Growth And Physiological Response Of Some Cultivated Species Under Allelopathic Stress Of Calotropis Procera (Aiton) W.T

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Introduction

Allelopathy is a natural ecological phenomenon in which different organisms affect the functioning of other organisms in their vicinity, negatively or positively (Rice 1984) by releasing secondary metabolites as byproducts called allelochemicals during different physiological processes in plants (Farooq et al. 2011). The unfavorable effects of allelochemicals on recipient plants are considered as biotic stress called 'allelochemical stress' (Cruz-Ortega et al. 2002). Allelochemicals are released by plants into the environment and caused allelopathic stress (Crutchfield et al., 1985). Such allelochemicals may be present in leaves, flowers, roots, fruits, or stems. They can also be found in the surrounding soil after decomposition of the plant residues. Allelochemicals are believed to be a joint action of several secondary metabolites including phenolic compounds (Dalton, 1999), flavonoids (Berhow and Voughn, 1999), juglone quinone (Jose and Gillespie, 1998), terpenoids (Langenheim, 1994) and non-protein amino acids like alkaloids (Harborne, 1988). In recent decades, many researchers such as Jabeen et al., 2011; Raoof and Siddiqui, 2012 and Mangal et al., 2013, 2014) have reported the effect of various allelochemicals of different plants on physiological and biochemical processes of the recipient plants.

Calotropis procera (Aiton) W.T (Milkweed) is a member of family Asclepiadaceae. It is a xerophytic perennial shrub or shallow tree that grows in many arid and semi-arid countries (Mascolo et al. 1988). In Egypt, it has very wide range of ecological amplitude and spread in many regions around the agricultural lands, desert regions and newly reclaimed areas. It possesses allelopathic interactions under field conditions (ex. Samreen et al. 2009, Abdel-Farid 2013, Hassan et al. 2015). A number of secondary metabolites or allelochemicals have been isolated from this plant that include many flavonoids, cardiac glycosides, triterpenes and sterols (Heneidak et al 2006). In the stands where it grows, bare patches of some crops and vegetables around its individuals were observed, especially in the newly reclaimed lands. Therefore, the present investigation focused on using an aqueous extract to study the hypothesis that water-soluble materials of Calotropis procera (Aiton) W. T. can be released from plant tissue and exert allelochemical stress on the

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neighboring species. To achieve this goal, a laboratory study was initiated to evaluate its effect on some growth and physiological parameters of wheat (Triticum aestivum), cucumber (Cucumis sativus) and tomato (Lycopersicum esculentum).

Materials and Methods
Collection of plant material
Leaves of matured C. procera plants were collected from its natural stands. With regards to test species, seeds of wheat, cucumber and tomato were collected from the field. Both seeds and leaves were stored in a room with a constant temperature of 25° at our laboratory ready for experiments. Leaves of C. procera were dried in air for 10 days and then crushed into powder form and packaged in paper bags for further use.

Preparation of leaf aqueous extract
The powdered sample from the leaves was weighed to get 500 g. A 1:1 w/v stock solution of leaf extract was obtained by soaking the crushed plant material in sterilized water (500 ml) for 48 h at room temperature to allow the auto extraction of plant metabolites. It was then passed through a muslin cloth and finally filtered through Whatman filter paper No. 1, according to El-Khatib and Abd-Elaah (1998), El-Khatib and Hegazy (1999) and El-Khatib (2000). From stock solution (100%), concentrations; 10%, 20%, 30%, 40% and 50% were then made. The extract was stored at 4°C in pre-sterilized flasks. To avoid contamination and prospective chemical alterations, the extract was ensured to be used within 3-4 days.

Pots experiment
Seeds of wheat, cucumber and tomato were sterilized by washing them in Sodium hypochlorite 5% for 2 minutes to avoid the effects of fungal contamination. Sterilized seeds were then washed thoroughly three times in distilled water before planting. Eight seeds from each tested species were then planted in pots (20 cm X 15 cm and 2 kg by weight) and then treated with different concentrations of leaf extracts prepared from C. proceca. The combination of treatments was laid out in a randomized complete block design (RCBD) with three replications. Treatments included six different concentrations of C. procera, leaf aqueous extract: 0 (distilled water), 10%, 20%, 30%, 40% and 50%. Each treatment had three replicates. Sandy clay loam soil for this pot experiment was collected from stands away from C. procera community. The soil is hydrated to the field capacity throughout the experiment period. After 10 days, the developing seedlings were thinned to five individuals / pot/ species. The experiments continued for one month under field conditions, then plants from each pot were harvested and final required measurements were taken.

Biophysical measurements.
Final measurements were taken after one month of growth of test species after termination of the experiment. Roots and shoot length of all plants in each replicate were measured in centimeter (cm) (Abdul-Baki and Anderson 1973). The fresh and dry weight of plants in each pot was determined and the averages were calculated (Agrawal 1994). The leaf area (cm²) of plants was recorded and the data obtained were analyzed (Watson and Watson 1953).

Biochemical measurement.
The chlorophyll and carotenoid contents were determined according to (Lichtenthaler 1987). Soluble sugar was extracted according to Homme et al. (1992). Soluble protein was determined according to the method described by El-Tayeb et al. (2006). Proline content of leaves was estimated according to Bates et al. (1973).

Statistical methods and data analysis
One Way Analysis of Variance (ANOVA) was used to analyze data recorded in the study. Significant means were separated according to Fishers least significant difference (LSD) at p = 0.05, and 0.01. The growth response to the concentrations of leaf aqueous extract of C. procera was measured by fitting the regression model (Y= α (X) + β). Determinant Coefficient value (R²) was computed to each tested species. The software program STATISTICA version 10 was used to perform statistical analysis. Based on the control measurements, relative percentage (%) of inhibition/stimulation in the measured growth parameters was computed.

Results and discussion
The importance of allelopathy in nature has attracted ecologists attention with the main goal of using the phenomenon in interpretation in many disciplines related plant community structure (El-Khatib 1998& 2000.). Allelopathy or the chemical mechanism of plant interference is characterized by a reduction in emergence or growth of some target species in the community. The allelopathic phenomenon has received much attention, as shown by numerous reports on the subject; khan et al., 2005;; Reigosa et al., 2006;; Kamal and Bano, 2009; Kamal, 2011). The activity of allelochemicals cannot be explained by just a single mode of action. The majority of effects such as reduction in seed germination and seedling growth, chlorosis, decreased ion uptake and other physiological, morphological and anatomically
abnormalities are caused by a variety of more specific interaction between allelochemicals and cellular or molecular systems (Einhellig 2002).

Many authors (ex. Abdel-Farid 2013, Farahat et al. 2015) studied the plant sociological characteristics of C. procera and reported its negative association with many species, even with those have similar ecological requirements. Also, the present investigation showed that aqueous extract inhibited growth parameters of the test species. It can, therefore, confirms the hypothesis that under natural conditions, water-soluble materials of C. procera can be released from plant tissue and exert allelochemical stress on the neighboring species. In the present investigation, responses of the bioassay species to the aqueous extract varied among the three test species. The allelopathic effects of C. procera were found to be dependent on the concentration of the extract. Parameters which were investigated include leaf pigmentation content, soluble protein content, free amino acids content, soluble carbohydrate content, proline content, leaf area, root and shoot length as well as fresh and dry weight.

Effects of the leaf aqueous extract on shoot and root lengths

Allelopathic effects of Calotropis leaf aqueous extract on root and shoot length of the test species have been shown in figure (1 a, b). The root and shoot lengths of the test species affected by allelopathic stress caused by the extract when compared with control. A significant decrease (P≥ 0.01) in the shoot and root length of all test species was observed with an increasing concentration of the C. procera leaf extracts. These results are lined with those of Ashrafi et al., (2009) and Batlang and Shushu, (2007), who reported that the inhibition of the plant depends on the concentrations of the allelochemicals. Interestingly the present results revealed that leaf aqueous extract was more capable for inhibiting root length of the test species than shoot length. Relative to controls, maximum percentages of inhibition of both root (75.61%) and shoot length (60%) were recorded in tomato. This may be due to the direct contact between the roots and phenolic compounds of the aqueous extract which may, in turn, inhibit cell division, which is highly active in meristematic tissues for the growing roots (Rietjens and Alink, 2003, Al-Watban and Salama 2012). Based on data of the regression analysis, the length of the shoot of tomato (α = -0.847) appeared to be more inhibited than those of cucumber (α = -0.923) and wheat (α = -0.925). In regards to root length, also cucumber was the most affected species (α = -0.787) than both tomato (α = -0.704) and wheat (α = -0.644). These results revealed that the growth response of plant parts to the allelopathic stress caused by allelochemicals in the leaf aqueous extract, where root length appeared to be more inhibited than those of shoot.

Effect of the leaf aqueous extract on root and shoot weights

The results presented (Fig. 2 a, b), clearly demonstrate the allelopathic effects of the leaf aqueous extract of C. procera, which has a phytotoxic influence on the test species. At higher concentrations of leaf extracts, a significant reduction in the fresh and dry weight of the test species was recorded. The study showed that an increase in treatment concentration, reduced the fresh and dry weight of the test species, except a cucumber which exhibited non-significant (P≥0. 05) increase in its fresh and dry weight at a concentration of 1.%. However, the reduction was more prominent at the highest extract concentration. These results are in agreement with An et al., (1993). They showed that any secondary compound with allelochemical activity can cause both stimulatory and inhibitory effects. This pattern is common and it related to concentration: stimulation at low concentrations and suppression for higher concentrations.

On the basis of fresh weight, the test species exhibited different degrees in their responses to the inhibitory effect of extracts as a tomato ≥ cucumber ≥ wheat, where the regression coefficient (α) values for the test species were -0.152, -0.248 and -0.253, respectively. Concerning dry weight, the test species has a pattern of reduction as tomato≥ wheat≥ cucumber, where the values of (α) were -0.051, -0.060 and -0.061, respectively (Fig 2 b). Relative to control, the maximum percentage of inhibition was recorded in tomato (fresh weight = 91.67%, dry weight = 90%), while the minimum one was recorded in cucumber (fresh weight = 64.69%, dry weight = 60.52%). According to the computed values of coefficient of determinant (R²), the leaf aqueous extract appeared to be a major factor controlled the plant growth, due to phytotoxic effect. However, the decrease in the fresh weight reflects the failure of roots to absorb an adequate amount of moisture from the soil. Resultantly, a drought like condition prevails which leads to its poor growth. Yasin et al., (2012) reported a similar reduction in the moisture content of the seedlings due to phytotoxic effects. This reduction suggests the interference by toxic substances from the extract with the cell division, causing a reduction in the root cell growth. This may lead to a decrease in mineral uptake, nutrient absorption and the transport of nutrients from the root to other plant parts. This reduction in growth resulted in the reduction in the biomass and consequently the dry weight.

Effect of the leaf aqueous extract on the leaf area

There was a wide variation in the average leaf area for each one of the test species as expressed in Cm² plant⁻¹ along the concentration gradient of the aqueous extract. Leaf area of the test species exhibited sensitivity to the inhibitory effect of C. procera extract. According to the sever effect of extract, the test species arranged in the following order: cucumber ≥ tomato ≥ wheat. The results of regression analysis showed that there is an inverse relationship between the leaf area of the test species as a response variable and extract concentration as an independent variable. The coefficients of regression (α) values were -0.886, -0.944 and -1.932 for cucumber, tomato, and wheat, respectively (Fig. 3). Except for the leaf area of cucumber at a concentration of 10%, where the non-significant increase was observed, the degree of

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inhibition in leaf area of all test species significantly (P≥0.01) increased with the increase of the extract concentration. Based on the control, the percentage of inhibition was 61.74%, 77.78% and 58.55% of cucumber, tomato, and wheat, respectively. The determinant coefficient (R²) in the regression model indicates the amount of variation in the leaf area of the test species with the increase of extract concentration, being 91.1%, 83.7% and 78.8% of tomato, cucumber, and wheat, respectively.

**Effect of the leaf aqueous extract on the pigment content**

In the present study, when pot plants were treated with different concentrations of C. procera leaf extract, the pigment content of all test species exhibited significant differences from control. In regards to chlorophyll content, both chlorophyll (a) and (b) exhibited the same pattern of reduction with the raising of the extract concentration, reflecting highly significant differences (P<0.01) in between and also between them and control (Fig 4 a, b). In comparison, a high concentration of extract (50%) was more determinant on the content of chlorophyll (a) in both tomato and cucumber than in wheat, where The coefficients of regression (α) values were -0.188, -0.524 and -0.678, respectively (Fig 4 a). Regarding chlorophyll (b) content, its value showed the same pattern of reduction as chlorophyll (a) with the raising of extract concentration, except at lower concentration (10%) wheat showed a non-significant (P≤0.05) increase from the control. The determinant effect of the extract appeared to be more obvious with tomato plant (R²= 91.88%). The results of the study are consistent with those of Einhellig and Rasmussen (1993) who reported that allelochemicals caused a marked reduction in the chlorophyll content of the test species. The reduction in chlorophyll contents (a and, b) observed in all the concentrations might be due to degradation of chlorophyll contents or reduction in their synthesis. Furthermore, Rice (1984) has suggested that the synthesis of porphyrin precursors of chlorophyll biosynthesis may be impeded by some allelopathic compounds. Allelochemicals may enhance the activities of enzymes such as chlorophyllase and Mg-dechelatase, responsible for the Chlorophyll derivatives pathway (Yang et al., 2004).

Concerning carotenoids, the test species showed different trends of variations. Carotenoid content of tomato significantly increase (P<0.05) with the raising of extract concentration, while those of wheat sharply decreases till 20%, after that, it increased. Cucumber showed significantly (P≥0.05) increase from control at concentrations of 10% and highly significant (P<0.01) decrease at 20% concentration. Generally, at higher concentration the carotenoid content of all test species exhibited the same pattern of increases. (Fig. 4 c). In comparison, carotenoid content of wheat (α = 0.013, percentage inhibition = 31.25%) was more negatively affected by the aqueous extract than that of cucumber (α = -0.0336, percentage inhibition = 12.5%). On the other hand, the positive effect of the aqueous extract on the carotenoid content was recorded in tomato (α = 0.008, the percentage of stimulation = 21.73%).

**Effect of leaf aqueous extract on soluble metabolites content**

Data presented in figure (5) show the effect of aqueous extract of C. procera on the content of soluble carbohydrates, soluble proteins, and free amino acids. It is clear that the aqueous extract has stimulus effect to increase the content of these metabolites in all test species. This stimulus effect was a concentration dependent, where highly significant (p≤0.01) differences between control and higher concentrations were recorded with all test species. Relatively, the species exhibited varying degrees of response, of which tomato was the least affected species.

In regards to soluble carbohydrates (Fig. 5 a), species arranged in order as cucumber ≥ wheat ≥ tomato (α = 6.475, 3.260 and 1.989, respectively). This positive relationship confirmed by the percentage of stimulation caused by the high concentration of the aqueous extract (50%), where its value in cucumber (92.13%) was higher than that of wheat (31.22%) and tomato (30.30%). In contrast to our results Ianzo et al., (2008) reported a decrease in the soluble sugars content in their test species. Meanwhile, the results obtained by El-Darier (2002) revealed that treatment of maize and bean seedlings with Eucalyptus rostrata powder leaf leads to accumulation of mono and polysaccharides. With a soluble protein (Fig. 5 b), species arranged as follows: wheat ≥ cucumber ≥ tomato (α = 2.899, 2.257 and 0.532, respectively). The determinant coefficient value reflects the role of the aqueous extract in the variance of this stimulation, where it was 99.11%, 86.84% and 94.80% for wheat, cucumber, and tomato, respectively. Al-Watban and Salama (2012) reported that aqueous extract of Artemisia monosperma aerial parts at concentration 2.0% and 4.0% w/v decrease the content of soluble sugars while increased the content of proteins in tissues of common bean seedlings. Moreover, Saleh (2013) demonstrated that treatment of corn seeds with Olive Processing Wastes (OPW) extract at concentration 3.0% w/v significantly increased the soluble sugars and proteins content in seedlings tissues, while the higher concentrations were inhibitory. It is well known that the soluble sugars can keep the intracellular osmosis pressure and play important roles in maintaining the normal physiological function of cells (Kohyama & Nishinari, 1991). The soluble proteins with colloidal properties are main components in cell matrix, and they can increase protoplasm hydration (Fisher et al., 1992). The results of the current investigation demonstrated that the soluble sugar content increased remarkably under the impact of aqueous extract concentration. This suggested that allelochemicals can hinder the ability of the test species to absorb water and cause a certain degree of water stress so that the accumulation of soluble sugar was increased in their tissues. Wang et al., (2005) also thought that allelochemicals can influence the plant roots’ absorption of water. It may also be that allelopathy stress caused degradation of macromolecular sugar and protein, and then transformed into sucrose, glucose, and other micromolecular soluble sugars.
Regarding free amino acids (Fig 5 c), the species arranged in the same manner as in the case of soluble proteins. The values of coefficient of determinant ($R^2$) reflect the positive role of aqueous extract in the recorded variances in the free amino acids content in all test species. The higher level of amino acids was assumed to the increasing in protein content which due to a great decline in the protease activity that plays an important role in the hydrolysis of reserve proteins. Similar results were observed by many authors (El-Khatib and hegazy 1999, Al-Watban and Salama 2012).

**Effect of leaf aqueous extract on proline content**

Figure (6) shows the data of the proline content of the test species under the effect of aqueous extract of C. procera. The accumulation of proline in all test species has the same trend and increase with the increase of the extract concentration. Highly significant differences ($p\geq 0.01$) between the treatments and control were recorded. The accumulation of proline was more pronounced in cucumber ($\alpha = 2.479$) followed by tomato ($\alpha = 1.308$) and then wheat ($\alpha = 1.150$). The percentages of stimulation were 581.09%, 250.45% and 240.745 folds of control for cucumber, tomato, and wheat, respectively. It is clear that the aqueous extract posed an allelochemical stress to the growing plants. The present results are in agreement with those of many authors (Abdulghadar and Nabat (2008), Al-Watban and Salama (2012) and Al-Taisan (2014), ) who also observed the similar type of results. Proline is the amino acid that is associated with different stresses in plants (Kumar et al., 2003). The magnitude of the increase was similar to that under drought stress, where proline protects proteins from denaturation by maintaining the hydration level. Under drought stress, in addition to its role as an osmoregulator, proline like other soluble organic compounds, may also act as osmoprotectants (Kamelie and Loseh, 1995). However, the present results are in contrast to those of Cattivelli et al., (2008) and Al-Taisan (2014) who reported the decrease in proline content in their test species under stressful.

In summary, it is familiar that both biotic and abiotic stresses during the growth period often cause to increase production of secondary metabolites (Weir et al., 2004) and other metabolic compounds as free amino acids, proline, sugars, organic solutes (Ercisli et al., 2005). The all test species in this study tended to accumulate amino acids, soluble sugars soluble proline and carotenoids which may be considered an adaptive mechanism to increase allelochemical stress tolerance.

![Figure 1. Effect of C. procera leaf aqueous extract on the length of shoot and root of the test species](image_url)
Figure 2. Effect of C. procera leaf aqueous extract on the fresh and dry weight of the test species

\[ y = -0.248x + 1.9123 \quad R^2 = 0.6367 \]

\[ y = -0.2525x + 1.6998 \quad R^2 = 0.7584 \]

\[ y = -0.1515x + 0.8386 \quad R^2 = 0.7607 \]

Figure 3. Effect of C. procera leaf aqueous extract on the leaf area of the test species

\[ y = -0.0598x + 0.3831 \quad R^2 = 0.7594 \]

\[ y = -0.0612x + 0.4643 \quad R^2 = 0.6329 \]

\[ y = -0.0511x + 0.2874 \quad R^2 = 0.7718 \]
Figure 4. Effect of C. procera leaf aqueous extract on the pigment content of the test species.
Figure 5. Effect of C. procera leaf aqueous extract on some soluble metabolites content of the test species.
References


