الذرة الرفيعة (*Sorghum bicolor* [L.] Moench) والدخن (*Pennisetum glaucum* [L.] R. Br.) والذرة الشامية (*Zea mays* L.) والقمح (*Triticum aestivum vulgare* L.) باستخدام تحليل «probit». أجريت التجارب في جامعة الجزيرة بالسودان في موسم 2015/2014، تم تحضير 10 تركيزات (2.11 إلى 21.05 جم / لتر) من المستخلص المائي لأوراق نبات العشر من المحلول الأساس (50 جم / لتر)، وتم تضمين المعاملة الشاهد المحتوية على الماء المقطر المعقم للمقارنة، وتم ترتيب المعاملات في تصميم كامل العشوائية بأربع مكررات، وتم فحص تأثير المستخلص المائي على تثبيط الإنبات في ثلاثة أيام بعد الإنبات الأولي، وخضعت البيانات لتحليل «Probit». أظهرت النتائج أن المستخلص المائي لأوراق نبات العشر له تأثير مثبط على إنبات بذور محاصيل الحبوب التي تم اختبارها، وكانت هناك علاقة موجبة مباشرة بين التركيز (جم / لتر) ونسبة التثبيط، كما أظهرت النتائج أيضاً أن قيمة التركيز القاتل النصفية ( $LC_{50}$ ) للذرة والقمح والدخن والذرة الرفيعة كان 17.6، 17.0، 19.2 و 16.7 جم / لتر، على التوالي. تم استنتاج أن المستخلص المائي لأوراق نبات العشر له تأثير سام على بذور محاصيل الحبوب التي تم اختبارها.

الكلمات المفتاحية: حشائش، سمية، عشبة، النجيليات.