Phytotoxic Effects of Aqueous Extracts of Apple of Sodom (*Calotropis procera* W.T) on Seed Germination of *Ischaemum afrum* (J.F.Gmel.) Dandy Using Probit Analysis

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Abstract
This study was carried out to investigate the phytotoxic effects of aqueous extracts of leaves, inflorescences, stems and roots of apple of Sodom (Calotropis procera W.T) on seed germination of Ischaemum afrum using probit analysis. Laboratory experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in season 2014/15. Ten concentrations of the aqueous extract of each part of apple of Sodom were prepared by sequential dilution of the stock extract (50g/l) 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. Treatments were arranged in completely randomized design with four replicates. The seeds were examined for inhibition (%) in germination at three days after initial germination. Data were subjected to probit analysis procedure (P 0.5).The results showed that the aqueous extracts of all tested parts of apple of Sodom suppressed seed germination of the I. afrum and there was direct positive relationship between concentration (g/l) and inhibition (%). The result also revealed that the leaves aqueous extract of apple of Sodom was more toxic (LD_{50} = 6.3 g/l) to the seeds of I. afrum followed by aqueous extract of inflorescences (LD_{50} = 7.2 g/l), stems (LD_{50} = 11.4 g/l) and roots (LD_{50} = 12.7 g/l). The results indicated that the aqueous extracts of apple of Sodom had toxic effect to the seeds of the I. afrum and could offer potential for the development of alternative herbicides.

Keywords: Allelopathy; Apple of Sodom; Calotropis; Ischaemum; Phytotoxic; Probit

Effets Phytotoxiques D'extraits Aqueux de la Pomme de Sodom (*Calotropis procera W.T*) sur la Germination des Graines *D'Ischaemum afrum* (J.F.Gmel.) *Dandy* en Ultisant l'analyse par la méthode de Probit

Résumé
Cette étude a été réalisée dans le but d'analyser les effets phytotoxiques d'extraits aqueux de feuilles, d'inflorescences, de tiges et de racines de la pomme de Sodome (Calotropis procera W.T) sur la germination des graines d'Ischaemum afrum, en utilisant l'analyse de probit. Des expériences de laboratoire ont été effectuées à la Faculté des Sciences Agricoles de l'Université de Gezira, au Soudan, pendant la saison 2014/15. Dix concentrations de l'extrait aqueux de chaque partie de pomme de Sodom ont été préparées par dilution séquentielle de l'extrait de réserve (50g/l) avec de l'eau distillée stérilisée pour donner 2,11, 4,21, 6,34, 8,42, 10,53, 12,63, 14,13, 16,84. 18,94 et 21,05 g/l. Un contrôl avec de l'eau distillée stérilisée a été inclus à des fins

de comparaison. Les traitements ont été organisés de manière totalement aléatoire avec quatre répétitions. Les graines ont été examinées pour l'inhibition (%) de la germination trois jours après la germination initiale. Les données ont été soumises à la procédure d'analyse probit (P < 0,5). Les résultats ont montré que les extraits aqueux de toutes les parties testées de pomme de Sodome supprimaient la germination des graines de I. afrum et il existait une relation positive directe entre la concentration (g/l) et la concentration inhibition (%). Le résultat a également révélé que l'extrait aqueux de feuilles de la pomme de Sodome était plus toxique (DL50 = 6,3 g/l) pour les graines de I. afrum suivi d'un extrait aqueux d'inflorescences (DL50 = 7,2 g/l), de tiges (DL50 = 11,4 g/l) et les racines (DL50 = 12,7 g/l). Les résultats ont indiqué que les extraits aqueux de la pomme de Sodom avaient un effet toxique sur les graines de I. afrum et pourraient offrir un potentiel pour le développement d'herbicides alternatifs.

Mots-clés: allélopathie; Pomme de Sodome; Calotropis; Ischaemum; Phytotoxique; Probit

Introduction

Food insecurity caused by rapid population growth has put pressure on researches to produce several synthetic agrochemicals to maximize the yield potentials of crops. However, these agrochemicals cause pollution and degradation of the natural environment (Dragoeva *et al*., 2015). A possible alternative to synthetic agricultural chemicals is *via* the use of allelopathy (Dragoeva *et al*., 2015). Allelopathy phenomenon has been defined as suppression of growth of one plant species by another through release of phytotoxic substances into the surrounding environment through litter decomposition, root exudation or direct volatilization (Bhadoria, 2011). These substances are known as allelochemicals (Singh and Chaundhary, 2011). Allelochemicals, secondary metabolites, are toxic to other plants at early growth stages, affecting seed germination and seedling growth (Farooq *et al*., 2008; Jabran *et al*., 2010). Several weed and crop species have been reported to possess allelochemicals which are present in many plants and in many plant parts, including roots, stems, buds, leaves, flowers, inflorescences, fruits and seeds (Yasin *et al.*, 2012; Gulzar and Siddiqui, 2017). In sustainable agriculture, allelochemicals are potential for the development of future herbicides and proposed for weed management (Dragoeva *et al*., 2015).

One of the plants noted for having some adverse effects on other plants through allelopathic interactions is apple of sodom [*Calotrois procera* (Aiton) W.T.] (Yasin *et al*., 2012). Apple of Sodom, is a member of the family Asclepiadaceae (Parihar *et al*., 2011). It is distributed throughout the tropical and sub-tropical regions in the world (Hassan, *et al*., 2015). It is mostly grown 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; Francis, 2003; Orwa *et al*., 2009). Apple of Sodom has a perennial growth habit with tall and erect stem having large number of branches thus assuming the shape of a shrub or sometimes small trees which can grow up to 2-3 m height (D'Souzaa *et al.*, 2010). It propagates mainly through the seed which are transported by wind and water, while asexual reproduction occurs through suckers from the roots (Parsons and Cuthbertson, 1992).

Ischaemum afrum (J.F.Gmel.) Dandy, belonging to the family poaceae, is a tall, tufted, erect or geniculate perennial grass with

linear, flat or glabrous leaves. Flowers are racemes and has 2-3 fascicled, yellow-brown or brownish color. It is widespread in tropical and semitropical regions in many countries of Africa and Asia (Tayalla *et al.*, 1988). *Ischaemum spp*. are noxious weeds in many countries of the world and to many crops (Holm *et al*., 1977). It is adapted to a wide variety of habitats. However, there is little information in literature on the biology and ecology of *Ischaemum afrum*. Because of long coleoptile length of this weed, it can even emerge when buried at 10 cm depth (Bakar and Nabi, 2003). *Ischaemum afrum* causes significant growth reduction, yield losses and contaminates crops. The plant has a high level of seed dormancy, because of the presence of glumes, which delay seed germination after shedding (Marenco and Santos, 1999). Controlling this weed by herbicides is difficult and the continuous use of herbicides for control has developed multiple resistances to herbicides belonging to different modes of action (Valverde, 2007). For the purpose of finding an effective and safe alternative way to combat *Ischaemum afrum*, there is a need to answer the following questions: Does apple of Sodom has a toxic effect on the germination of the seeds of weed? If the answer is yes, which part of the apple has a higher toxicity and what is the value of the lethal concentration (LC_{50}) for each part.

Considering the economic importance of the weed *Ischaemum afrum*, this study was carried out to investigate the phytotoxic effects of aqueous extracts of leaves, inflorescences, stems and roots of apple of Sodom (*Calotropis procera* W.T) on seed germination of *Ischaemum afrum* using probit analysis.

Materials and Methods

Experimental site

Germination tests were conducted in the biology laboratory at the Faculty of Agricultural Sciences (FAS), University of Gezira (UofG), Sudan in season 2014/15. The laboratory has an average temperature range between 25 - 30°C and the relative humidity ranging between 60 - 65 %.

Materials collection

Leaves, inflorescences, stems and roots of mature plants of apple of Sodom were collected from Experimental Farm (EF) of the FAS in season 2014/15. The plants parts were transferred to the biology laboratory of the FAS. Then, each part of the plant was washed with sterilized-distilled water, air dried on bench for 20 days at room temperature and in a dark room to avoid the direct sun light that might cause undesired reactions. The seeds of *I. afrum* that have a germination percentage of 98±1 and purity of 100% were collected from the EF of the FAS in season 2014/05. 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-distilled water for several times and stored at room temperature till used.

Preparation and calculation of the actual concentration of the aqueous extract

Fifty grams, initial weight (IW), of the powder of each part of apple of Sodom were placed in a conical flask, sterilized-distilled water was added to give a volume of 1000 ml and then the flasks were shaken for 24 hours at room temperature (28±2°C) by an orbital shaker (160 rpm). The aqueous extract of each part of the plant was filtered by a muslin cloth and the leachate was dried and the final weight (FW) of the filter or the weight of the precipitation (cake) was calculated by a sensitive balance. The final volume (FV) of the aqueous extract for each part of apple of Sodom was calculated by measuring cylinder. The actual concentration (AC) of the aqueous extract of each part of the plant was calculated

using the following equation:

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

Bioassay procedure

Ten concentrations (n) of the aqueous extract of each part of apple of Sodom 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. Seeds of *I. afrum* (100 seeds) were put on Glass Fiber Filter Paper (GFFP) placed in a glass Petri-dish (GPD), 9 cm internal diameter. Each GPD moistened with 20 ml of each part of apple of Sodom aqueous extract, sealed with Parafilm, covered with black polyethylene bag and incubated at 30^0C in the dark. The treatments, of each part of the plant, were arranged in completely randomized design with four replicates (r). The seeds were examined for germination at three days after emergence. The percentage of the inhibition of seed germination was calculated using the following equation:

Statistical analysis

Data collected were subjected to probit analysis and the results were expressed as a concentration to inhibit a certain portion of the tested seeds (LC_{10}, LC_{50} and Lc_{90}). The concentration (g/l) was transformed to log_{10}-concentration, (independent variable, X) and the corrected inhibition (%) was transformed to probits (dependent variable, Y) by using Finney's table (Randhawa, 2009). The simple linear regression equation is:

$$Y = \alpha + \beta X$$

Where:

Y: Probit value
X: Log_{10}- concentration
α: intercept
β: regression coefficient, the slope

The regression coefficient 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).

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

The inhibition (%) was corrected using Abbotts formula. It is given by:

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

Where:

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

Results

The results showed that the aqueous extracts of the leaves, inflorescences, stems and roots apple of Sodom inhibited the seed germination of the *I. afrum* and there was direct positive relationship between concentration (g/l) and inhibition (%) (Fig. 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. Probit analysis transformed the sigmoid concentration-response curve to a straight line. Hence, the LC_{10}, LC_{50} and LC_{90} were accurately estimated.

Phytotoxic effects of the leaves aqueous extract

The simple linear regression equation was *Probit* = 2.54 *log_{10} concentration* - 2.04. The value of the coefficient of simple determination (R^2) was 0.92. The LC_{10}, LC_{50}

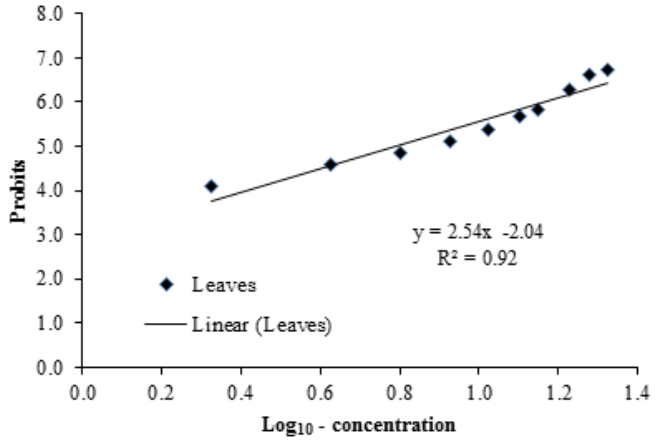

Fig. 1. Relationship between Log_{10} of concentration of leaves aqueous extracts of apple of Sodom ©. *procera*) and probit of inhibition (%) of seed germination of *I. afrum*

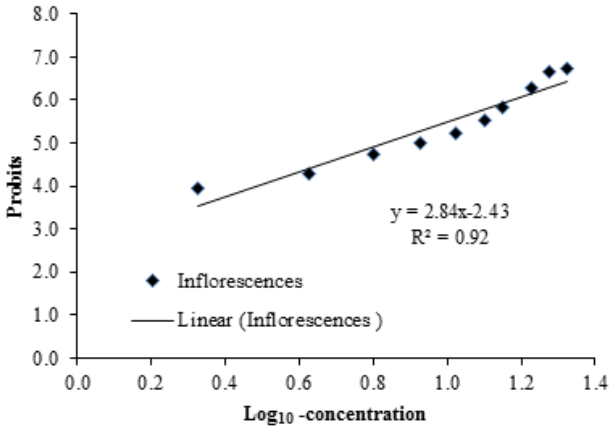

Fig. 2. Relationship between Log_{10} of concentration of inflorescences aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of *I. afrum*

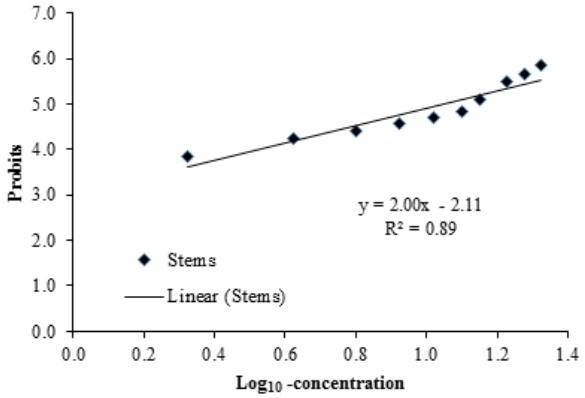

Fig. 3. Relationship between Log_{10} of concentration of stems aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of *I. afrum*

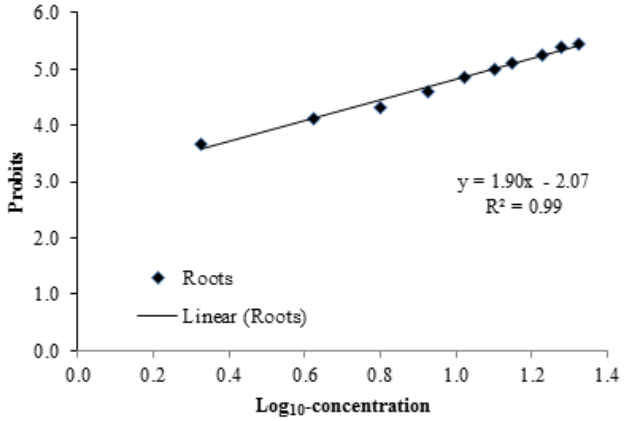

Fig. 4. Relationship between Log_{10} of concentration of roots aqueous extracts of apple of Sodom (*C. procera*) and probit of inhibition (%) of seed germination of *I. afrum*

and LC_{90} were 2.0, 6.3 and 20.2 g/l, respectively (Table 1).

Phytotoxic effects of the inflorescences aqueous extract

The simple linear regression equation for the relationship between the inhibition and the concentration of the inflorescences aqueous extract was estimated as follows: Probit = 2.48 $\log_{10}$ concentration - 2.43. The value of the (R^2) was 0.89, whereas the Lc_{10} LC_{50} and LC_{90} were 2.6, 11.4 and 49.7 g/l, respectively (Table 1).

Phytotoxic effects of the stems aqueous extract

The relationship between the inhibition and the concentration of the stems aqueous extract was described by the following formula: Probit = 2.00 $\log_{10}$ concentration - 2.11. The value of the (R^2) was 0.89, whereas the LC_{10} LC_{50} and LC_{90} were 2.6, 11.4 and 49.7 g/l,

Table 1. Phytotoxic effects of the leaves aqueous extracts of apple of Sodom *(C. procera*) on inhibition (%) of seed germination of *I. afrum* using probit analysis

Plant Parts	No of Tested Seeds (Rep)	Inhibition % values (95% Confidence limits for concentration)			Chi^2	Df [a]	Sig.
		Lc_{10}	Lc_{50}	LC_{90}			
Leaves	400	2.0	6.3	20.2	84.0	8	0.00 [b]
	(4)	(1.2 - 2.7)	(5.2 - 7.4)	(16.3 - 27.5)			
Inflorescences	400	2.5	7.2	20.3	109.8	8	0.00 [b]
	(4)	(1.6 - 3.4)	(5.9 - 8.4)	16.4 - 28.3)			
Stems	400	2.6	11.4	49.7	94.1	8	0.00 [b]
	(4)	(1.3 - 3.8)	(9.3 - 14.1)	(32.9 - 106.4)			
Roots	400	2.6	12.7	60.9	8.1	8	0.42 [c]
	(4)	(2.3 - 3.0)	(12.0 - 13.4)	(52.5 - 72.0)			

respectively (Table 1).

Phytotoxic effects of the roots aqueous extract

The simple linear regression equation for the relationship between the inhibition and the concentration of the roots aqueous extract was Probit = 1.90 $\log_{10}$ concentration - 2.07. The value of coefficient of simple determination (R^2) was 0.99. The LC_{10} LC_{50} and LC_{90} were 2.6, 12.7 and 60.9 g/l, respectively (Table 1).

Discussions

The results showed that the aqueous extracts of different parts of the apple of Sodom inhibited the seed germination of the *I. afrum* and there was direct positive relationship between concentration and inhibition. Also, the result revealed that the leaves aqueous extract of apple of Sodom was more toxic (LD_{50} = 6.3 g/l) to the seeds of *I. afrum* followed by aqueous extract of inflorescences (LD_{50} = 7.2 g/l), stems (LD_{50} = 11.4 g/l) and roots (LD_{50} = 12.7 g/l).

These findings were similar to the results obtained by Gulzar and Siddiqui (2017) who studied the effects of soaking the seeds of *Brassica oleracea* in aqueous extract containing 20%, 40%, 60% and 80% concentrations of leaf, fruit and flower of apple of Sodom on germination percentage, seedling growth, dry biomass, and relative water content. It was reported that higher concentrations of extract (60% and 80%) significantly decreased germination percentage, radicle length, plumule length, dry matter accumulation, and relative water content of the seedlings of *B. oleracea*. The inhibitory effect increased with the increase in the concentration of three types of extract used, with more pronounced effect noticed by leaf extract followed by fruit and flower extracts. There were significant interactions among the different concentrations of extracts used, type of extract with respect to germination percentage, seedling length, dry biomass, and relative water content. The same authors also pointed out that the delayed germination and low germination rate of *B. oleracea* after treatment by extracts could be due to the fact that extracts damaged the membrane system of the seeds and apple of Sodom might have released phenolic compounds into the soil and these are probably involved in the growth retardatory

effect of test species.

The results are consistent with that of Shah *et al.* (2017) who studied the phytotoxic effects of different concentrations of aqueous extracts of leaves, stems and root of milkweed (*Calotropis procera*) on the wheat crop. Their results showed that the reduction of all studied parameters, including weeds, growth and yield of wheat was proportional to the concentration of aqueous extracts of milkweed in use. The level of inhibition was concentration-dependent as inhibition increased with an increase in the concentration of aqueous extracts of all three plant parts of milkweed. They concluded that application of maximum concentration (40%) of aqueous leaf, stem and root extracts correspondingly reduced all the studied parameters. It was concluded that the *Calotropis procera* should be removed near the cultivating wheat fields because it contains some phytotoxic substances, which may be leached out by the roots and cause serious losses to the growers and crop species.

Allelochemicals interfere with the seed germination, plant development and cause morphological change at cellular or molecular level. Alterations in the mitotic activity and disturbances in different phases of mitotic division are indicators of cytotoxic effect (Dragoeva *et al*., 2015). The leaves aqueous extract of *C. procera* at 5, 10, 20, 40 and 60 % has phytotoxic effect on the seed germination of tomato and eggplant. The germination % was reduced 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, this plant has strong allelopathic potential and might have a potential for biological control of weeds and insects (Samreen *et al*., 2009).

The phytochemical screening of the aerial parts of the plant showed that the leaves contain amyrin, amyrin acetate, ß-sitosterol, ursolic acid, cardenolides, calotropin and calotropagenin, and that might contribute to its allelopathic effect (Sharma *et al*., 2011). Suppression in seed germination of *I. afrum* weed as a result of allelochemical stress may be due to suppression of water uptake, cell division, elongation and alteration in the activity of gibberellic acid (Tawaha and Turk, 2003; Gholami *et al.,* 2011) which is known to regulate de novo amylase production during germination process. The suppression of seed germination was found to be concentration-dependent (Gulzar and Siddiqui, 2017).

Conclusion

The research findings indicated that the aqueous extracts of all tested parts of apple of Sodom suppressed seed germination of the *I. afrum*. The leaves aqueous extract recorded the highest toxicity of LD_{50} = 6.3 g/l whereas the root extract recorded the lowest toxicity of LD_{50} = 12.7 g/l. The apple of Sodom could therefore offer potential for the development of alternative herbicides. Subsequent studies are recommended to reveal the extent to which the active substances that present in different parts of the plant that can be used to produce rational measures to combat weeds.

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