RESPONSE OF *Zea mays* L. TO COGERMINATION AND AQUEOUS EXTRACTS OF *Datura inoxia* Mill.

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Abstract: The present study was undertaken to assess the allelopathic effect of *Datura inoxia* Mill. in relation to the germination and initial growth of *Zea mays* L. Cogermination of maize with *D. inoxia* seeds and aqueous extracts from fresh and dry *D. inoxia* biomass in concentrations of 7.5%, 10% and 20% were examined under laboratory conditions using Petri dishes. The results showed that seeds cogermination had not significant effect on maize germination, but had significant effects on seedlings growth. Inhibition of root length of maize seedlings was of 61.9% and inhibition of shoot length was of 40.0%. The aqueous extracts of *D. inoxia* showed significantly deterioration in germination and seedlings growth of maize. The inhibitory effects of the extracts on germination ranged from 23.2% to 57.1%, on root length – from 19.8% to 92.6%, and on shoot length – from 33.2% to 69.6%. In conclusion, since this weed had strong allelopathic potential which suppressed germination and initial growth of maize. Moreover, there is an urgent need of integrated *D. inoxia* management strategy to stop further spread of this alien weed into cultivable areas.

Keywords: allelopathy; cogermination; Downy thorn-apple; inhibition; maize.

INTRODUCTION

Infestation of agricultural fields with perennial and annual weeds leads to lower crop yields. Weeds influence crop growth by causing phytotoxicity from fallen seeds, flowers, leaves, decomposition of plant residues, etc. Mechanisms underlying these effects of weeds may displace many species through competition or allelopathy. The development of allelopathy in agricultural crops represents a challenge for bioengineering and biotechnology [5].

Datura inoxia Mill. is an annual wild plant, ornamental, extremely toxic plant for animals and humans, belonging to the family Solanaceae and originates from Central America. It has been introduced to many areas beyond its natural range (Africa, Asia, Australia and Europe), due to its attractive flowers and increased growing in the gardens for decorative purposes. However, in many places, *D. inoxia* has escaped from cultivation and is being established as an alien species while in other European countries its alien status is unknown. *Datura* sp. is commonly known as Downy thorn-apple, Jimsonweed, Angel's trumpet, Moonapple, Sacred datura [14] and is a most important medicinal plant and plant hallucinogen. The chemical compounds in *Datura* sp., important for pharmaceutical industry, include: hyoscyamine, scopolamine (hyoscine), atropine, withanolides (lactones) and other tropane alkaloids [8, 17]. These biochemicals, are known as allelochemicals, have allelopathic effects on survival of plants [8].

Although the alkaloids are recognised as a means of plant defence against pathogens and predators, recent investigations suggest that plant-produced alkaloids contribute to interference with other plants and that primary allelopathic activity may disrupt a biochemical pathway in plants which is analogous to one more usually associated with animals [15].

Many authors consider that allelochemicals in plants are released into the soil by decomposition of plant residues and exudates from plant tissues, and act as allelopathic substances, inhibiting seed germination and neighboring plant growth, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's source of a nutrient [9, 13, 24].

Downy thorn-apple spreads by seeds, which are transported as contaminants of crops seeds, in soil and irrigation water, and in hay, straw and plant trash. The seeds have long dormancy in the soil and are probably allelopathic to the germination on other plants. This species is usually a minor weed of summer crops, pastures and fodders. Apart from competing with crops and pastures the seeds contaminate crops, pasture and fodder seed, the plants are unpalatable, the spiny fruits contaminate wool, and the plants and their seeds are poisonous [20].

In Romania, *D. inoxia* is distributed along Black Sea coastal area (part of the region of Dobrudja), mainly in habitats close to harbors [3] and in other parts of the country. The sandy areas along Danube and continental sands are the most vulnerable habitats for *D. inoxia* [14].

Maize (*Zea mays*. L), is an important agricultural crop for Romania with about 2,5 million hectares of maize being cultivated and with a total production of about 10,02 million tons in 2016 [16].

The allelopathic interactions showed that the *Datura* sp. is dangerous and can cause high economic losses in the agricultural crops and its presence in the cultivating fields is a matter of concern [1]. According to Oerke [18], the potential yield reductions by weeds are: maize 40%, wheat 23%, soybeans 37%, rice 37%, cotton 35%, and potatoes 30%.

On the other hand, the excessive use of pesticides in agriculture cause environmental pressures and influences the level and quality of life [22] and reduces the germination and the mitotic index induced a large number of chromosomal anomalies [6, 7].

In order to minimize the weeds effects on agricultural crops and to identify effective management ways to prevent further infestations, it is necessary to understand the mechanism of allelopathic interactions between weeds and crops.

The purpose of this study was to determine the possible allelopathic effects of *D. inoxia* on germination and growth of maize.

MATERIALS AND METHODS

The experiments were conducted in 2018 in the Laboratory of Breeding plants at the Faculty of Agronomy in Craiova. Seeds of maize (P0937 hybrid) were purchased from seed company DuPont Pioneer (Pioneer Hi-breed, Romania) and weed seeds of *D. inoxia* were collected during 2018 (September) from Craiova area.

The all seeds were surface-sterilized for 20 min with 1% NaOCl (4% NaOCl commercial bleach), then rinsed three times with distilled water [26].

In the first experiment the effect of cogermination was investigated according to Dikic [10]. In each treatment 30 seeds of maize and 30 seeds of Downy thorn-apple were placed in Petri dishes on top of filter paper soaked in distilled water. Control treatments consisted of 30 seeds of a single species per dish, respectively maize. The Petri dishes were kept at room temperature (23 °C ± 2) for 7 days. All treatments had three replications. Additional extract/water was added to each as needed.

In the second experiment, the effects of *D. inoxia* aqueous extracts on maize were evaluated on filter paper in sterilized Petri dishes. Aqueous extracts were from 200 g fresh and dry aboveground biomass of plants that was mixed with 1000 ml of distilled water and kept for 24 h at room temperature. This mixture was filtered through filter paper and the obtained extracts were diluted with distilled water to obtain three final concentrations of 20%, 10% and 7.5%. Twenty seeds were placed in Petri dishes on top of filter paper. In each Petri dish was added 15 ml of aqueous extract, while distilled water was used in control. Petri dishes were kept at room temperature (23 °C ± 2) for 7 days. All treatments had three replications.

The allelopathic effect was evaluated at the end of the experiments by measuring the root and shoot length (cm) of seedlings.

The final seeds germination (G%) was calculated using the formula:

$$G\% = (\text{Germinated seeds} / \text{Total seeds}) \times 100$$

The inhibition (I%) of extracts was calculated applying the following formula:

$$I\% = [1 - (T/C)] \times 100$$

where: T= treatment and C = control.

The obtained data was analysed statistically with analysis of variance (ANOVA) and differences between treatment means were compared using the Least Significant Difference test at $p \leq 0.05$.

RESULTS

Effect of cogermination of *D. inoxia* seeds on germination, root and shoot lengths of maize

The results of variance analysis (ANOVA) for cogermination showed that were significantly influenced only root and shoot length of maize. The seeds germination was not significantly affected (Table 1).

Table 1. ANOVA of studied traits of maize at cogermination with *D. inoxia* seeds

Traits	SS	df	MS	F
Germination (%)	6.25	1	6.25	0.2^{ns}
Root length (cm)	49.21	1	49.21	33.19*
Shoot length (cm)	4.33	1	4.33	17.84*

Note: * = Significant at $p \leq 0.05$; ns = non-significant; SS = sum of squares; df = degrees of freedom; MS = mean squares; F = F test

The cogermination with downy thorn-apple seeds strongly inhibited the shoot and root length of maize (61.9% and 40.0%, respectively) (Figure 1 and Table 3).

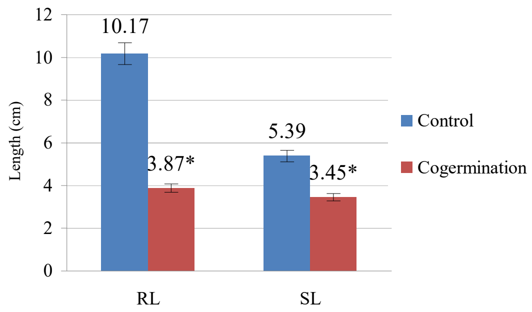

Figure 1. Effect of cogermination on maize root length (RL) and shoot length (SL)
Note: * = Significant at $p \leq 0.05$

Effect of aqueous extracts of *D. inoxia* on germination, root and shoot lengths of maize

The results from the analysis of the variance (ANOVA) for the aqueous extracts of *D. inoxia* showed a significant influence on the germination, the root length and the shoot length of maize (Table 2).

Table 2. ANOVA of studied traits of maize at treatment with aqueous extracts of *D. inoxia*

Traits	SS	df	MS	F
Germination (%)	3384.37	3	1128.12	6.81*
Root length (cm)	100.46	3	33.48	50.61*
Shoot length (cm)	14.65	3	4.88	9.85*

Note: * = Significant at $p \leq 0.05$; SS = sum of squares; df = degrees of freedom; MS = mean squares; F = F test

The results presented in Figure 2 and Table 3, revealed that the increase of extracts concentration resulted in reduction of germinated seeds percentage (G%). As compared to the control, *D. inoxia* extracts had no significant effects on seeds germination of the maize, except at 20% extract concentration, where seeds germination was significantly inhibited by 57.1%.

Both, the shoot and root lengths were found to be approximately ten and five cm, respectively in the control. *D. inoxia* extracts caused significant inhibition of maize seedling growth in the concentration range 7.5 - 20%. As shown in Figure 3 and Table 3, the extracts of *D. inoxia* inhibited maize root growth with I% at 19.8, 63.9 and 92.6%; while the I% of shoot inhibition were at 33.2, 42.8 and 69.6%.

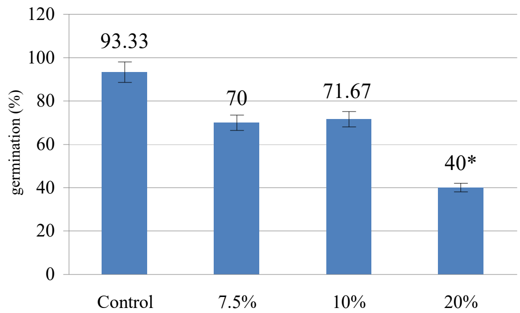

Figure 2. Effect of different concentrations of aqueous extracts of *D. inoxia* on maize germination (G%).
Note: * = Significant at p≤0.05

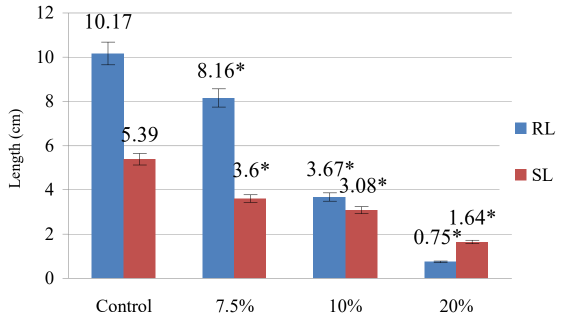

Figure 3. Effect of different concentrations of aqueous extracts of *D. inoxia* on maize root length (RL) and shoot length (SL)
Note: * = Significant at p≤0.05

Table 3. Inhibition (I%) of aqueous extracts and of cogermination on germination (G %) and seedling growth of maize

Treatments	I% of G	I% of root length	I% of shoot length
Control	-	-	-
7.5%	24.9	19.8	33.2
10%	23.2	63.9	42.8
20%	57.1	92.6	69.6
Cogermination (*D. inoxia* + *Zea mais* seeds)	0	61.9	40.0

DISCUSSION

There are no previous experimental results representing the cogermination effect of *Datura sp.* on the germination and growth in maize seedlings. However, it is known from the literature that the cogermination of weeds with various agricultural species can affect differently by stimulating or inhibiting germination, and growth depending on the donor and target species. For example, Ravlik [21] showed that the weeds seeds cogermination (*Cirsium arvense, Amaranthus retroflexus, Tripleurospermum inodorum, Solanum nigrum*) had not significant effect on agricultural crop germination, namely: on wheat, barley, carrot and soybean.

In this study, inhibition of germination and seedlings growth were significantly for all aqueous extracts concentrations. Some authors consider that reducing the shoot length can be caused by a reduction in auxin level that causes inhibition of cell division and cell elongation [2, 12, 28]. According to Turk et al. [29], an indirect association between lower seed germination and allelopathic inhibition may be the consequence of the inhibition of water uptake and enzyme activity.

Seedling growth was more sensitive to the aqueous extracts compared to seed germination. Similar results have been obtained in other studies with *D. stramonium*. The seedling growth of the *Triticum aestivum* and *Vigna unguiculata* species (root length) was more suppressed than the germination in the aqueous extract of *D. stramonium* at 2%, 4%, 6% and 8% concentrations [23]. The higher sensitivity of early seedling growth to phytotoxic extracts than to germination could be due to (i) the presence of seed coat which acts as a barrier in between the embryo and its surrounding environment, (ii) the selective permeability of seed coats [4, 31].

In our case of *D. inoxia*, an inhibitory effect was observed, similarly to the previous results of Szabo et al. [27]. They reported that the 5% and 7.5% concentration of *D. stramonium* plant extracts inhibited the shoot and root development of germinating maize. Pacanoski et al. [19] showed that the aqueous leachates of *D. stramonium* roots and shoots, did not produced any significant effect on the germination and the shoot length of maize, but significantly reduced the root length at the highest (1/1) *D. stramonium* roots leachate compared to control. Dafaallah et al. [9] showed that the aqueous extract of Jimsonweed significantly reduced seed germination of the tested poaceous crops (sorghum, millet, maize and wheat) and there was direct negative relationship between concentration seeds germination. On the contrary, the underground extract of *D. stramonium* increased maize radicle length by 35.3% compared to control, and decreased maize shoot length and germination by 28.4% and 10.2%, respectively compared to control [25]. Gella et al. [11] reported that aqueous extracts of *D. stramonium* root and stem, have stimulated elongation of wheat seedlings, but did not significantly affect the germination.

The results obtained in our study, suggest that the reduction in germination and root and shoot lengths of maize might be attributed by allelochemicals present in aqueous extracts of *D. inoxia*.

Our results corroborate with You and Wang [30] who found the inhibitory effect of allelochemicals (essential oil and its aqueous saturated solution) from

D. stramonium on germination and growth of maize, lettuce, radish, common bean and cucumber. According to Butnariu [8], presence of tropane alkaloids from *D. stramonium* extracts causes damage on *Sorghum halepense* seed germination and seedling growth. Ahmad et al. [1] found that the aqueous extracts of the aerial parts of Jimsonweed inhibited germination of wheat and this plant should be eradicated even if found near to the growing fields because the seeds contain the allelochemicals 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 cultivating fields toxic for the crop species.

In conclusion, the seeds cogermination had not significant effect on maize germination, but had significant effects on seedlings growth. Therefore, since this weed had strong allelopathic potential which suppressed germination and initial growth of maize. Further research is therefore need to identify arable crops which are not negatively affected by allelochemicals from *D. inoxia*. Moreover, there is an urgent need of integrated *D. inoxia* management strategy to stop further spread of this alien weed into cultivable areas. Farmers shall to give special attention in avoiding or minimizing those weed of *D. inoxia* from farm to contain their adverse effects on the maize.

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