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Phytotoxic Effects of Basil (*Ocimum basilicum* L.) Aqueous Extract on Seed Germination of Some Cereal Crops

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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 aqueous extract of aboveground parts of basil (*Ocimum basilicum* 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 during the season 2017/2018. 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 aqueous extract of aboveground parts of basil were prepared from the stock solution (100 g / l). A control containing 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. Collected data were transformed using Abbott's formula and subjected to probit analysis ($P \leq 0.5$). The aqueous extract of aboveground parts of basil inhibited the seed germination of the tested cereal crops and there was direct positive relationship between concentration (g/l) and inhibition (%). Also, the result showed that the seeds of maize were most sensitive to the aqueous extract of aboveground parts of basil followed by the seeds of millet, wheat and sorghum. The LC_{50} for maize, wheat, millet and sorghum was 34.1, 46.3, 46.7, and 59.1, g/l, respectively. It was concluded that the aqueous extract of aboveground parts of basil had toxic effect to the seeds of the tested cereal crops
Keywords: basil, cereal, *Ocimum*, maize, millet, phytotoxic, probit, sorghum, wheat.

Introduction

Several medicinal and ornamental plants are screened for their allelopathic potential worldwide (Fujii *et al.*, 2003; Miri *et al.*, 2013). These plants are phytotoxic in nature as they produce and release many chemicals into the surrounding ecosystem (Delabays *et al.*, 2004). These chemicals, known as allelochemicals, are secondary metabolites and synthesized by plants (Farooq *et al.* 2011). Allelochemicals are present in different parts of the plant such as roots, stem, leaves, flowers,

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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). Also, it influences the cell division, cell elongation, membrane permeability, enzyme activity, etc. (Dragoeva *et al.*, 2015).

One of the plants noted for having some adverse effects on other plants through allelopathic interactions is Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family. It is one of the most important species of the genera *Ocimum*, native to India, Southern Asia and Middle East. The genus *Ocimum* comprised about 60 species and numerous varieties that are widely distributed over the warm regions of the world (Evans, 2001; Kumar, 2009; Bączek *et al.*, 2019). Basil is an annual and herbaceous plant with a square branched stem and numerous opposite, stalked, ovate, slightly toothed and glabrous leaves, often reddish in color. The small white, yellowish or pinkish flowers are arranged in whorls in the upper leaf axils. The fruit consists of four nutlets. It is cultivated extensively in different countries in Africa, Europe and USA (Singh and Panda, 2005). Basil is the most widely grown either for the fresh market or for essential oil production. It is considered as an important source of volatile aromatic oils, medicinal and ornamental chemical compounds (Evans, 2001; Kumar, 2009). Extracts of basil plant that are used in traditional medicines contain biologically active constituents (Nahak *et al.* 2011). The main constituents of basil were linalool, α-muurolol, methyl chavicol, eugenol, γ-cadinene, α-bergamotene, eucalyptol, germacrene D, cubenol, β-elemene and α-bulnesene (Onofrei, *et al.* 2018).

Phytotoxic activity of *Ocimum* spp. plant extracts was investigated against the germination and seedling growth of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa *(Medicago sativa*), Italian ryegrass (*Lolium multiflorum*), barnyard grass (*Echinochloa crus-galli*) and timothy (*Phleum pratense*) at four different concentrations. The plant extracts at concentrations greater than 1 mg dry weight equivalent extract mL−1 reduced significantly the total germination percent, germination index, germination energy, speed of emergence, seedling vigour index, and coefficient of the rate of germination of all test species except barnyard grass and germination percent of lettuce. The shoot and root growth of all test species were significantly inhibited by the extracts at concentrations greater than 10 mg dry weight equivalent extract mL−1 (Islam and Noguchi, 2014). In addition, *Ocimum basilicum* plants (leaf, root and seed) reduce germination and alter root/shoot developments of many commercial crops; gram (*Cicer arietinum* L.), lentil (*Lens esculenta* L.), mustard (*Brassica campestris* Linn.) okra (*Abelmoschus esculentus* L.) and pea (*Pisum sativum* L.) (Verma *et al.*, 2012).

Cereal crops like Sorghum, Millets, Wheat, Maize and Rice are particularly important to humans because of their role as staple food crops in many areas of the world. Cereals are also used to produce animal feed, oils, starch, flour, sugar, syrup, processed foods, malt, alcoholic beverages, gluten and renewable energy (Sarwar *et al.,* 2013). Considering the economic importance of cereal crops, this study was carried out to investigate the phytotoxic effects of the aqueous extract of aboveground parts of basil 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 2018. The laboratory has an average temperature range between 25 - 30°C and the relative humidity ranging between 60 - 70 %.

Materials collection

Mature plants of basil plants were collected from Experimental Farm of the FAS during the season 2017/2018. The plants were transferred to the biology laboratory of the FAS. The aboveground parts of plants were collected, washed with sterilized distilled water and air dried on bench for 21 days at room temperature in a dark room to avoid the direct sun light that might cause undesired reactions. The dried aboveground parts 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 of Wed Medani city, Gezira state, Sudan. The seeds were surface sterilized with 1% (v/v) sodium hypochlorite (NaOCl) solution, for 3 min. Subsequently the seeds were washed with sterilized distilled water for several times and stored at room temperature.

Preparation of the actual concentration of the aqueous extract of the aboveground parts of basil plants:

Hundred grams, initial weight (IW), of the aboveground parts of basil plants were placed in a conical flask, sterilized distilled water was added to give a volume of 1000 ml. Flasks were shaken for 24 hours at room temperature (27±3°C) by an orbital shaker (160 rpm). The aqueous extract was filtered by a muslin cloth, the leachate was dried and the precipitation (cake) weight (*PW*) was determined by a sensitive balance. The final volume (*FV*) of the water extract was measured by measuring cylinder.

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

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

The actual concentration (AC) of the aqueous extract of the aboveground parts of basil plants was calculated using the following equation:

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

Bioassay procedure:

Ten concentrations (n) of the aqueous extract of the aboveground parts of basil 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 containing 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 aboveground parts of basil, 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:

$$Inhibition\ (\%) = \frac{TS - TGS}{TS} \times 100 \qquad (3)$$

where *TS* is the total number of seeds and *TGS* is the total number of germinated seeds. The inhibition (%) was corrected using Abbott's formula. It is given by:

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

Where: *X* is the % survivorship of the control group and *Y* is the % survivorship in the experimental group.

Statistical analysis

Data were transformed using Abbott's formula 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 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 and discussion

The results showed that the aqueous extract of aboveground parts of basil 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.

Phytotoxic effect on sorghum

The relationship between the inhibition in seed germination of sorghum and the concentration of the aqueous extract of the *Ocimum basilicum* was described by the following simple linear regression equation:

$$\Pr obit = 1.718 \log_{10} concnetration - 3.047 \qquad (5)$$

The value of coefficient of simple determination (R^2) was 0.8954. The LC_{10}, LC_{50} and LC_{90} were 10.7, 59.1 and 330.3 g/l, respectively (Table 1).

Phytotoxic effect on millet

The relationship between the inhibition of seed germination of millet and the concentration of the aqueous extract of the *Ocimum basilicum* was described by the following simple linear regression equation:

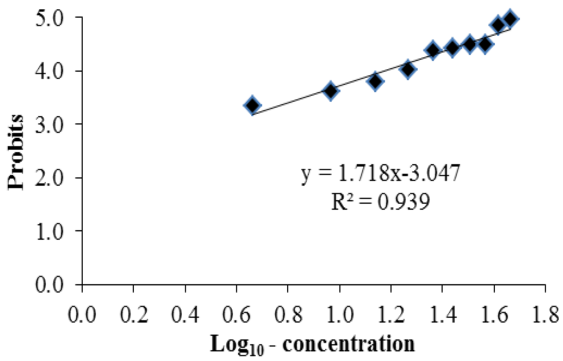

Figure 1. Relationship between Log_{10} of concentration of the aqueous extract of aboveground parts of basil (*Ocimum basilicum*) and probit of inhibition (%) of seed germination of sorghum.

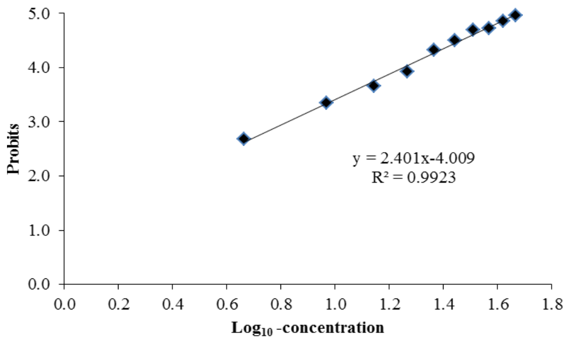

Fig. 2. Relationship between Log_{10} of concentration of the aqueous extract of aboveground parts of basil (*Ocimum basilicum*) and probit of inhibition (%) of seed germination of millet.

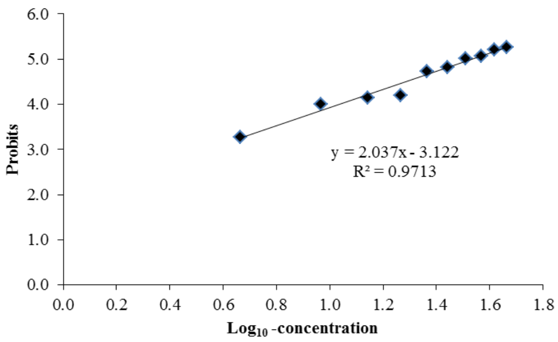

Fig. 3. Relationship between Log_{10} of concentration of the aqueous extract of aboveground parts of basil (*O. basilicum*) and probit of inhibition (%) of seed germination of maize.

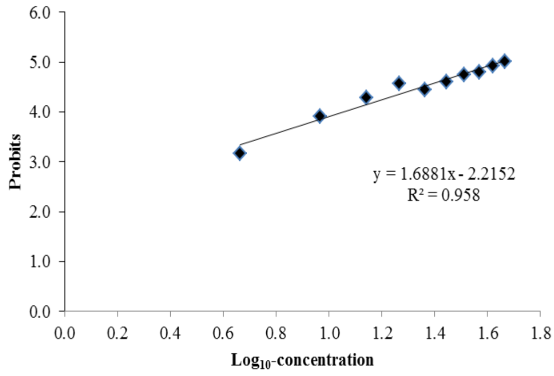

Fig. 4. Relationship between Log_{10} of concentration of the aqueous extract of aboveground parts of basil (*Ocimum basilicum*) and probit of inhibition (%) of seed germination of wheat.

$$Probit = 2.401 \log_{10} concnetration - 4.009 \quad (6)$$

The value of coefficient of simple determination (R^2) was 0.9458. The LC_{10}, LC_{50} and LC_{90} were 13.7, 46.7 and 169.7 g/l, respectively (Table 1).

Phytotoxic effect on maize

The relationship between the inhibition of seed germination of maize and the concentration of the aqueous extract of the *Ocimum basilicum* was described by the following simple linear regression equation:

$$Probit = 2.037 \log_{10} concnetration - 3.122 \quad (7)$$

The value of coefficient of simple determination (R^2) was 0.8629. The LC_{10}, LC_{50} and LC_{90} were 8.0, 34.1 and 145.1 g/l, respectively (Table 1).

Phytotoxic effect on wheat

The relationship between the inhibition of seed germination of wheat and the concentration of the aqueous extract of the *Ocimum basilicum* was described by the following simple linear regression equation:

Table 1. Relationship between Log_{10} of concentration of the aqueous extract of aboveground parts of basil (*Ocimum basilicum*) and probit of inhibition (%) of seed germination of wheat.

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)	10.7 (7.8-13.1)	59.1 (48.6-79.9)	330.3 (199.9-738.39)	28.2	8	0.000 [b]
Millet	400 (4)	13.7 (12. 6-14.7)	46.7 (44.0-50.1)	169.7 (137.0-191. 7)	6.7	8	0.574 [c]
Maize	400 (4)	8.0 (6.3-9.6)	34.1 (30.9-38.3)	145.1 (111.6-207.6)	21.4	8	0.006 [b]
Wheat	400 (4)	7.1 (5.1-8.9)	46.3 (39.9-56.3)	302.9 (198. 7-562.0)	19.3	8	0.013 [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.**

c. **Since the significance level is greater than .150, no heterogeneity factor is used in the calculation of confidence limits.**

$$Probit = 1.688 \log_{10} concnetration - 2.215 \ (8)$$

The value of coefficient of simple determination (R^2) was 0.93.75. The LC_{10}, LC_{50} and LC_{90} were 7.1, 46.3 and 302.9 g/l, respectively (Table 1).

The results showed that the aqueous extract of Above ground parts of basil inhibited the seed germination of the tested cereal crops. There was direct positive relationship between the concentration and the inhibition. Also, the result revealed that the seeds of maize were most sensitive to the aqueous extract of aboveground parts of basil followed by the seeds of millet and wheat, however, the extract was less toxic to the seeds of sorghum.

These results are in line with the findings Verma *et al.*, (2012) who conducted a bioassay experiment to explore the phytotoxic potential of aqueous extracts of leaf, root and seeds of basil on seed germination, root and shoot elongation of selected important cereal crops; maize, wheat and barley. The inhibitory effect was displayed by all tested extracts with maximum in leaf followed by root and seed extract. Moreover, basil aqueous extracts, significantly, adversely influenced the root and shoot elongation of all tested crops. The variation in toxicity of the plant species to the aqueous extracts could be due to (i) the presence of seed coat which acts as a barrier in between the embryo and its surrounding environment, and (ii) the selective permeability of seed coats which may protect the inhibitory activity of phytotoxic extract/substances if they cannot pass through seed coats (Islam and Noguchi, 2014).

These results were in line with the findings made by Sharmal and Singh (2003) that evaluated the phytotoxic effects of basil on seed germination of some weed species. The authors found that the seed germination of redroot pigweed and hairy beggarticks was totally inhibited with the incorporation of 7.5 g of basil leaf powder into 100 g of sand. Also, the germination of seeds was significantly inhibited in tested weeds as grown in aqueous extract of basil leaf at concentration of 10% (w/v). Baličević *et al.*, (2015) investigated the phytotoxic effect of basil on seed germination and early growth of selected weed species under laboratory conditions. The results indicated that basil reduced germination of hoary cress up to 13.8 %. The toxic effects of basil extract were eventually due to the release of allelochemicals after decaying (Chou and Patrick, 1976). Also, Dhima *et al.,* (2009) reported that aqueous extracts of aboveground parts of basil reduced seed germination and growth of barnyardgrass [*Echinochloa crus-galli* (L.) PB.]. Gulzar and Siddiqui (2017) carried out an experiment to explore the effect of aqueous extract of apple of Sodom on the seed germination of *Brassica oleracea*. Seeds of brassica were steeped in solutions containing concentrations (20%, 40%, 60% and 80%) 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. Gulzar and Siddiqui (2017) 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.

Phytochemical screening of aqueous extract and elemental analysis of basil showed the presence of some chemicals such as saponins, tannins and cardiac glycosides. There were potassium, calcium, sodium and magnesium in the concentration. Khair-ul-Bariyah *et al.*, (2012) concluded that basil contains biologically active compounds and minerals. The aerial part of the basil plant contains basically the amyrin, amyrin acetate, sitosterol, ursolic acid, cardenolides, calotropin and calotropagenin that might contribute its toxic effect (Sharma *et al.*, 2011). Suppression of seed germination of the tested cereal crops caused by allelochemical stress could be due to inhibition of water uptake, cell division, cell elongation and changing 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 inhibition of seed germination was found to be concentration-dependent (Oudhia, 1999).

Conclusion

- It was concluded that the aqueous extract of basil (Ocimum basilicum L.) was phytotoxic to the seed germination of the tested cereal crops.
- The seeds of maize were most sensitive to the aqueous extract of basil followed by the seeds

of wheat and millet. However, the extract was less toxic to the seeds of sorghum.

- It is necessary to take into account the effective management of basil in the fields of cereal crops so as not to adversely affect the seed rate and therefore crop density.
- Further studies are expected to reveal the extent to which active substances in different parts of the plant can be used to produce rational weed control measures.

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المجلة الليبية للعلوم الزراعية
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ورقة أصيلة

تأثير السمية النباتية للمستخلص المائي لنبات الريحان (*Ocimum basilicum* L.) على إنبات بذور بعض محاصيل الحبوب

عوض الله بلال دفع الله و سارة الأحمد
قسم وقاية النبات – كلية الزراعة – جامعة الجزيرة– السودان

المستخلص

العديد من النباتات ذات طبيعة سمية نباتية لأنها تنتج وتطلق العديد من المركبات الكيميائية في البيئة. أجريت هذه الدراسة للتحقق في تأثير السمية النباتية للمستخلص المائي للأجزاء فوق سطح الأرض لنبات الريحان (*Ocimum basilicum* L.) على تثبيط إنبات بذور الذرة الرفيعة (*Sorghum bicolor* [L.] Moench) و الدخن (*Pennisetum glaucum* [L.] R. Br.) و الذرة الشامية (*Zea mays* L.) و القمح (*Triticum vulgare* L.) باستخدام تحليل "probit". أجريت التجارب المعملية في كلية العلوم الزراعية ، جامعة الجزيرة ، السودان في موسم 2018/2017. تم تحضير 10 تراكيز (4.62 ، 9.26 ، 13.87 ، 18.51 ، 23.12 ، 27.74 ، 32.36 ، 36.98 ، 41.61 و 46.28 جم / لتر) من المستخلص المائي للأجزاء فوق سطح الأرض لنبات الريحان من المحلول الأساس (100 جم / لتر). استعمل الماء المقطر المعقم للمعاملة الشاهد للمقارنة. استعمل التصميم كامل العشوائية بأربعة مكررات. فحصت البذور للتثبيط (٪) في الإنبات في ثلاثة أيام بعد الإنبات الأولي. حولت البيانات المجمعة باستخدام صيغة "Abbott" وأُخضعت لتحليل "Probit". ثبط المستخلص المائي للأجزاء فوق الأرض لنبات الريحان إنبات بذور محاصيل الحبوب المختبرة وكانت هناك علاقة موجبة مباشرة بين التركيز (جم / لتر) والتثبيط (٪). كما أظهرت النتائج أن بذور الذرة الشامية كانت أكثر حساسية للمستخلص المائي للأجزاء فوق سطح الأرض لنبات الريحان متبوعة ببذور الدخن والقمح والذرة الرفيعة. كانت قيمة التركيز القاتل النصفي (LC_{50}) للذرة والقمح والدخن والذرة الرفيعة هي 34.1 ، 46.3 ، 46.7 ، و 59.1 جم / لتر، على التوالي. استنتج أن المستخلص المائي للأجزاء فوق سطح الأرض لنبات الريحان لها تأثير سام على بذور محاصيل الحبوب التي تم اختبارها.

الكلمات الدالة: ريحان، *Ocimum* ، حبوب، ذرة، دخن، ، سم نباتي، تحليل Probit، ذرة رفيعة، قمح.

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