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Phytotoxic Effects of *Eucalyptus camaldulensis* Dehnh Leaves Aqueous Extracts on Seed Germination of some Cereal Crops using Probit Analysis

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Abstract: *Several agro-forestry trees may have the ability to retard germination and growth of other plants by releasing many chemical substances into the environment. This study was carried out investigate the phytotoxic effects of the leaves aqueous extract of Eucalyptus camaldulensis Dehnh 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. A laboratory experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in season 2014/15. Ten concentrations (4.44, 8.89, 13.33, 17.78, 22.22, 26.66, 31.11, 35.55, 40.00 and 44.44 g/l) of the leaves aqueous extract of Eucalyptus were prepared from the stock solution (100 g / l). A control with sterilized-distilled water was included for comparison. Treatments were arranged in a completely randomized design with four replicates. The seeds were examined for inhibition (%) in germination at three days after initial germination. Collected data were transformed using Abbott's formula and subjected to probit analysis procedure ($P \leq 0.5$). The results revealed that the leaves aqueous extract of Eucalyptus inhibited the seed germination of the tested cereal crops and there was a direct positive relationship between concentration (g/l) and inhibition (%).The results also revealed that the seeds of wheat were most sensitive (LC50 = 18.7 g/l) to the leaves aqueous extract of Eucalyptus followed by the seeds of sorghum (LC50 = 21.3 g/l). However, the extract was less toxic to the seeds of millet (24.1 g/l) and maize (25.9 g/l). It was concluded that the leaves aqueous extract of Eucalyptus had a toxic effect on the seeds of the tested cereal crops.*

Keywords: Eucalyptus; LC_{50}; maize; millet; phytotoxic; probit; sorghum; tomato; wheat

1. INTRODUCTION

Nemours flowering plants may have the ability to suppress germination and growth of other plant species by delivering some chemical substances into the rhizosphere [1]. These chemicals or allelochemicals are in fact toxic byproducts (secondary metabolites) of the plant [2]. The effect of the phytotoxicity of these chemical compounds on other adjacent plants is often predominant early in the life cycle and the most noticeable effect is the reduction of seed germination and seedling development [3]. The allelochemicals also have been usually considered as the compounds, which are synthesized by plants in order to manipulate the different biological processes [4]. These allelochemicals are exist in various parts of the plant such as leaves, stems, fruits rhizomes, seeds and are delivered into the soil by means of degradation, deterioration, exudation, leaching and volatilization of plant stuff [5]. Allelochemicals disrupt the physiological and metabolic functions of plants such as nutrient and water uptake, photosynthesis, respiration and DNA synthesis [6]. The allelochemicals can reduce cell division or auxin that stimulates the growth of shoot and roots [7]. Allelochemicals such as phenolic compounds inhibit root and shoot length [8]. Growth inhibition caused by these allelochemicals is likely to be due to its interference with the plant growth processes [7]. Allelochemicals released to the environment can either inhibit shoot and/or root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby demolish the plant's source of a nutrient. The allelochemicals could come up with a potential source for reasonable herbicides and biological control means [9].

One of the plants noted for having some adverse effects on other plants through allelopathic interactions is Eucalyptus (*Eucalyptus camaldulensis* Dehnh), a member of the Myrtaceae family. It is known with various names such as long beak eucalyptus, murray red gum, red gum, river gum, river red gum and red river gum [10]. Eucalyptus tree is a large evergreen tree growing rapidly to 125-160 meters [11]. It is native to Australia and has been introduced into many countries around the world because of its rapid growth and high demand for paper and plywood [12]. To fill the widening gap between the supply and demand of forest raw materials, many *Eucalyptus* species are even grown in agricultural fields with crops, because of their rapid growth, adaptability and high productivity [12, 13]. Eucalyptus occupies agricultural land intended for food crops cultivation; and may adversely affect indigenous plant species (including crops). Also, Eucalyptus can compete with crops underlying light, water and soil nutrient or by changing the soil pH [14, 15]. Moreover, there is a constant debate about the ecological functions of *Eucalyptus*, it reduces the diversity of understorey species and the productivity of understory crops [16], because its allelochemicals have allelopathic effects on other plant species [17]. Considering the economic importance of cereal crops, this study was carried out to investigate the phytotoxic effects of the leaves aqueous extract of Eucalyptus 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.

2. MATERIALS AND METHODS

2.1. Experimental Site

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

Fully grown leaves of Eucalyptus tree were gathered from Experimental Farm of the FAS in season 2014/15. The leaves were transferred to the biology laboratory of the FAS. The Leaves were washed with sterilized distilled water, air dried on bench for 15 days at room temperature in a dark room to avoid the direct sun light that might cause undesired reactions. The dried leaves were then crushed into powder and kept in brown 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 at 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. Then, the seeds were washed with sterilized distill water and stocked 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 leaves powder of Eucalyptus 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 Eucalyptus leaves 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 leaves 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 leaves aqueous extract were prepared by sequential dilution of the stock extract with sterilized-distilled water to give 4.44, 8.89, 13.33, 17.78, 22.22, 26.66, 31.11, 35.55, 40.00 and 44.44 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 leaves aqueous extract of Eucalyptus, sealed with Parafilm, covered with black polyethylene bag and incubated at 30^0C in the dark. The treatments, of each crop, were arranged in a 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:

$$Corrected\ Inhibition\ (\%) = \frac{X-Y}{X} \times 100 \quad (4)$$

Where:

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

2.5. 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 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). The simple linear regression equation is:

$$Y = \alpha + \beta X \quad (5)$$

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 leaves aqueous extract of Eucalyptus 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). Plotting probits against $\log_{10}$-concentration straightened the cumulative distribution line and the curve was transformed to more accurately to describe the data. Hence, the LC_{10}, LC_{50} and LC_{90} were accurately estimated.

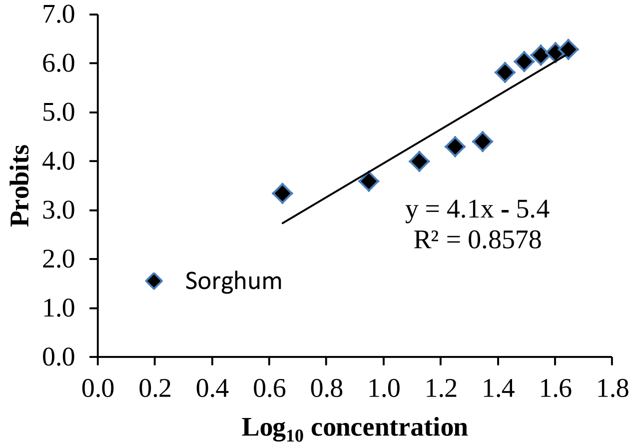

Figure 1. Relationship between Log_{10} of concentration of leaves aqueous extracts of Eucalyptus (*E. camaldulensis*) and probit of inhibition (%) of seed germination of sorghum

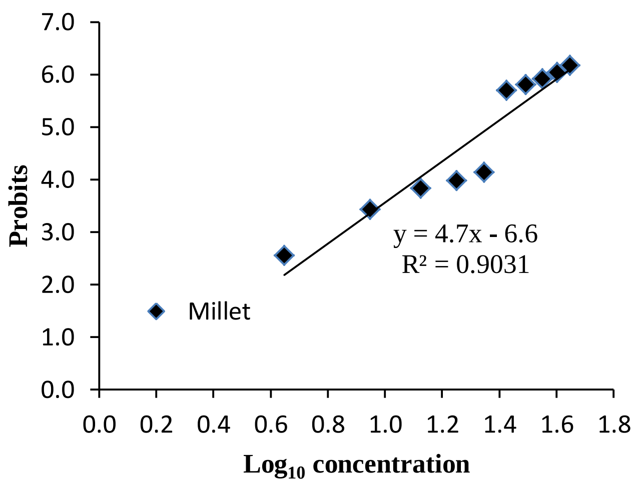

Figure 2. Relationship between Log_{10} of concentration of leaves aqueous extracts of Eucalyptus (*E. camaldulensis*) and probit of inhibition (%) of seed germination of millet

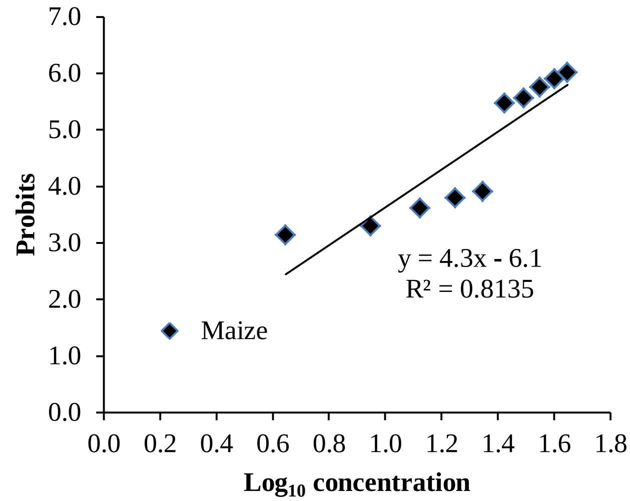

Figure 3. Relationship between Log_{10} of concentration of leaves aqueous extracts of Eucalyptus (*E. camaldulensis*) and probit of inhibition (%) of seed germination of maize

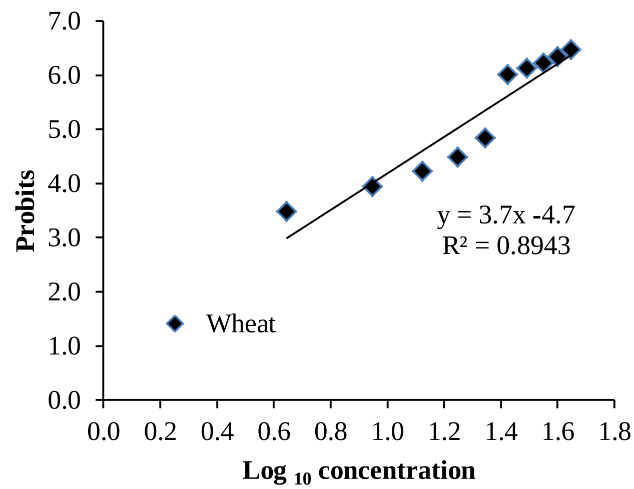

Figure 4. Relationship between Log_{10} of concentration of leaves aqueous extracts of Eucalyptus (*E. camaldulensis*) and probit of inhibition (%) of seed germination of wheat

3.1. Phytotoxic Effect on Sorghum

The simple linear regression equation was "$Probit = 4.1\ log_{10}concentration - 5.4$". The value of coefficient of simple determination (R^2) was 0.86.The LC_{10}, LC_{50} and LC_{90} were 10.3, 21.3 and 44.1 g/l, respectively (Table 1).

3.2. Phytotoxic Effect on Millet

The simple linear regression equation was "$Probit = 4.7\ log_{10}concentration - 6.6$". The value of coefficient of simple determination (R^2) was 0.90. The LC_{10}, LC_{50} and LC_{90} were 13.0, 24.1 and 44.9 g/l, respectively (Table 1).

Table 1. Phytotoxic effects of the leaves aqueous extracts of Eucalyptus (*E. camaldulensis*) 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)	10.3 (4.0-14.6)	21.3 (15.3-27.8)	44.1 (32.6-94.2)	503.6	8	0.000 [b]
Millet	400 (4)	13.0 (7.8-16.6)	24.1 (19.8-28.7)	44.9 (36.1-69.8)	297.9	8	0.000 [b]
Maize	400 (4)	13.1 (1.3-19.5)	25.9 (15.4-42.9)	51.3 (34.6-511.5)	992.8	8	0.000 [b]
Wheat	400 (4)	8.4 (4.5-11.5)	18.7 (14.6-22.8)	41.5 (32.3-66.0)	270.5	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 Maize

The simple linear regression equation was "$Probit = 4.3\ log_{10}concentration - 6.1$". The value of coefficient of simple determination (R^2) was 0.81. The LC_{10}, LC_{50} and LC_{90} were 13.1, 25.9 and 51.3 g/l, respectively (Table 1).

3.4. Phytotoxic Effect on Wheat

The simple linear regression equation was "$Probit = 3.7\ log_{10}concentration - 4.7$". The value of coefficient of simple determination (R^2) was 0.89. The LC_{10}, LC_{50} and LC_{90} were 8.4, 18.7 and 41.5 g/l, respectively (Table 1).

4. DISCUSSIONS

The results showed that the leaves aqueous extract of Eucalyptus was toxic to the seed germination of the tested cereal crops and there was direct positive relationship between concentration (g/l) and inhibition (%). The seeds of wheat were most sensitive to the aqueous extract of Eucalyptus followed by the seeds of sorghum. However, the extract was less toxic to the seeds of millet and maize. These findings were consistent with those of [18] who conducted a greenhouse experiment to determine the allelopathic effect of Eucalyptus site soil on the germination and growth of maize, wheat and sorghum. It was found that the shoot length, and shoot and root dry weights of wheat was the most affected in polybag containing soil collected from 3 m distance. Maize was relatively tolerant to the allelopathic effect of the eucalyptus site soil. Reference [19] verified the allelopathic effect of leaf leachate of *E camaldulensis* on germination, growth, morphological and physiological parameters of sorghum. Leaf leachate was tested at 5, 10 and 20% concentrations. Sterilized distilled water used as control. Germination of seeds, length of shoots and root length and seedlings dry matter were significantly reduced by all tested concentrations. Reduction in chlorophyll content, soluble sugar content and consequently protein content is proportional to the increase in the leachate concentration.

Similar results were obtained by [20] who studied the allelopathic effect of soaked, grounded and boiled in tape water *E. camaldulensis* extracts on seed germination and growth of maize var. 'Kissan'. Results showed that all the extracts greatly reduced maize seed germination, root and shoot length, as well as fresh and dry weight. Reference [21] conducted a pot experiment to determine the effects of different leaf-powder ratios of *E. camaldulensis* and *E. grandis* on maize as well as change in soil reaction. Results suggested that leaves powder of each tree species had a significant inhibitory effect on germination and seedling growth of maize crop compared to the control treatments. Application of low-dose leaves powder of either tree species had low effect on maize crop. It is concluded that maize should not be planted very close to *Eucalyptus* trees and the rate of crop seeds should be increased to obtain maximum germination.

The results of this study were also consistent with the observation by [22] who assessed the allelopathic effect of Eucalyptus leaves extract on germination and growth of three wheat varieties and reported the decreased germination rate, leaf and root lengths, dry and wet weights of both roots and shoots. Reference [23] studied the allelopathic effect of aqueous extracts of *E. camaldulensis* on the germination percentage and seedling growths (fresh and dry weight) of wheat have been determined. It was noted that aqueous extracts at a concentration of 10, 15 and 20% had inhibitory effect on wheat germination and the effect was significantly higher than control treatment. Fresh and dry weight of seedling was also reduced significantly compared to the control. The inhibitory effects were increased as the extract concentration increased. These findings indicate that wheat sown in fields which had leaf litter of *E. camaldulensis* will be negatively affected regarding germination, growth and ultimately leading to lower wheat productivity. Reference [24] studied the allelophatic effects of leaves methanolic extract of *E. camaldulonsis* on some morphological and physiological characteristics of wheat (*T. aestivum* L.) in a growth chamber. They found that the leaves methanolic extract significantly reduced germination percentage, radicule and plumule length, wet and dry weight.

The inhibition of germination is dependent on the concentration of the extract, it may be due to the entry of water soluble allelochemicals in to the seed, which retards the germination and growth [25]. The phytochemical studies on the aerial parts of the Eucalyptus showed that the plant release volatile compounds such as benzoic, cinnamic and phenolic acids, which inhibit growth of crops and weeds growing near it [26]. These chemical compounds such phenolics, terpenoides and alkaloids and their derivatives found in different parts of Eucalyptus plant are potential inhibitors of germination, seedlings growth, fresh weights and dry weights of seedlings. They have been reported to inhibit cell division, changes in phytohormones and their balance, water uptake and germination of seeds also reported that the aqueous leaves extract of Eucalyptus has allelopathic properties including germination inhibition and yield reduction [27].

CONCLUTION

It was concluded that that the leaves aqueous extract of Eucalyptus had toxic effect to the seeds of the tested cereal crops and there was a direct negative relationship between concentration germination. The seeds of wheat were most sensitive to the aqueous extract of Eucalyptus followed by the seeds of sorghum. However, the extract was less toxic to the seeds of millet and maize. Eucalyptus produces abundant litter in the form of fallen leaves and a layer of bark naturally. If allelochemicals are released from Eucalyptus leaf litter, its accumulation in the soil may lead to poor development of neighboring plant growth. Therefore, more studies related to the effects of Eucalyptus allelochemicals over cultivated plants and other weed plants are required.

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