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Original article

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

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Abstract

Solanaceae plants are strong allelopathic 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 seeds aqueous extract of thorn apple (*Datua stramonium* L.) on seed germination of sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetum glaucum* [L.] R. Br.), maize (*Zea mays* L.) and wheat (*Triticum vulgare* 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 transformed using Abbott's formula and subjected to probit analysis ($p \leq 0.05$). The results showed that the seeds aqueous extract of *D. stramonium* inhibited the seed germination of the tested cereal crops and there was direct positive relationship between concentration (g/l) and inhibition (%). The results also showed that the seeds of wheat (LC_{50} = 22.6 g/l) were most sensitive to the seeds aqueous extract of thorn apple followed by the seeds of sorghum (LC_{50} = 26.5 g/l) and maize (LC_{50} = 27.9 g/l). However, the extract was less toxic to the seeds of millet (LC_{50} = 32.2 g/l). It was concluded that that the aqueous extract of thorn apple (*D. stramonium* L.) was phytotoxic to the seed germination of the tested cereal crops.

Keywords: allelopathy, *Datura*, LC50, maize, millet, Poeaeae, sorghum, wheat.

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INTRODUCTION

Solanaceae plants are strong allelopathic in nature as they produce and release many chemical compounds into the environment. These plants are phytotoxic in nature as they produce and release many chemicals into the surrounding ecosystem (Mushtaq and Siddiqui, 2018). These chemicals, known as allelochemicals, are secondary metabolites and synthesized by plants in order to manage the various biological processes (Farooq et al., 2011). 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 (Chou, 1990). The toxic effect of allelochemicals on other plant species is usually noticeable at stages of seed germination and seedling growth (Farooq et al., 2008; Jabran et al., 2010). 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 (Verma et al., 2012). Also, it influences the cell division, cell elongation, membrane permeability, enzyme activity, etc. (Dragoeva, 2015).

Several studies conducted by many researchers showed that thorn apple (*Datua stramonium* L.) causes both, direct and indirect damage throughout allelopathic substances (El-Shora al., 2018; Novak et al., 2018; Sakadzo et al., 2018). *D. stramonium* is a strong-scented annual plant of Solanaceae family. The stem is erect, branched, with smooth toothed leaves and single, trumpet-shaped white flowers. The fruit is egg-shaped spiny capsule, filled with small, black seeds. It is native to Central America and is present as an alien invasive plant species in almost all temperate and tropical regions of the world. 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 (Bikic et al., 2017). Considering the economic importance of cereal 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 sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetum glaucum* [L.] R. Br.), maize (*Zea mays* L.) and wheat (*Triticum 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

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 distilled 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 tinted-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.

Preparation of the leaves aqueous extract

Hundred grams, initial weight (I W), 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). The aqueous extract of the seed was filtered by a muslin cloth and the leachate was dried and the precipitation (cake) weight (P W) was determined by a sensitive balance. The final volume (F V) of the water extract for the D. stramonium seeds was measured by measuring cylinder. The final weight (F W), dissolved powder, was calculated using the following equation:

$$F\,W = I\,W - P\,W$$

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

$$AC\ (g/l) = \frac{F\,W}{F\,V} \times 1000$$

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 sorghum, millet, maize and wheat (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 the aqueous extract of the seed powder of D. stramonium, sealed with Parafilm, covered with black polyethylene bag and incubated at 300C 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:

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

The inhibition (%) was corrected using Abbott's formula (Abbott, 1987). It is given by:

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

Where:

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

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

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).

Results and Discussions

The results showed that the aqueous extract of the seed powder of *D. stramonium* inhibited the seed germination of the tested cereal crops; sorghum, millet, maize and wheat 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.

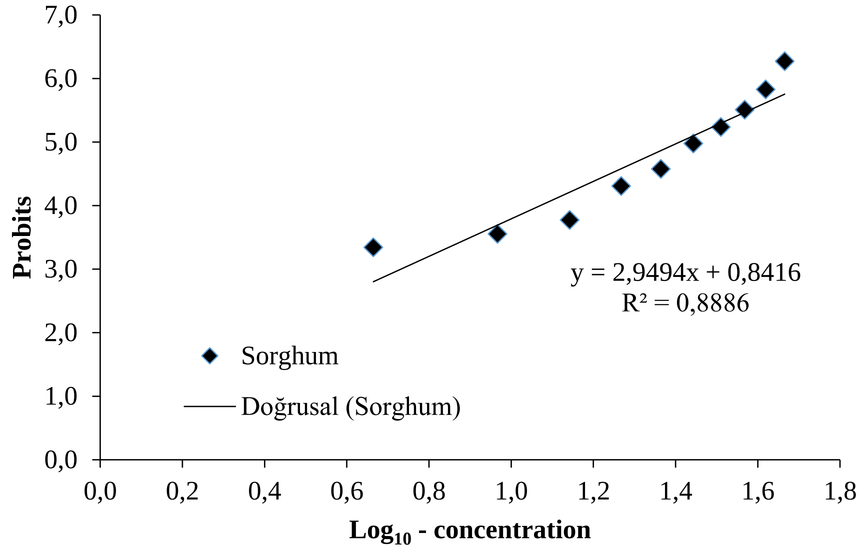

Figure 1. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of sorghum

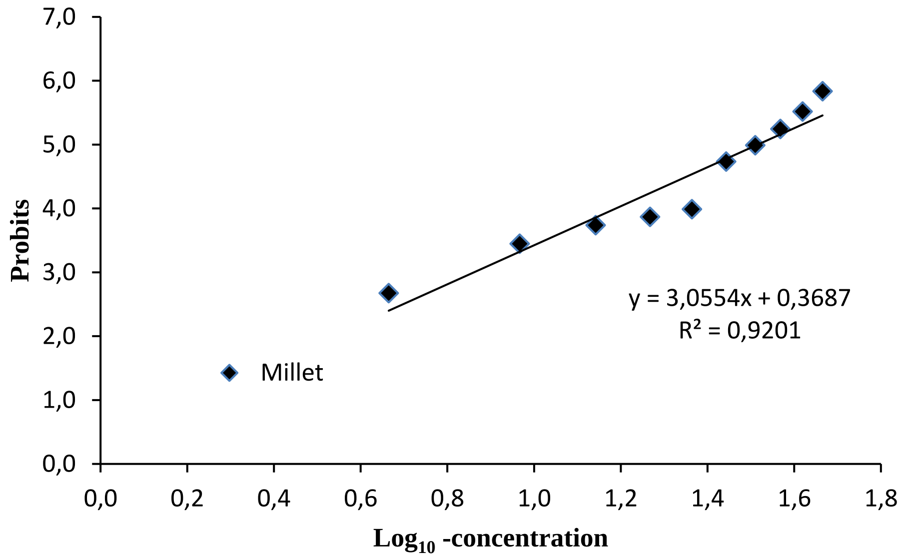

Figure 2. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of millet

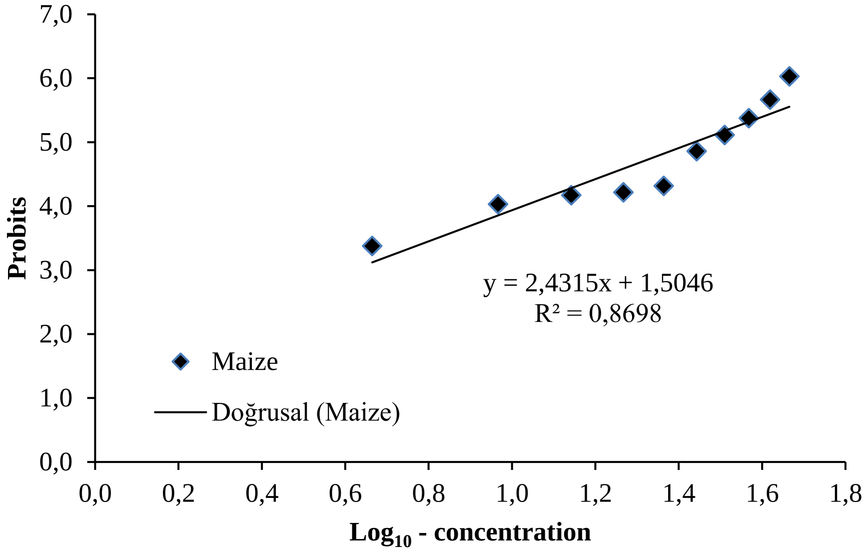

Figure 3. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of maize

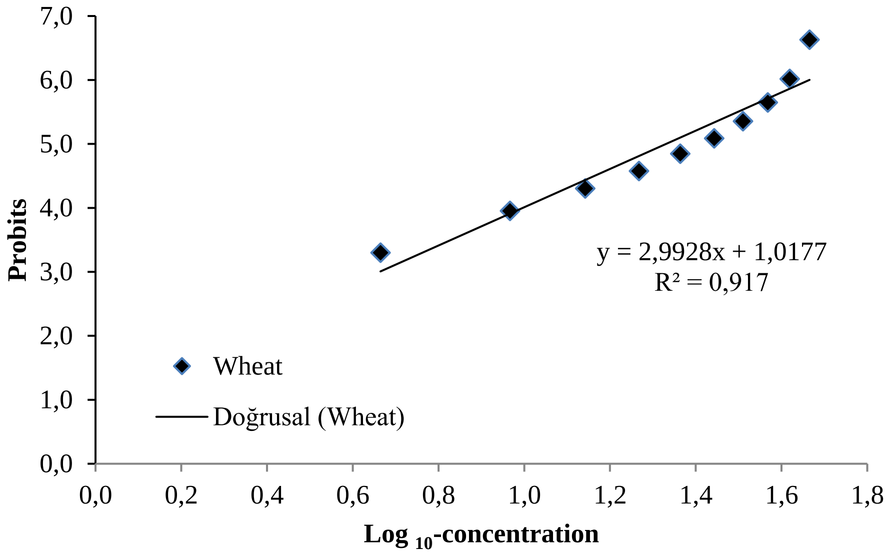

Figure 4. Relationship between Log_{10} of concentration of the aqueous extract of the seed powder of *D. stramonium* and probit of inhibition (%) of seed germination of wheat

Phytotoxic effect of on sorghum

The relationship between the inhibition of seed germination of sorghum and the concentration of the seed aqueous extract of the D. stramonium was described by the following the simple linear regression equation; $Probit = 2.95\ log_{10}concentration + 0.84$. The value of coefficient of simple determination (R2) was 0.888. The LC10, LC50 and LC90 were 11.1, 26.3 and 63.2 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

Cereal crops	No. of tested seeds (Rep.)	Inhibition % values (95% Confidence limits for concentration)			Chi^2	Df [a]	Sig.
		LC_{10}	LC_{50}	LC_{90}			
Sorghum	400 (4)	11.1 (6.3-14.7)	26.5 (21.7-32.6)	63.2 (47.0-115.5)	217.2	8	0.000 [b]
Millet	400 (4)	14.5 (10. 2-17.7)	32.2 (28.2-37.9)	71.5 (55.5-113.4)	126.7	8	0.000 [b]
Maize	400 (4)	9.2 (5.0-12.5)	27.9 (22.9-35.1)	85.0 (58.9-176.4)	154.1	8	0.000 [b]
Wheat	400 (4)	8.8 (6.0-11.1)	22.6 (19.6-25.9)	58.5 (47.0-82.2)	97.0	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.

Phytotoxic effect on millet

The relationship between the inhibition of seed germination of millet and the concentration of the seed aqueous extract of the *D. stramonium* was described by the following the simple linear regression equation; $Probit = 2.40\ log_{10}concentration - 4.01$. The value of coefficient of simple determination (R^2) was 0.992. The LC_{10}, LC_{50} and LC_{90} were 14.5, 32.2 and 71.5 g/l, respectively (Table 1).

Phytotoxic effect on maize

The relationship between the inhibition of seed germination of maize and the concentration of the seed aqueous extract of the *D. stramonium* was described by the following the simple linear regression equation; $Probit = 2.43\ log_{10}concentration + 1.505$ The value of coefficient of simple determination (R^2) was 0.870. The LC_{10}, LC_{50} and LC_{90} were 9.2, 27.9 and 85.0 g/l, respectively (Table 1).

Phytotoxic effect on wheat

The relationship between the inhibition of seed germination of wheat and the concentration of the seed aqueous extract of the *D. stramonium* was described by the following the simple linear regression equation; $Probit = 2.99\ log_{10}concentration + 1.018$. The value of coefficient of simple determination (R^2) was 0.917. The LC_{10}, LC_{50} and LC_{90} were 8.8, 22.6 and 58.5 g/l, respectively (Table 1).

The results showed that the aqueous extract of thorn apple (*D. stramonium*) seed inhibited the seed germination of the tested cereal crops and there was direct positive relationship between concentration and inhibition. The result also revealed the seeds of wheat (LC_{50} = 22.6 g/l) were most sensitive to the seeds aqueous extract of thorn apple followed by the seeds of sorghum (LC_{50} = 26.5 g/l) and maize (LC_{50} = 27.9 g/l). However, the extract was less toxic to the seeds of millet (LC_{50} = 32.2 g/l). These findings were consistent with those of Sakadzo et al. (2018) who conducted laboratory and greenhouse trials to investigate the allelopathic effects of *D. stramonium* weed on seed germination, early seedling growth and dry biomass of wheat (*Triticum aestivum*). Results from the study indicated that germination, shoot length and dry weight significantly decreased proportionally ($p < 0.001$) as the concentration of the aqueous 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 wheat, hence cannot be used as a bio-herbicide to control weeds on the wheat since it is non-selective to the crops studied.

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 (Maibam et al., 2011). *Datura stramonium* expresses the allelopathic effect on several species, reducing the yield of cereal crops, especially sorghum, millet, maize and wheat (Oljaca et al., 2002; Scepanovic et al., 2008) and biodiversity of local flora. The aqueous extracts of *D. stramonium* seeds contain high concentration of saponins, steroids, alkaloids and glycosides (Shagal et al., 2012). Saponins, flavonoids, alkaloids, glycosides and phenol are common among crude aqueous extract of the plant (Nain et al., 2013). The primary biologically active substances in *D. stramonium* are the alkaloids atropine and scopolamine (Ivancheva et al., 2006). 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 Oyun (2006), allelochemicals inhibit water uptake which is a precursor to physiological processes that should occur in seed before germination is triggered hence affecting germination (Altikat et al., 2013; Ullah et al., 2015). 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 (Mushtaq and Siddiqui, 2018) 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

It was concluded that that the aqueous extract of thorn apple (*D. stramonium*) seed was phytotoxic to the seed germination of the tested cereal crops. The seeds of wheat (LC_{50} = 22.6 g/l) were most sensitive to the seeds aqueous extract of thorn apple followed by the seeds of sorghum (LC_{50} = 26.5 g/l) and maize (LC_{50} = 27.9 g/l). However, the extract was less toxic to the seeds of millet (LC_{50} = 32.2 g/l).

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