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Phytotoxic Effects of Thorn Apple (*Datua stramonium* L.) Seed Aqueous Extract on Seed Germination of some Leguminous Crops Using Probit Analysis

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Abstract*: Solanaceae plants are phytotoxic in nature as they produce and release many allelochemical compounds into the ecosystem. This study was carried out to investigate the phytotoxic effects of the seeds aqueous extract of thorn apple (Datua stramonium L.) on seed germination of common bean (Phaseolus vulgaris L.), cowpea (Vigna sinensis [L.] Walp.), pigeon pea (Cajanus cajan [L.] Millsp.) and alfalfa (Medicago sativa L.) using probit analysis. Laboratory experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in season 2014/15. Ten concentrations (4.62, 9.26, 13.87, 18.51, 23.12, 27.74, 32.36, 36.98, 41.61 and 46.28 g/l) of the seeds aqueous extract of D. stramonium were prepared from the stock solution (100 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 ($P \leq 0.5$). The results showed that the seeds aqueous extract of D. stramonium inhibited the seed germination of the tested leguminous crops and there was direct positive relationship between concentration (g/l) and inhibition (%).The results also showed that the LC50 for common bean, cowpea, pigeon pea and alfalfa was 20.7, 21.9, 22.2 and 22.2 g/l, respectively. It was concluded that that the aqueous extract of thorn apple (D. stramonium L.) was phytotoxic to the seed germination of the tested leguminous crops.*

Keywords: Alfalfa; Allelopathy; Common bean; Cowpea; *Datura*; LC_{50}; Legume; Pigeon pea

1. INTRODUCTION

Solanaceae plants are phytotoxic in nature as they produce and release many allelochemical compounds into the ecosystem. These plants are phytotoxic in nature as they produce and release many chemicals into the surrounding ecosystem [1]. These chemicals, known as allelochemicals, are secondary metabolites and synthesized by plants in order to manage the various biological processes [2]. Allelochemicals are present in different parts of the plant such as roots, stem, leaves, flowers, inflorescences, fruits and seeds and could be released into the environment by root exudation, leaching, volatilization and decomposed plant material [3]. The toxic effect of allelochemicals on other plant species is usually noticeable at stages of seed germination and seedling growth [4, 5]. Aqueous extract of plants may interfere with crop germination and seedling growth by causing plant growth inhibition, causing nutrient transformation and/or by influencing the microbial population that can affect the crop seedlings [6]. Also, it influences the cell division, cell elongation, membrane permeability, enzyme activity, etc. [7].

Several studies conducted by many researchers showed that thorn apple (*Datua stramonium* L.) causes both, direct and indirect damage throughout allelopathic substances [8, 9, 10]. *D. stramonium* is a strong-scented annual plant of Solanaceae family. The plant is an annual and grows to 1.2 m high. It has a simple or bifurcated, round, erect glabrous stem. The leaves are 20 cm long, long-petioled, ovate, dentate, glabrous and dark green. The flowers are large, white, solitary, terminal or in the branch bifurcations. The calyx has a long 5-edged and short 5-tipped tube. The corolla is funnel shaped and folded with a short 5-sectioned border. There are 5 free stamens and 1 superior ovary. The fruit is a 5 cm long 4-valved capsule, which is densely thorny and walnut-sized. The numerous seeds are 3.5 mm long, flat, reniform and black [11]. It is often found along roadsides, wastelands, garbage dumps, but also in parks, gardens and other sites on nitrogen-rich soils. It is also among troublesome invasive alien species that releases allelochemicals to the environment, suppressing the development of native plants [12]. Considering the economic importance of leguminous crops, this study was carried out to investigate the phytotoxic effects of the aqueous extract of the seed powder of the thorn apple (*Datua stramonium* L.) on seed germination of common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna sinensis* [L.] Walp.), pigeon pea (*Cajanus cajan* [L.] Millsp.) and alfalfa (*Medicago sativa* L.) using probit analysis.

2. MATERIALS AND METHODS

2.1. 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 %.

2.2. Materials Collection

Mature fruits of thorn apple plants were collected from Experimental Farm of the FAS in season 2014/15. The fruits were transferred to the biology laboratory of the FAS. Seeds were collected from the fruits and 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 seeds were then crushed into powder and kept in brown bottles till used. Certified commercial seeds of common bean, cowpea, pigeon pea and alfalfa, that having a germination percentage of 98-100% and purity of 100%, were obtained from the central market of Wed Medani city, Gezira stat, 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.

2.3. Preparation and calculation of the actual concentration of the leaves aqueous extract

Hundred grams, initial weight (IW) of the seed powder of *D. stramonium* plants 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 (27±3°C) by an orbital shaker (160 rpm). Subsequently, the aqueous extract of the seeds 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 aqueous extract of the *D. stramonium* seeds was measured by measuring cylinder.

The final weight (FW), dissolved powder, was calculated using the following equation:

$$FW = IW - PW \quad (1)$$

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

$$AC\ (g/l) = \frac{FW}{FV} \times 1000 \quad (2)$$

2.4. Bioassay Procedure

Ten concentrations (n) of the aqueous extract of the seed powder of *D. stramonium* were prepared by sequential dilution of the stock extract with sterilized-distilled water to give 4.62, 9.26, 13.87, 18.51, 23.12, 27.74, 32.36, 36.98, 41.61 and 46.28 g/l. A control with sterilized-distilled water was included for comparison. Seeds of common bean, cowpea, pigeon pea and alfalfa (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 100 ml of the aqueous extract of the seed powder of *D. stramonium*, sealed with Parafilm, covered 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 at three days after initial germination. The percentage of the inhibition of seed germination was calculated using the following equation:

$$Inhibition\ (\%) = \frac{Total\ number\ of\ seeds - number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100 \quad (3)$$

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

2.5. 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 [13]. The simple linear regression equation is:

$$Y = \alpha + \beta X \quad (4)$$

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).

3. RESULTS

The results showed that the aqueous extract of the seed powder of *D. stramonium* inhibited the seed germination of the tested leguminous crops; common bean, cowpea, pigeon pea and alfalfa and there was direct positive relationship between concentration (g/l) and inhibition (%) (Fig. 1, 2, 3 and 4). Probit analysis transformed the sigmoid concentration-response curve to a straight line. Hence, the LC_{10}, LC_{50} and LC_{90} were accurately estimated.

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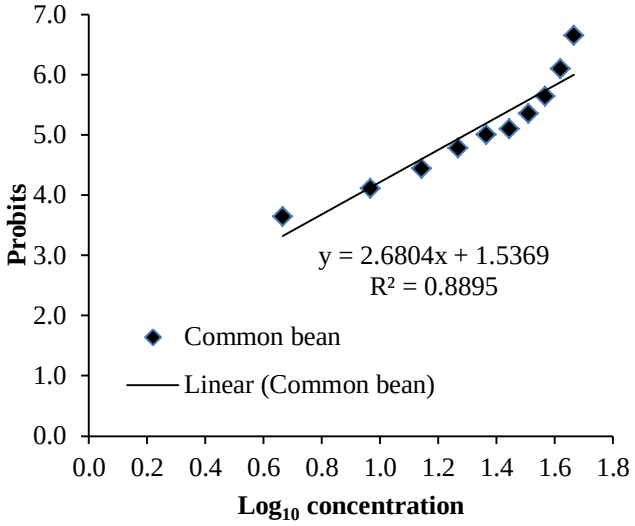

Figure 1. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of Common bean

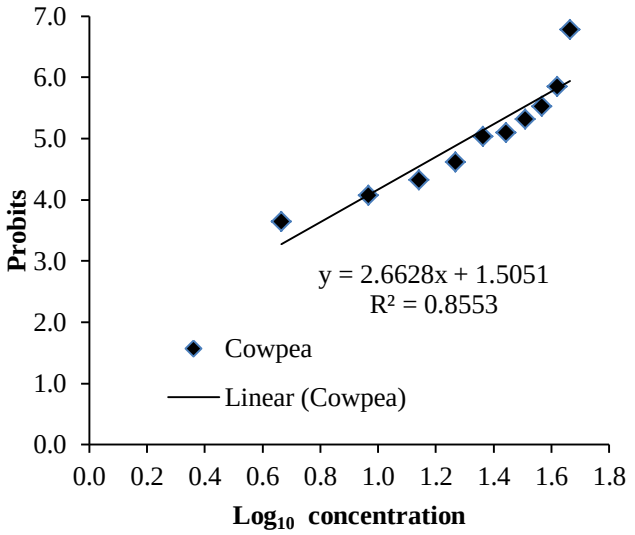

Figure 2. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of Cowpea

3.1. Effect on common bean

The simple linear regression equation was $Probit = 2.68\ log_{10}concentration + 1.54$. The value of coefficient of simple determination (R^2) was 0.89. The LC_{10}, LC_{50} and LC_{90} were 6.9, 20.7 and 62.1 g/l, respectively (Table 1).

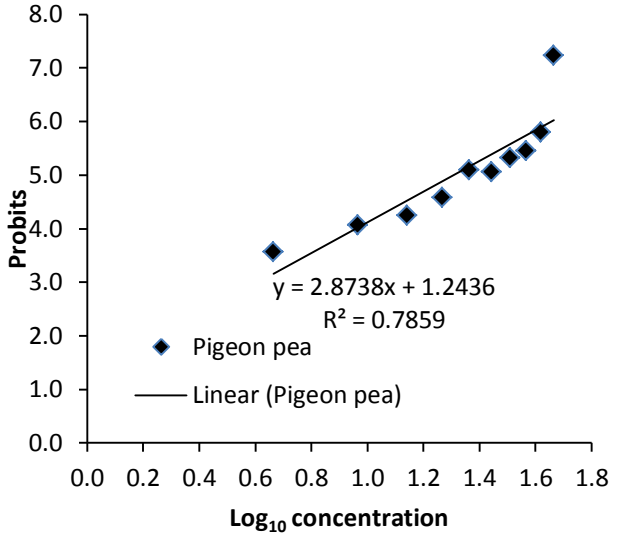

Figure 3. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of Pigeon pea

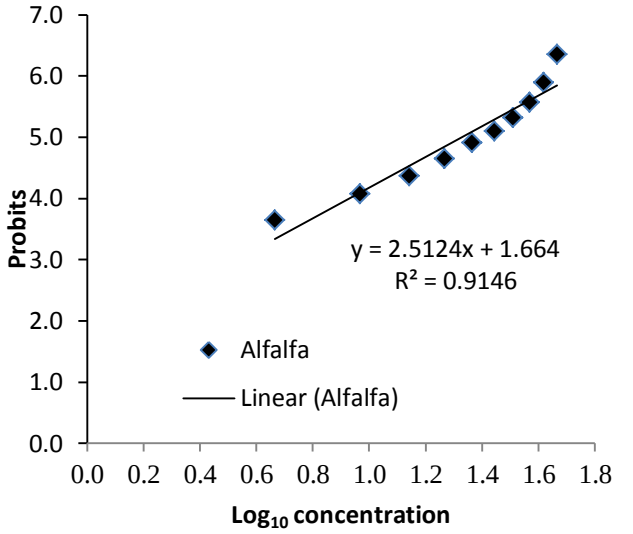

Figure 4. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of Alfalfa

3.2. Phytotoxic effect on cowpea

The simple linear regression equation was $Probit = 2.66\ log_{10}concentration + 1.51$. The value of coefficient of simple determination (R^2) was 0.86. The LC_{10}, LC_{50} and LC_{90} were 7.8, 21.9 and 66.1 g/l, respectively (Table 1).

Table 1. Phytotoxic effects of the aqueous extract of the seed powder of *D. stramonium* on inhibition (%) of seed germination of some cereal crops using probit analysis

Leguminous crops	No. of tested seeds (Rep.)	Inhibition % values (95% Confidence limits for concentration)			Chi^2	Df [a]	Sig.
		LC_{10}	LC_{50}	LC_{90}			
Common bean	400 (4)	6.9 (4.2-9.3)	20.7 (17.4-24.4)	62.1 (47.8-95.8)	101.1	8	0.000 [b]
Cowpea	400 (4)	7.8 (4.3-9.8)	21.9 (18.2-26.1)	66.1 (49.7-108.1)	1.22.8	8	0.000 [b]
Pigeon pea	400 (4)	7.6 (4.2-10.5)	22.2 (18.2-26.9)	64.8 (48.0-111.9)	150.4	8	0.000 [b]
Alfalfa	400 (4)	7.1 (4.6-9.4)	22.2 (19.0-25.8)	69.1 (53.3-104.3)	87.1	8	0.000 [b]

a. Statistics based on individual cases differ from statistics based on aggregated cases.
b. Since the significance level is less than .150, a heterogeneity factor is used in the calculation of confidence limits.

3.3. Phytotoxic effect on pigeon pea

The simple linear regression equation was $Probit = 2.87\ log_{10}concentration + 1.24$. The value of coefficient of simple determination (R^2) was 0.79. The LC_{10}, LC_{50} and LC_{90} were 7.6, 22.2 and 69.1 g/l, respectively (Table 1).

3.4. Phytotoxic effect on alfalfa

The simple linear regression equation was $Probit = 2.51\ log_{10}concentration + 1.66$. The value of coefficient of simple determination (R^2) was 0.92. The LC_{10}, LC_{50} and LC_{90} were 7.1, 22.2 and 69.1 g/l, respectively (Table 1).

4. Discussions

The results showed that the aqueous extract of thorn apple (*D. stramonium*) seed inhibited the seed germination of the tested leguminous crops and there was direct positive relationship between concentration and inhibition. The results also showed that the LC_{50} for common bean, cowpea, pigeon pea and alfalfa was 20.7, 21.9, 22.2 and 22.2 g/l, respectively. These findings were consistent with those of [10] who conducted laboratory and greenhouse trials to study the allelopathic effects of *D. stramonium* weed on seed germination, early seedling growth and dry biomass of *Vigna unguiculata*. Results from the study indicated that germination, shoot length and dry weight significantly decreased proportionally ($P < 0.001$) as the concentration of the aqueous leaf extracts *of D. stramonium* at 2, 4, 6 and 8% increased from 2 to 8%. The results showed that *D. stramonium* has allelopathic effects on cowpeas, hence cannot be used as a bio herbicide to control weeds on the cowpeas since it is non-selective to the crops studied.

The results were consistent with those of [14] who carried out a laboratory experiment to investigate the allelopathic effects of the *D. stramonium* extracts on seed germination, plant height and dry mass of soybean (*Glycine max* (L.) Merrill.) plant. The researchers showed that the *D. stramonium* aqueous extracts, applied in concentrations of 10%, 40% and 60%, inhibited the soybean plants. The maximum allelopathic effects were detected in the experimental variants treated with the highest concentration of aqueous extracts (60%). This finding was in line with observation made by [15] who reported that the aqueous leachates of different parts of thorn apple produced a significant inhibition effect on germination of chickpea. Reference [16] found that the aqueous extracts of the aerial parts of thorn apple pose significant and varying allelopathic effects on seed germination and seedling vigor of neighboring plants. Germination was inhibited as well as delayed according to the extract concentrations. Reference [16] also pointed out that the extracts inhibited germination process and the plant should be eradicated even if found near to the growing fields because the seeds contain the dangerous alkaloids and can be dispersed in the fields during seed dispersal. These seeds remain in dormant stage for years and when their dormant stage is over they grow vigorously and could make the precious fields toxic for the crop species.

It has been reported that sixty-four tropane alkaloids have been detected in *D. stramonium* plant, whereby the highest alkaloid concentration being found in seeds [17]. *Datura stramonium* expresses the allelopathic effect on several species, reducing the yield of cereal crops, especially sorghum, millet, maize and wheat [18, 19] and biodiversity of local flora. The aqueous extracts of *D. stramonium* seeds contain high concentration of saponins, steroids, alkaloids and glycosides [20]. Saponins, flavonoids, alkaloids, glycosides and phenol are common among crude aqueous extract of the plant [21]. The primary biologically active substances in *D. stramonium* are the alkaloids atropine and scopolamine [22]. Although many allelopathic substances have been extracted and identified from *D. stramonium*, the biochemists believe there are still many allelopathic substances that have not been yet identified. According to [23], allelochemicals inhibit water uptake which is a precursor to physiological processes that should occur in

seed before germination is triggered hence affecting germination [24, 25]. Reduced water uptake resulted in reduced imbibition leading to delayed germination and emergence. Suppression of seed germination of the tested cereal crops caused by allelochemical stress could be also due to inhibition of water uptake, cell division, cell elongation and changing in the activity of gibberellic acid [1] which is known to regulate de novo amylase production during germination process. The inhibition of seed germination was found to be concentration-dependent [26].

CONCLUTION

The seeds aqueous extract of *D. stramonium* inhibited the seed germination of the tested leguminous crops and there was direct positive relationship between concentration (g/l) and inhibition (%). The LC_{50} for common bean, cowpea, pigeon pea and alfalfa was 20.7, 21.9, 22.2 and 22.2 g/l, respectively. It was concluded that that the aqueous extract of thorn apple (*D. stramonium* L.) was phytotoxic to the seed germination of the tested leguminous crops.

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