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Phytotoxic Effects of Apple of Sodom [*Calotropis procera* (Aiton) W.T.] Leaves Aqueous Extract on Seed Germination of some Solanaceous Crops Using Probit Analysis

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Abstract:
Several plants are phytotoxic in nature as they produce and release many chemical compounds 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 sweet paper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.) and hot paper (*Capsicum frutescens* L.) using probit analysis**.** A laboratory experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in 2017. Ten concentrations (2.11, 4.21, 6.34, 8.42, 10.53, 12.63, 14.13, 16.84, 18.94 and 21.05 g/l) of the leaves aqueous extract of apple of Sodom were prepared from the stock solution (50 g / l). A control with sterilized-distilled water was included for comparison. Treatments were arranged in completely randomized design with four replicates. Inhibition of seeds germination was examined at consecutive three days after initial germination. Collected data were transformed using Abbott's formula and subjected to probit analysis procedure ($P \leq 0.5$). The leaves aqueous extract of Apple of Sodom inhibited the seed germination of tested solanaceous crops. The results displayed direct positive relationship between concentration (g/l) and inhibition (%). Also, the result revealed that the leaves aqueous extract of Apple of Sodom was more toxic to the seeds of the hot pepper compared to the seeds of the other tested solanaceous crops. There were no differences in inhibition of seed germination among the rest of tested crops. The LC_{50} for sweet pepper, tomato, eggplant and hot pepper was 14.88, 13.52, 13.46 and 5.35 g/l, respectively. It was concluded that that the leaves aqueous extract of Apple of Sodom had toxic effects to the seeds of the tested solanaceous crops.
Keywords: Allelo-chemicals; Apple of Sodom; *Calotropis*; eggplant; hot paper; phytotoxic; probit; sweet paper; tomato.

Introduction

Several plants are phytotoxic in nature as they produce and release many chemical compounds into the environment (Delabays *et. al.*, 2004). They are known as allelochemicals (Singh and Chaundhary, 2011). These allelochemicals are actually secondary metabolites and phytotoxic (Farooq et *al*., 2011). Phytotoxic impact of these compounds on other plants is usually dominant at early growth stages, causing inhibition of seed germination and seedling growth (Farooq et *al*., 2008; Jabran *et al*., 2010). The allelochemicals also have been traditionally considered as the compounds, which are synthesized by plants in order to manage the various biological processes (Viter *et al*., 2015). They are released from

plant parts by means of leaching, root exudation, volatilization from their foliage, residue decomposition and other processes in both natural and agricultural systems (Chou, 1990). Allelochemicals disrupt the numerous physiological and metabolic functions of plants such as nutrient and water uptake, photosynthesis, respiration and DNA synthesis (Einhelling, 2002). They can reduce cell division or auxin that induces the growth of shoot and roots (Gholami *et al.,* 2011). Allelochemicals such as phenolic compounds inhibit root and shoot length (Hussain and Reigosa, 2011). Growth inhibition caused by these allelochemicals may probably be due to its interference with the plant growth processes (Gholami *et al.,* 2011). Allelochemicals released to the environment can either inhibit shoot and/or root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's source of a nutrient. The allelochemicals could be 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 xerophytic perennial shrub or small tree. It is native to tropical and subtropical Africa, Asia and common in the Middle East. It grows on a variety of soils, range from fine to coarse texture, with varying degrees of salinity (Hassan, *et al*., 2015). It grows abundantly in arid and semiarid regions without irrigation, chemical fertilizers, pesticides or other agronomic practices (Sharma *et al*., 2012). The plant produces deep roots and root suckers, and rarely grows in shallow soils over unfractured rock (D'Souzaa *et al.*, 2010). It grows in open habitats and is particularly common in overgrazed pastures and poor soils, where there is little competition with grasses. It is also found along roadsides, watercourses, river flats and coastal dunes (Parsons and Cuthbertson, 2001; Francis 2003; Orwa *et al*., 2009). The importance of Apple of Sodom in the functioning of ecosystems is reflected in its hosting of butterflies, while it also acts as a food plant for arthropods. In addition, it is used for medicinal purposes in many arid countries. Potential new uses of this species in semi-arid regions include the phytoremediation of soils contaminated with trace elements and the use of biomass as a source of renewable energy (Hassan, *et al*., 2015). Apple of Sodom has widespread and persistent occurrence near sorghum, maize, cotton and sugarcane crop fields, which suggests some adverse effects on these crops through allelopathic interactions. Therefore, there is always a threat that it may become a major weed of cropping systems (Yasin *et al*., 2012).

Data obtained from bioassays are generally in percent response (germination % or inhibition %) at the corresponding concentration. The response is always binomial and the relationship between the response and the various concentrations is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship. Once a regression is run the LC_{50}, which represent concentration at which 50 % of the seeds inhibited, could be accurately measured. Considering the economic importance of solanaceous crops, this study was carried out to investigate the phytotoxic effects of the leaves aqueous extract of apple of Sodom seed germination of paper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), eggplant (*Solanum melongena* L.) and hot paper (*Capsicum frutescens* 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 2017. The laboratory has a temperature and relative humidity range between 25 - 30°C and 60 - 70 %, respectively.

Materials collection

Leaves of mature plants of Apple of Sodom were collected from Experimental Farm of the FAS in 2017. The leaves were transferred to the biology laboratory of the FAS. Then, they were washed with sterilized distill water and air dried on bench for 21 days in dark at room temperature, to avoid the direct effects of sun light that might cause undesired reactions. The dried leaves were then crushed into powder, by a commercial spice grinder, and kept in brown bottles till used. Certified seeds of sweet pepper, tomato, eggplant and hot pepper, that have a germination percentage of 95-100% and purity of 100%, were obtained from the central market at 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.

Preparation 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) by an orbital shaker (160 rpm). The aqueous extract of the leaves was filtered by a 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:

$$AC\ (g/l) = \frac{FW}{FV} \times 1000$$

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. The seeds of sweet pepper, tomato, eggplant and hot pepper (100 seeds each) were put 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 30 ml of tested concentrations of aqueous extract, sealed with Parafilm, ~~covered~~ wrapped with black polyethylene bag and incubated at 30^0C in the dark. The treatments, of each crop, were arranged in completely randomized design with four replicates (r). The seeds were examined for germination inhibition at consecutive three days after initial germination. The percentage of the inhibition of seed germination was calculated using the following equation:

$$Inhibition\ (\%) = \frac{Total\ number\ of\ seeds - number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100$$

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

$$Corrected\ Inhibition\ (\%) = \frac{X - Y}{X} \times 100$$

Where:

X is the % survivorship in the control group (germination % in the control treatment)

Y is the % survivorship in the experimental group (germination % in the concentration treatment)

Statistical analysis: Data were transformed using Abbott's formulaand subjected to probit analysis procedure. Results from probit analysis were reported as a concentration to inhibit a certain portion of the tested seeds to germinate (LC_{10}, LC_{50} and LC_{90}). The regression coefficient (slope) and intercept of the regression line of the probit transformed data were also reported. Goodness-of-fit of the regression line was indicated by the chi-square. Probit transformed data were converted back to the original units. The statistical analysis was done using the Microsoft Excel and SPSS software v.16 (SPSS, 2007).

Results

The results showed that the leaves aqueous extract of apple of Sodom inhibited the seed germination of the tested solanaceous crops; sweet paper, tomato, eggplant and hot paper. Moreover, the results displayed a direct positive relationship between concentration (g/l) and inhibition (%) (Figures 1, 2, 3 and 4). Plotting probits against log_{10}-concentration straightened the cumulative distribution line and the curve was transformed to more accurately to describe the data.

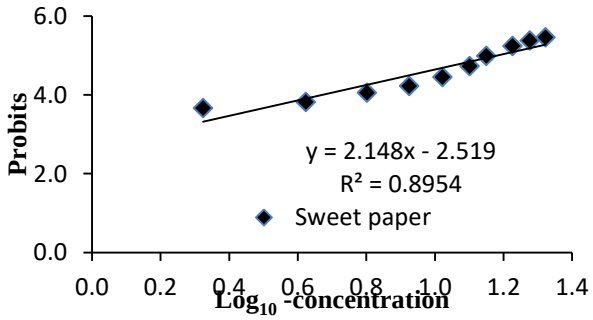

Figure 1. Relationship between Log_{10} of concentration of leaves aqueous extracts of apple of Sodom (*Calotropis procera* W.T) and probit of inhibition (%) of seed germination of sweet paper

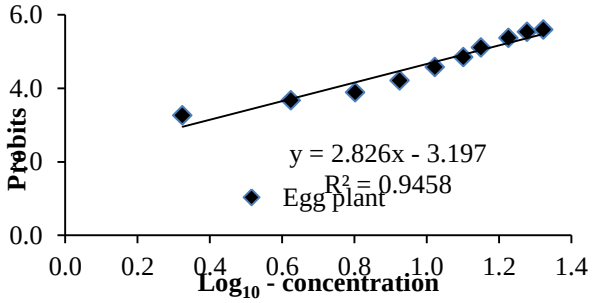

Figure 2. Relationship between Log_{10} of concentration of leaves aqueous extracts of apple of Sodom (*Calotropis procera* W.T) and probit of inhibition (%) of seed germination of eggplant

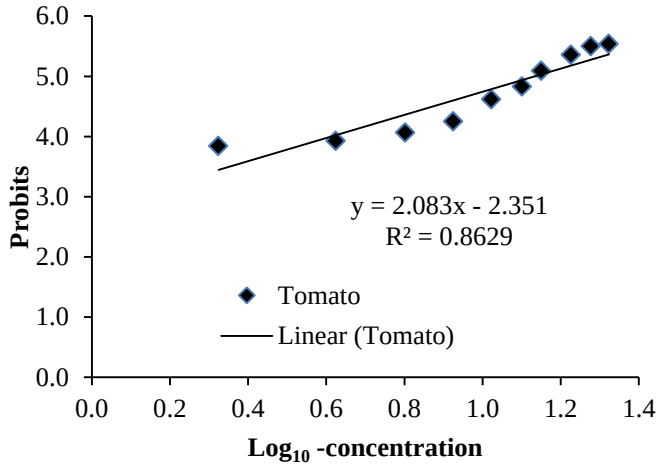

Figure 3. Relationship between Log_{10} of concentration of leaves aqueous extracts of apple of Sodom (*Calotropis procera* W.T) and probit of inhibition (%) of seed germination of tomato

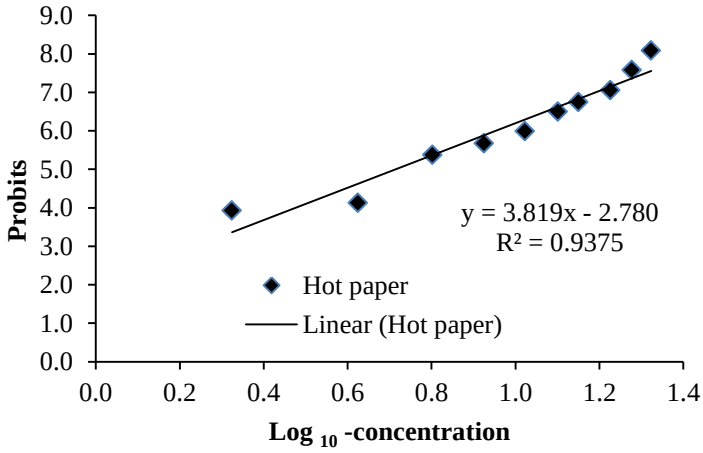

Figure 4. Relationship between Log_{10} of concentration of leaves aqueous extracts of apple of Sodom (*Calotropis procera* W.T) and probit of inhibition (%) of seed germination of hot paper

Phytotoxic effects of the leaves aqueous extract on:

a. sweet paper:

The simple linear regression equation was $Probit = 2.15\ log_{10}concentration - 2.52$. The value of coefficient of simple determination (R^2) was 0.8954.The LC_{10}, LC_{50} and LC_{90} were 3.8, 14.9 and 58.8 g/l, respectively (Table 1).

b. eggplant

The simple linear regression equation was $Probit = 2.83\ log_{10}concentration - 3.20$. The value of coefficient of simple determination (R^2) was 0.9458. The LC_{10}, LC_{50} and LC_{90} were 4.8, 13.5 and 38.4 g/l, respectively (Table 1).

b. tomato:

The simple linear regression equation was $Probit = 2.083\ log_{10}concentration - 2.351$. The value of coefficient of simple determination (R^2) was 0.8629. The LC_{10}, LC_{50} and LC_{90} were 3.3, 13.5 and 55.5 g/l, respectively (Table 1).

d. hot paper:

The simple linear regression equation was $Probit =$

3.319 $log_{10}concentration - 2.780$. The value of coefficient of simple determination (R^2) was 0.93.75. The LC_{10}, LC_{50} and LC_{90} were 2.5, 5.4 and 11.6 g/l, respectively (Table 1).

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

Solanaceous crops	No. of Tested seeds (Rep.)	Inhibition % values (minimum - maximum)			Chi^2	Df [a]	Sig.
		LC_{10}	LC_{50}	LC_{90}			
Sweet paper	400 (4)	3.8 (2.3-5.1)	14.9 (12.5-18.8)	58.8 (38.8-124.8)	83.3	8	0.00 [b]
Tomato	400 (4)	3.3 (1.7-4.7)	13.5 (11.0-17.5)	55.5 (35.1-137.4)	113.3	8	0.00 [b]
Eggplant	400 (4)	4.8 (3.6-5.8)	13.5 (12.1-15.4)	38.41 (30.3-55.2)	57.8	8	0.00 [b]
Hot paper	400 (4)	2.5 (1.7-3.1)	5.35 (4.5-6.2)	11.6 (10.0-14.2)	103.4	8	0.00 [b]

a. Statistics based on individual cases differ from statistics based on aggregated cases.
b. b. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.
c. Data between two bracts are 95% confidence limits for concentration (minimum - maximum).

Discussion

The results showed that the leaves aqueous extract of Apple of Sodom inhibited the seed germination of the tested solanaceous crops and the relationship between concentration (g/l) and inhibition (%) was directly positive. Also, the result revealed that the leaves aqueous extract of Apple of Sodom was more toxic to the seeds of the hot pepper compared to the other solanaceous crops, since the lowest LD_{50} (5.4 g/l) and highest R^2 (3.319) was recorded by of hot pepper. However, there were no differences in the degree of the toxicity among sweet pepper, tomato and eggplant. These findings are in lined with those of Al-Zahrani and Al-Robai (2007) who pointed out that the leaves aqueous extract of apple of Sodom at 5, 10, 20, 40 and 60 % has allelopathic effect on the seed germination of tomato and eggplant. The germination percentage was decreased with increasing concentration of the aqueous extract (Al-Zahrani and Al-Robai, 2007; Ghasemi *et al*., 2012). 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). Moreover, the plant has strong allelopathic potential and might be a candidate for biological control of weeds and insects (Samreen *et al*., 2009).

These results are in lined with Gulzar and Siddiqui (2017) whom carried out an experiment to investigate the effect of aqueous extract of apple of Sodom on the seed germination of *Brassica oleracea* var. botrytis. Seeds of brassica were soaked in solutions containing 20%, 40%, 60% and 80% concentrations of leaves aqueous extract of apple of Sodom. The results revealed that the higher concentrations (60% and 80%) of the aqueous extract significantly reduced seed germination of the brassica in comparison to untreated control. The inhibitory effect increases with the increase in the concentration of aqueous extract. They concluded that the delayed germination and low germination rate of the brassica 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 apple of Sodom had varying

degrees of inhibition on the seed germination of mustard plant (*Brassica nigra*). 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 (Olofsdotter, 1998; Tawaha and Turk, 2003).

The phytochemical studies on the aerial parts of the plan showed that the leaves contain mainly the amyrin, amyrin acetate, ß-sitosterol, ursolic acid, cardenolides, calotropin and calotropagenin that might contribute its allelopathic potential (Sharma *et al*., 2011). The inhibition in seed germination of the tested solanaceous 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; Olofsdotter, 1998; Tawaha and Turk, 2003) which is known to regulate de novo amylase production during germination process. The results displayed that the inhibition of seed germination of tested crops were increased with concentration of leaves aqueous extract of Apple of Sodom. These findings are in agreed with those of Oudhia (1999) who stated that the inhibition of seed germination was found to be concentration-dependent.

Conclusion

- It was concluded that that the leaves aqueous extract of Apple of Sodom had toxic effect to the seeds of the tested solanaceous crops. The toxicity is increased with concentration of leaves aqueous extract. Also, the leaves aqueous extract was more toxic to the seeds of the hot pepper compared to the seeds of the other tested solanaceous crops. The plant has allelopathic behavior and–therefore, could be used extensively for the control of many plants.

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تأثير السمية النباتية للمستخلص المائي لاوراق نبات العشر [*Calotropis procera* (Aiton) W.T] على إنبات بذور بعض المحاصيل الباذنجانية باستخدام تحليل "Probit"

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المستخلص

العديد من النباتات ذات طبيعة سمية نباتية لأنها تنتج وتطلق العديد من المركبات الكيميائية السامة في البيئة. أجريت هذه الدراسة للتحقق في تأثير السمية النباتية للمستخلص المائي لأوراق نبات العشر [*Calotropis procera* (Aiton) W.T] على تثبيط إنبات بذور الفلفل الحلو (*Capsicum annuum* L) والطماطم (*Lycopersicon esculentum* Mill) والباذنجان (*Solanum melongena* L) الفلفل الحار (الشطة) (*Capsicum frutescens* L) باستخدام تحليل "probit". أجريت هذه الدراسة في معمل كلية العلوم الزراعية ، جامعة الجزيرة ، السودان في عام 2017. تم تحضير 10 تراكيز (2.3 ، 4.6 ، 7.0 ، 9.3 ، 11.6 ، 13.9 ، 16.2 ، 18.5 ، 20.8 ، 23.2 جم / لتر) من المستخلص المائي لأوراق نبات العشر من المحلول الاساس (50 جم / لتر). تم تضمين المعاملة الشاهد المحتوية على الماء المقطر المعقم للمقارنة. تم ترتيب المعاملات في تصميم كامل العشوائية باربعة مكررات. تم فحص البذور لمعرفة النسبة المئوية للتثبيط (٪) في الإنبات في ثلاثة أيام بعد الإنبات الأولي. تم تحويل البيانات المجمعة باستخدام صيغة "Abbott" وأُخضعت لتحليل "Probit". ثبط المستخلص المائي لأوراق نبات العشر إنبات البذور للمحاصيل الباذنجانية المختبرة. أوضحت الدراسة أن هناك علاقة موجبة مباشرة بين التركيز (جم / لتر) والتثبيط (٪). كما أوضحت النتائج أن المستخلص المائي لأوراق نبات العشر كان أكثر سمية لبذور الشطة الحارة مقارنة ببذور المحصولات النباتية الأخرى التي تم اختبارها ، ولم تكن هناك فروق بين باقي النباتات. كانت قيمة التركيز القاتل النصفي (LC_{50}) للفلفلية وللطماطم وللباذنجان وللشطة الحارة هو 14.88 ، 13.52 ، 13.46 و 5.35 جم / لتر ، على التوالي. يستنتج من هذة الدراسة أن المستخلص المائي لأوراق نبات العشر له تأثير سام على بذور المحصولات النباتية التي تم اختبارها.