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Thorn apple (*Datura stramonium* L.) allelopathy on cowpeas (*Vigna unguiculata* L.) and wheat (*Triticum aestivum* L.) in Zimbabwe

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***Datura stramonium* extracts have allelopathic properties. The study was conducted to investigate the allelopathic effects of *D. stramonium* weed on seed germination, early seedling growth and dry biomass of crop plants (*Triticum aestivum* and *Vigna unguiculata)*. Laboratory and greenhouse trials were arranged as completely randomised design and the field pot experiment was arranged as a randomised complete block design. Aqueous leaf extracts of *D. stramonium* at 2, 4, 6 and 8% concentrations were applied to determine their effects on seed germination, early seedling growth of crops under laboratory, field and greenhouse conditions. Distilled water (0%) acted as a control. Results from the study indicated that germination, shoot length and dry weight significantly decreased proportionally ($p<0.001$) as the concentration increased from 2 to 8%. The results showed that *D. stramonium* has allelopathic effects on wheat and cowpeas, hence cannot be used as a bio herbicide to control *Tagetes minuta* and *Amaranthus hybridus* on the selected crops since it is non selective to the crops studied. There is therefore need for further research on screening of arable crops against the allelopathic effects of *D. stramonium.* This will help to identify arable crops which are not negatively affected by allelochemicals from *D. stramonium* weed so that it can be used as a selective bio herbicide against other weeds.**

Key words: Allelopathy, aqueous leaf extract*, Datura stramonium, Triticum aestivum, Vigna unguiculata*.

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is an important food legume and an essential component of cropping systems in the drier and marginal areas of the tropics and subtropics (Fatokan et al., 2000). Cowpea is a summer annual legume that grows under extreme drought conditions. It is grown for vegetable or grain consumption (Saidi et al., 2007) and has numerous advantages ranging from high nutrition (25% protein, 1.4% fat, 60.8% carbohydrates and 3.4% ash), soil fertility improvement and weed control (Aliyu and Emechebe, 2003).

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Wheat (*Triticum aestivum* L.) is grown throughout the world and is adaptable to the wide range of environmental conditions. Wheat is used for human and livestock consumption. In Zimbabwe, it is mainly used as human food in the form of bread. Wheat is used in the making of pasta products, breakfast cereals, cake and tortilla.

Allelopathy involves harmful or beneficial effects of one plant upon another through the production of secondary chemical compounds that technically escape into the environment in sufficient quantity and with enough persistence to cause the enrolled effects (Khalid et al., 2002; Thi et al., 2015). Allelochemicals emancipated as residues, exudates and leaches by many plants from leaves, stem, roots, fruits and seeds were reported to interfere with the growth of other plants (Asgharipour and Armini, 2010). Allelopathy in this era is recognized as appropriate potential technology to control weeds using chemicals released from decomposed plant parts of other species (Naseem et al., 2009).

If allelopathy is to be exploited in weed management then information has to be generated on the effects of the allelochemicals on food crops. Thakur and Bhardwai (1992) reported that leachates from *Eucalyptus globulus* leaves significantly reduced maize germination but were ineffective on wheat germination. Studies have also demonstrated the harmful influence of the application of *Eucalyptus camaldulensis* plant extracts to sorghum seed germination, seedlings emergence and biomass gain (Mohamadi and Rajaie, 2009). Aqueous extracts of leaves notably inhibited seed germination of sorghum with application of *Parthenium hysterophorus* (Murthy et al., 1995), *Ipomoea carnea*, *Commelina benghalensis* and *Cyprus rotundus* (Channappagoudar et al., 2003) and *Eucalyptus camaldulensis* (Mohamadi and Rajaie, 2009). However, these allelochemicals sometimes have positive effects on sorghum growth; *Moringa oleifera* leaf extracts enhanced germination of sorghum by 29% (Phiri, 2010). The same kind of germination promotion behaviour was also observed in extract of *Cassia angustifolia* (Hussain et al., 2007).

Thorn apple (*D. stramonium*) is an annual poisonous plant that grows to approximately 1.5 m high. It is characterised by solitary white, trumpet-shaped flower (Fatoba et al., 2011). It has been said that several chemicals have been identified and phytochemical investigators believe that there are still many other chemicals which have not been identified to be exploited as bioherbicides (Elisante and Ndakidemi, 2014). Allelochemicals found in *D. stramonium* have allelopathic effects on survival of native plants. *D. stramonium* contains a series of allelochemical in form of alkaloids, atropine, hiosciamine and scopolamine (Butnariu, 2012), which inhibits the growth and development of root and shoots of *Trigonella* and *Lepidium* in a concentration dependent manner (EL-shora and Abd EL-Gawad, 2014, EL-shora et al., 2015a; An et al., 1996). These allelochemicals are said to reduce cell division or auxin that induces the growth of shoot and root (Gholami et al., 2011). Furthermore allelochemicals affect the root system of the plant through reduction in shoot extension, number of roots, curling of the root axis, swelling or necrosis of root tips and lowered reproductive capacity of the plant (An et al., 1998). Inhibition of growth caused by these allelochemicals may probably be due to its interference with the plant growth processes (Gholami et al., 2011).

There is limited research on *D. stramonium* allelopathy on arable crops. Therefore, the objective of this study was to determine *D. stramonium* allelopathy on *V. unguiculata* and *T. aestivum*.

MATERIALS AND METHODS

Experiment 1: Effects of *D. stramonium* concentration on the germination and early establishment of *T. aestivum* and *V. unguiculata*

Study site

The laboratory experiment was carried out at Midlands State University, located in Midlands province of Zimbabwe. The geographical location is 19°45' S (line of latitude) and 29°85' E (line of longitude). The site is in agro-ecological region III, at an altitude of 1428 m. The mean annual temperature was 18°C.

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times. Treatments were 20 ml of distilled water (control) and aqueous *D. stramonium* applied at 2, 4, 6 and 8% concentration as a ratio of plant extract powder to 100 ml distilled water. Two grams of extract powder was added to 100 ml of distilled water to give 2% concentration of aqueous and similarly, 4, 6 and 8% concentrations were prepared.

Preparation of concentrations

Leaves of fully grown plants were washed to remove soil particles. The material was then cut into pieces and shade dried for one month. After drying, the material was crushed into powder form manually using a traditional mortar and pistil. Further grinding was done by using an electric mortar.

The material (powder and distilled water) was mixed and poured into a conical flask with its mouth closed and kept for 24 h in the dark at room temperature according to the method used by Dhavan and Narwal (1994). The four flask were marked with stickers according to the *D. stramonium* concentrations (2, 4, 6 and 8%). This was followed by filtration process in two steps, in the first step muslin cloth was used and later the filtrate was allowed to pass through Whatman filter paper No. 1. The prepared aqueous concentrations were kept in a refrigerator to prevent conversions of some of the compounds upon exposure to light and high temperature.

Experimental procedure

Two hundred and twenty five seeds of the wheat and cowpeas were surface sterilized with 0.1% mercuric chloride solution for

2 min and washed twice with distilled water. The petri dishes were labeled with a permanent marker in relation to concentration level. Fifteen seeds of each weed were placed in petri dishes on Whatman filter paper No. 1. 20 ml of each *D. stramonium* aqueous concentration (2, 4, 6 and 8%) was added to each Petri dish. 20 ml of distilled water was used as a control. Watering was done after every three days and the petri dishes were kept in an incubator at 24°C room temperature for 10 days.

Data collection

Seed germination was recorded on the 7th day; seeds with emerged radical were counted and recorded. Seedling plumule length and radicle length was also measured using a 30 cm ruler on the 10th day after sowing in the petri dishes.

Experiment 2: Pot experiment: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of weeds in the field

Experimental design

The experiment was arranged as a complete randomised design with five treatments replicated three times and two crops were tested.

Experimental procedure for the field and greenhouse experiments

Two hundred and twenty five seeds of the selected crops were surface sterilized with 0.1% mercuric chloride solution for 2 min and washed twice with distilled water. Five litres pots were used and they were filled with mixtures of soil (loamy sand). Fifteen seeds of each of the tested weeds were sown in each pot at 0.5 cm and then irrigated with various solutions to field capacity every three days

Data collection for the field and greenhouse experiments

Data on seed emergence, shoot, and root length; seedling fresh and dry weight was recorded. Seed emergence was determined by physically counting the number of seedlings on the 8th day after planting. During the experiment period (after 30 days after planting), shoot and root length was also measured using a 30 cm ruler. The dry weight was determined by placing the tested samples in the oven to a temperature of 110°C for 48 h until a constant weight was realised.

Experiment 3: Effects of different *D. stramonium* aqueous concentrations on germination and early seedling growth of crops and weeds in the greenhouse

Study site

The greenhouse experiment was carried out at Morningside in Masvingo Province of Zimbabwe at a geographical location of latitude 20° 7’ 17S and longitude 30° 49’ 58 E. The site is in agro-ecological zone 4, at an altitude of 1034 m above the sea level. It receives an average of 600 mm of rain annually with a mean annual temperature of 28°C.

Experimental design

The experiment was arranged as a complete randomized design with five treatments replicated 3 times. Sixty pots were used and each replication had twenty pots. Two hundred and twenty five seeds of the selected crops were surface sterilized with 0.1% mercuric chloride solution for 2 min and washed twice with distilled water. Five litres pots were used, filled with mixtures of soil (loamy sand). Fifteen seeds of each of the tested weeds and crops were sown in each pot at 0.5 cm depth, and then irrigated to field capacity every 3 days with plant extracts at 2, 4, 6, and 8%.

Data analysis

Collected data was subjected to Analysis of Variance at 5% significance level using Genstat 4.0 version 2013. Fishers protected least significance test at 5% was used to separate the means where significant differences were noted.

RESULTS

The results show that there was a significant $P<0.001$ of the leaf extract concentration with water having the highest germination percentage, radicle length, root length, plumule length, shoot length, fresh and dry biomass of wheat and cow peas as compared to the rest of the treatments in the laboratory, field and greenhouse.

Seed germination and emergence

The results showed that an increase in thorn apple concentration led to the decrease in germination and emergence as shown in Figures 1 to 3. The highest germination (100) was recorded where distilled water was applied in all tested species in all the experiments. In the laboratory, wheat and cowpeas recorded a decrease of germination of 55.3 % and 55.65 % at 8% of aqueous thorn apple, respectively. The lowest germination percentage was recorded where 8% concentration of *D. stramonium* was applied on wheat and cowpeas across all the three environments.

Radicle and root length

The results indicated that as the concentration of thorn apple aqueous extract was increased from 0 to 8%, this led to a decrease in root length in all tested species across all the environments. Results from the laboratory and field showed highly significant reductions ($p<0.001$) effects of *D. stramonium* on root length across all the concentrations for both crops as shown in Table 1.

Plumule and shoot length

Results from the study showed that the plumule and shoot length of both tested species were reduced significantly ($p<0.001$) by aqueous leaf extracts of *D. stramonium* concentrations across all the environments shown in Table 2.

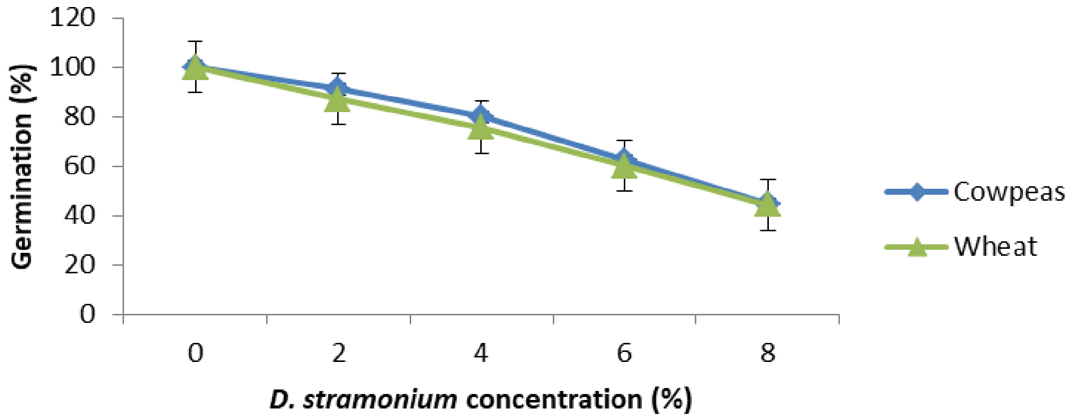

Figure 1. Effects of aqueous concentration of thorn apple on seed germination of crops in the laboratory

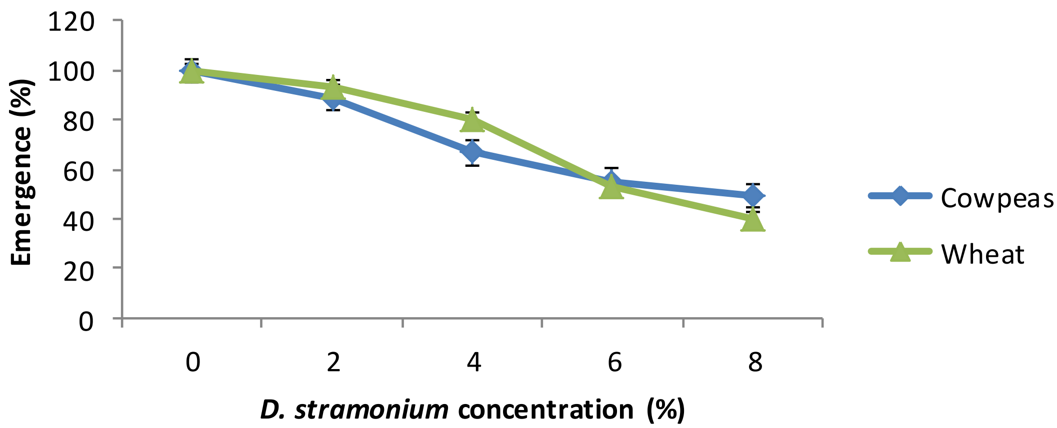

Figure 2. Effects of aqueous concentrations of thorn apple on seed emergence of crops in the field.

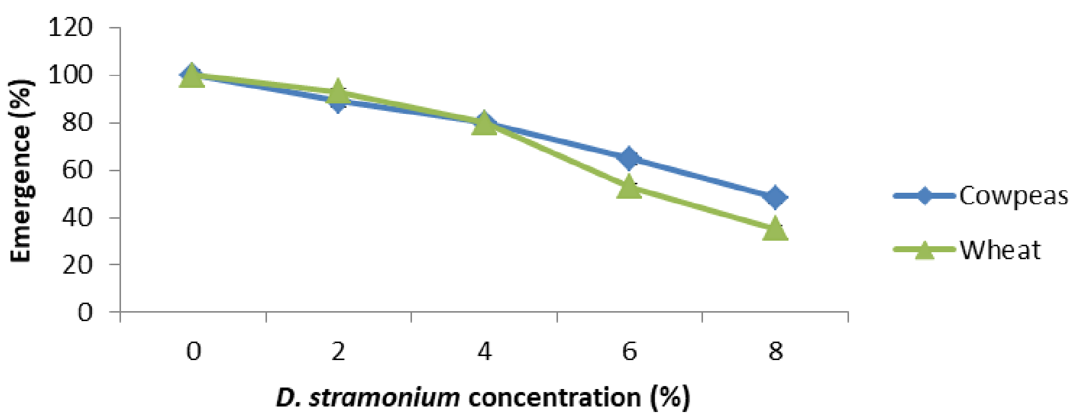

Figure 3. Effects of thorn apple aqueous concentrations on seed emergence of crops in the greenhouse.

Seedling fresh weight

Results indicated that the effect of the *D. stramonium* aqueous extracts was concentration dependent; it significantly ($p<0.001$) reduced seedling fresh biomass shown in Table 3. The highest reduction was recorded at

Table 1. Effects of aqueous concentrations of thorn apple on radical length of tested crops in the laboratory and field.

Concentration (%)	Laboratory		Field		Green house	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	51.03^{a}	43.23^{a}	65.23^{a}	74.70^{a}	65.03^{a}	74.90^{a}
2	42.23^{b}	41.17^{b}	62.93^{b}	69.53a^{b}	62.87^{b}	68.97^{b}
4	43.1^{c}	37.33^{c}	58.87^{c}	66.50^{bc}	58.83^{c}	65.07^{c}
6	40.1^{d}	35.13^{d}	54.23^{d}	61.70^{c}	53.47^{d}	59.43^{d}
8	38.43^{e}	34.10^{e}	51.03	55.37^{d}	50.90^{e}	51.67^{e}
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	0.8	0.2	1.7	4.5	2	0.7

*Means followed by the same letter in the same column are not significantly different.

Table 2. Effects of different aqueous concentrations on early seedling growth (plumule length) in the laboratory, field and greenhouse.

Concentration (%)	Laboratory		Field		Green house	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	21.38^{a}	43.23^{a}	79.03^{a}	85.60^{a}	79.10^{a}	85.73^{a}
2	19.78^{b}	41.17^{b}	76.87^{ab}	83.80^{b}	76.90^{ab}	83.67^{b}
4	18.12^{c}	37.33^{c}	75.10^{b}	79.03^{c}	74.80^{b}	78.93^{c}
6	17.12^{d}	35.13^{d}	69.57^{c}	76.20^{d}	69.83^{c}	75.90^{d}
8	16.40^{e}	34.10^{e}	66.50^{d}	70.60^{e}	66.67^{d}	70.40^{e}
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV (%)	3.2	0.2	1.6	0.5	1.8	0.7

*Means followed by the same letter in the same column are not significantly different.

Table 3. Effects of different aqueous concentrations of thorn apple on fresh weight of crops in the greenhouse and field.

Concentration (%)	Greenhouse		Field	
	Triticum aestivum	*Vigna unguiculata*	*Triticum aestivum*	*Vigna unguiculata*
0	2.537^{a}	5.527^{a}	2.510^{a}	5.497^{a}
2	2.407^{b}	5.390^{b}	2.370^{b}	5.370^{b}
4	2.273^{c}	5.237^{c}	2.217^{c}	5.30^{c}
6	2.177^{d}	5.060^{d}	2.160^{d}	5.047^{d}
8	2.017^{e}	4.957^{e}	2.017^{e}	4.947^{e}
P value	<0.001	<0.001	<0.001	<0.001
CV (%)	0.5	0.6	1.1	0.5

*Means followed by the same letter in the same column are not significantly different.

8% concentration in both field and greenhouse environments.

Dry weight

From the study, results indicated that as the concentration of thorn apple, aqueous extract increased from 0 to 8%; seedling dry weight was significantly ($p<0.001$) reduced in both field and greenhouse environments shown in Table 4.

DISCUSSION

Results in this study indicated that where distilled water

Table 4. Effects of different aqueous concentrations of thorn apple on dry weight of crops in the field and greenhouse.

Concentration (%)	Greenhouse		Field	
	Triticum aestivum	***Vigna unguiculata***	***Triticum aestivum***	***Vigna unguiculata***
0	1.0867[a]	2.277[a]	1.500[a]	2.230[a]
2	0.9233[b]	2.140[b]	0.9100[b]	2.103[b]
4	0.823[c]	1.987[c]	0.7567[c]	1.963[c]
6	0.7267[d]	1.810[d]	0.700[c]	1.780[d]
8	0.5667[e]	1.707e	0.5567[d]	1.680[e]
P value	<0.001	<0.001	<0.001	<0.001
CV (%)	3.7	1.5	4.2	4.6

*Means followed by the same letter in the same column are not significantly different.

was applied germination was very high due to lack of germination inhibition. This shows that water is necessary for germination to take place; it triggers biochemical reactions. Seed germination on all tested species might have been inhibited by the presence of aqueous thorn apple extracts; these results were in line with those by Hassannejad and Ghafarbi (2013). This could be attributed to reduced water uptake by the seeds in the presence of allelochemicals found in thorn apple (EL-shora et al., 2015a). According to Oyun (2006), allelochemicals inhibit water absorption which is a precursor to physiological processes (hydrolysis of food nutrients) 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 imbibitions leading to delayed germination and emergence. Results from the field and greenhouse experiments showed the same trend as compared to the laboratory; however, seed emergence was not affected at large. In the laboratory, seed were always exposed directly to aqueous extracts which might have caused significant effects as compared to the pots which did have soil. When a comparison is made in terms of seeds that germinated in petri dishes and pots results showed that seeds germinated better in pots as compared to petri dishes. Allelochemicals present in *D. stramonium* might have been inactivated in the soil by several factors such as complexation with soil colloids, chelation with ions and decomposition by micro-organisms. In an experiment by Wasim et al. (2008) on effects of allelochemicals on vigour of *T. aestivum* (variety GW273), results showed that allelochemicals are known to disturb the activity of enzymes that break down starch that nourish the growing embryo during germination. Results obtained were also in agreement to with those by Alam and Islam (2002), Altikat et al. (2013), and Ullah et al. (2015) who found that allelochemicals disturb activities of peroxidase alpha-amylase and acid phosphates which aid the breaking down of starch in wheat. From the study results observed, they were in agreement with those obtained by EL-Shora and Abd EL-Gawad (2014), EL-Shora et al., (2015a) and Singh et al. (1992) who reported that biological activities of receiver plants to allelochemicals are known to be concentration dependent with inhibition increasing as the concentration of *D. stramonium* increased from 0 to 8%.

Results on root length from the laboratory showed highly significant effects of *D. stramonium* on root length on both crops. This might have been promoted by similar physiological processes that occur during cell division, cell expansion and auxin production. When results of root length in petri dishes and pots were compared, they showed that seedlings in pots had greater root length than those in petri dishes. From the study, this trend might have been brought by continuous exposure of roots directly to free aqueous *D. stramonium* in the laboratory which might have reduced root length. In pot experiments, however, allelochemicals present in *D. stramonium* aqueous extract might have been inactivated in the soil thereby significantly reducing root length to a higher extent. From this study, results indicated that plant roots exposed to *D. stramonium* became brownish and shorter in length. The results were in agreement with those by Butnariu (2012) who concluded that *D. stramonium* contains a series of alkaloids such as atropine (d-1-hiosciamine), hiosciamine, and scopolamine which inhibit the growth and development of roots. The allelochemicals present in *D. stramonium* might have interfered with auxin production on root tips which promotes root elongation and expansion (Gholami et al., 2011). Allelochemicals from thorn apple in this study exhibited allelopathic potential on seedling growth of tested species. From the study, results indicated that these extracts might have some inhibitory alkaloids which might have reduced seedling growth of the tested species by hindering cell division, development and cell elongation on root tips. Results from the study showed that seedling growth (radical and root length) was reduced and plant roots exposed to allelochemicals became brownish. This might have been the mode of action of allelochemicals which interfered with hormone production on root tips. Cowpea and wheat became shorter and showed signs of necrosis when they were exposed to *D. stramonium* aqueous extract. The results were in agreement with those by Lorber and Muller (1976) who observed that allelochemicals caused structural damage to root tips of target plants leading to reduced root size and length. From the study, these allelochemicals might have interfered with hormones that encourage growth processes, development, cell elongation, cell division and cell enlargement on younger active root tips where mitosis is active (EL-Shora et al., 2015a). These findings were similar to those by Gholami et al. (2011) who concluded that alkaloids present in *D. stramonium* (hiosciamine and scopolamine) can reduce cell division and auxin synthesis that induces the growth of shoots and roots.

Results from the study showed that the plumule and shoot length of both tested species was reduced by aqueous leaf extracts of *D. stramonium.* The results obtained suggest that the reduction in shoot length might be attributed to the presence of *D. stramonium* aqueous extracts. Reduction in plumule length and shoot length might be due to the presence of allelochemicals in *D. stramonium* which might have inhibited mitosis. The presence of allelochemicals might have caused disassembling of microtubules leading to shortened spindle fibres essential for cell division to take place. *D. stramonium* aqueous extract might have inhibited protein synthesis in shoot meristems in tested species leading to reduced shoot length. The results corroborate with those by Hussain and Reigosa (2011) who observed the inhibitory effects of allelochemicals (phenolic compounds) on root and shoot length of *Dactylusglomerata*, *Loliumperenne* and *Rumexacetosa*. The allelopathic effects of *D. stramonium* on shoot growth of tested species increased as their concentration was increasing.

Results in the greenhouse and field experiments indicated that the effect of the *D. stramonium* aqueous extracts on cowpeas and wheat was concentration dependent (EL-Shora and Abd EL-Gawad, 2014; EL-Shora et al., 2015a). The presence of *D. stramonium* aqueous extracts might have interfered with photosynthesis leading to drastic changes in the physiology of plants. Reduction in photosynthesis might have led to reduced plant growth and reduced fresh biomass accumulation due to reduced water content in the tested species (EL-Shora et al., 2018). According to Oyun (2006), allelochemicals inhibit water absorption which is a precursor to physiological processes like photosynthesis. Reduced water uptake might have promoted reduced fresh biomass accumulation on tested species. Photosynthesis encourages vegetative growth and rapid accumulation of fresh biomass. The effects of allelopathy on seedling fresh weight and growth of plants may occur through a variety of mechanisms including reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced water uptake, inhibited photosynthesis, respiration, inhibited protein formation and decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1984).

Reduction in total biomass dry matter correlated with root and shoot length; this is in line with a study by Garcia et al. (2002) who observed that reduction in biomass might be due to stunted and reduced seedling growth.

Conclusion

It can be concluded from the results that allelochemicals present in the aqueous extract of *D. stramonium* suppressed germination and early seedling growth of *Triticum aestivum* and *Vigna unguiculata.* Sensitivity of the tested species differed; the seedling growth of the tested species (root length) was more suppressed than the germination. Wheat and cowpeas are susceptible to *D. stramonium* allelochemicals hence cannot be used as bio herbicides in these two arable crops. However, further research is necessary for comparison of more than one season in different agro-ecological zones. The results showed that *D. stramonium* has allelopathic effects on wheat and cowpeas, hence cannot be used as a bio herbicide since it is non selective to the crops studied. There is therefore need for further research on screening of arable crops against the allelopathic effects of *D. Stramonium.* This will help to identify arable crops which are not negatively affected by allelochemicals from *D. stramonium* weed, so that it can be used as a selective bio herbicide against other weeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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