



Full Length Article

Allelopathic Potential of *Calotropis procera* and *Morettia philaeana*

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Abstract

The allelopathic potential of different concentrations of aqueous leaf and flower extracts of *Calotropis procera* and *Morettia philaeana* were investigated in laboratory experiments for germination and seedlings growth of *Triticum aestivum* (wheat), *Raphanus sativus* (radish) and *Brassica napus* (canola). The germination of wheat and radish was delayed and its percentage was reduced significantly after treatment with leaves extracts of *C. procera* and *M. philaeana*. Shoot and root length was significantly reduced after treatment. The reduction of seed germination percentage and shoot and root length was proportional to the concentration of the extracts used. Flower extract of *C. procera* delayed the germination in wheat and reduced significantly the root and shoot lengths, where as the extract delayed and reduced significantly the germination percentage and reduced significantly shoot length in canola. Distinct stress on germinated seeds appeared as proline content increased significantly in wheat plants after treatment with *C. procera* or *M. philaeana* extracts particularly at higher concentration of the extracts. *Morettia philaeana* aqueous extract was stronger than that of *C. procera* on wheat and radish. © 2013 Friends Science Publishers

Keywords: Allelopathic potential; *Morettia philaeana*; Proline content; Seed germination; Shoot and root length

Introduction

Allelopathy refers to the direct or indirect, harmful or beneficial effects of one plant on another through the production of chemical compounds escaping into the environment (Rice, 1984). Allelochemicals are released into soil through leaching, root exudation, volatilization and decomposition of plant residues (Rice, 1984), and are often water soluble. These are secondary metabolites secreted in very low amounts by plants but have very important role as plant defense against microorganisms or other abiotic factors. There are two types of these secondary metabolites; the first, phytoanticipins are found during normal condition of plant and represent its first arsenal defense against different biotic and abiotic stresses. The second type, phytoalexins are low molecular weight compounds that are formed *de novo* when plants are subjected to any stress conditions. Allelopathy involves the mechanism by which chemicals from one plant are released into the environment that affects other plant's biological processes. Allelopathic responses are species specific and may vary during developmental stages of the same plant. Allelochemicals may be present in leaves, flowers, roots, fruits, or stems. They can also be found in the surrounding soil after decomposition of the allelopathic plant residues. In sensitive species, the allelochemicals may inhibit seed germination, shoot/root growth, disturb nutrient uptake, or they may alter a naturally occurring symbiotic relationship, and thereby destroying the plant's usable source of a nutrient.

Allelopathy has been employed widely in crop protection as an environment friendly method instead of using herbicides, fungicides and insecticides (Khanh *et al.*, 2005; Bhadoria 2011).

In Egypt, wheat is one of the most important crops of economic significance. Egyptian government imports millions of tons of wheat every year which cost millions of dollars (11.5 million tones in the 2011/2012 (July/June). Wheat yields are restricted due to a number of factors. Competition between wheat crop and associated harmful weeds such as *Avena fatua* L. and *Brassica nigra* L., and take off disease caused by some soil fungi have been common phenomena. The allelopathic potential of some wild plants may be explored as an effective approach to overcome the problems of pathogen attack and weed competition to this important crop in Egypt. Exploiting such allelopathic plants and their effective secondary metabolites as a strategy for weed management demands lot of efforts.

Calotropis procera Decne., a member of family Asclepiadaceae, has very wide range of ecological amplitude. It has spread in many regions of Egypt around the agricultural lands and farms. It is also widely distributed in desert regions in Egypt. A number of secondary metabolites have been isolated from this plant that include many flavonoids (Heneidak *et al.*, 2006), cardiac glycosides (Hanna *et al.*, 2002), triterpens (Bhutani *et al.*, 1992) and sterols (Chundattu *et al.*, 2012), but the allelopathic potential of this plant for managing weeds still remains to be explored. *Morettia philaeana* (Delile) DC., a

member of family Brassicaceae, is also distributed widely in the Egyptian deserts and newly reclaimed lands. Like all other Brassicaceae members, *M. philaeana* contains glucosinolates and their hydrolyzed by-products (isothiocyanates). The isothiocyanates have very strong allelopathic potential against weeds and rhizosphere microorganisms (Jafarihyazdi and Javidfar, 2011). Very little is known about allelopathic potential of *M. philaeana* that may have other compounds such as saponins, phenylpropanoids and phenolics. Recently, some flavonoids had been isolated and identified from *M. philaeana* and their antimicrobial activity was assessed (Kawashty *et al.*, 2012).

In these studies, we investigated the allelopathic potential of aqueous extracts of *C. procera* and *M. philaeana* on the germination and seedling growth of wheat, radish and canola.

Materials and Methods

Plant Materials and Samples Collection

C. procera and *M. philaeana* plants were collected at flowering stage from the campus of Aswan University, South West bank of Lake between two dams, and transferred to the laboratory. Leaves and flowers of *C. procera* and leaves of *M. philaeana* were separated, dried at room temperature in a dark place and ground to powder using electrical mixer.

Preparation of Aqueous Plant Extracts

Ground powder of leaves of *C. procera* and *M. philaeana* and flowers of *C. procera* was used to prepare stock solution (20% w/v) by soaking the plant powder in sterilized distilled water for one day at room temperature. The extract was filtered through Whatman No. 2 filter paper. Series of concentrations from plant extract were prepared using the sterilized distilled water; 2, 5 and 10% for *C. procera* leaves, 2.5, 5, 7.5 and 10% for *C. procera* flowers and 2, 5 and 7.5% for *M. philaeana* leaves. For pots experiments, extract concentration of 2, 3.5, 5 and 7% were prepared from *C. procera* and *M. philaeana* leaves powder.

In Vitro Seed Germination and Seedlings Growth

Seeds of wheat, radish and canola were surface sterilized using diluted solution of sodium hypochlorite (5%) for 10 min, then using 70% ethyl alcohol for one minute and rinsed several times with sterilized distilled water. For *in vitro* test, 10 seeds from each tested species were placed on sterilized filter paper in 9-cm Petri dishes. In every Petri dish 8.5 mL of extract as per treatment was added at the time of germination, and another 8.5 mL added on fourth day of germination so that each treatment received a total of 17 mL. Sterilized distilled water was used as control. Three

replicates were prepared for each concentration. Petri dishes were placed in an incubator at 26°C±2 with 14/10 h of light/darkness. Seeds were considered as germinated, when the radicle protruded was visible to naked eye (El-Khatib and Abd-Elaah, 1998). Petri plates were monitored daily for seed germination and the measurements of shoot and root lengths was recorded at the seventh day from germination in case of *C. procera* and *M. philaeana* leaves and at the tenth day from germination in case of *C. procera* flower. The data presented for seedling growth (root and shoot length) were based on the randomly selected eight seedlings from replicates of each treatment.

In Vivo Pots Experiment

Seeds were sown in pots filled with soil and kept for two days in a refrigerator (4°C) to get homogenized seedling (seedlings with the same size and length). Every 7 day-old seedling was transferred to a separated plastic pot of 7 cm diameter and 8 cm depth containing 250 g soil each. Different concentration (2, 3.5, 5, and 7%) of leaf extracts of *C. procera* and *M. philaeana* were prepared in sterilized distilled water and 25 mL added to each pot as per treatment. Another 25 mL of extract was applied on the seventh day after plantation, and there after distilled water was added to keep the pots moist. Distilled water was used as control. After two weeks from planting, the seedlings were harvested, dried at room temperature for several days and dry weight was recorded.

Determination of Free Proline Content in Wheat Leaves

Free proline was determined from the leaves of wheat according to the method of Bates *et al.* (1973). For this 25 mg of dried ground leaf powder was suspended in 4 mL of 3% aqueous sulphosalicylic acid and shaken overnight. The homogenate was passed through Whatman filter paper No. 2 to collect the filtrate. Two mL of the filtrate was mixed with 4 mL acidic ninhydrin reagent and boiled in a water bath for 1 h at 100°C. After cooling in an ice bath, 4 mL of toluene was added to the mixture and vortex for 20 s. After separation of the two phases, the absorbance of the colored toluene layer was measured at 520 nm with spectrophotometer (Thermo Spectronic Genesys 5). The proline concentration was determined from a standard curve and calculated on a dry weight basis (mg g⁻¹ dry weight of leaves). The proline content was determined in wheat, but not in radish because of the shortage of plant materials.

Statistical Analysis

One way analysis of variance (ANOVA) (F-test) from Minitab version 12.21 was used to test the significant difference of all the data recorded in the studies. The data are presented in the form of mean with standard deviation and considering *p* values < 0.05 as significant.

Results

Effect of Aqueous Extracts on Seed Germination:

The aqueous leaf extracts of *C. procera* significantly delayed and reduced the seed germination of wheat and the reduction was proportional to the concentration used along the experimental period from third to seventh day after germination and the highest reduction was noticed with the highest extract concentrations (5% and 10%). Four days after treatment the aqueous extract of leaves at 5 and 10% concentration suppressed germination to 66 and 43%, respectively (Table 1). Aqueous extracts of *M. philaeana* leaves reduced the germination of wheat significantly at the highest concentrations (5 and 7.5%) which showed 30 and 36% suppression in the percentage of germination, respectively. Aqueous extracts of *C. procera* significantly delayed and reduced the seed germination of radish along the experimental period particularly at the highest concentrations (5 and 10%). Four days after treatment radish showed 70 and 13% of seed germination at concentrations of 5 and 10%, respectively. *M. philaeana* after four days from treatment suppressed the percentage of germination of radish to 40 and 16% at concentrations of 5 and 7.5%, respectively. Aqueous extract from flowers of *C. procera* had a moderate inhibition of germination of wheat but delayed the germination especially at the highest concentrations used (Table 2). Whereas the flower extract of *C. procera* not only delayed the germination of canola, but also reduced it significantly at the highest concentrations used (5 and 7.5%) so that there was no germination at 10% concentration (Table 2). From the seed germination experiments in Petri dishes, it is obvious that leaf extracts of both *C. procera* and *M. philaeana* reduced germination percentage of wheat and radish and such an inhibition increased with increasing concentration of extracts. Although germination was inhibited in both the test species, but the effect was more pronounced in radish especially at the highest concentrations used.

Effect of Aqueous Extracts on Shoot and Root Growth

The effect of different concentrations of *C. procera* and *M. philaeana* leaf extracts on shoot and root length of wheat and radish was evaluated after 7 day from treatment (Table 1). Data revealed that shoot and root length of wheat was significantly decreased at all concentrations of leaf extracts of both weeds. The shoot and root length of radish was significantly reduced at the highest concentration of *C. procera*, where these were reduced significantly after treatment with all concentrations of *M. philaeana* (Table 1). Although different concentrations from both *C. procera* and *M. philaeana* significantly reduced the shoot and root lengths of wheat and radish, the reduction was more prominent at the highest extract concentration.

Effect of Aqueous Extracts on Dry Weight of Plants

Dry weight is a function of the genetic as well as environmental factors (Kamal, 2010). As the shoot and root length decreased significantly after treatment with different concentrations of *C. procera* and *M. philaeana* extract, we had not determined either the fresh or the dry weight of the receiver plants. Dry weight of wheat seedlings was determined after treatment with different concentrations from *C. procera* and *M. philaeana* leaf extracts after growing these plants in pots. *C. procera* and *M. philaeana* extracts had none significant effect on the dry weight of wheat seedlings (Table 3).

Effect of Aqueous Extracts on Proline Content

Proline is the amino acid that is associated with different stresses in plants (Kocheva and Georgiev, 2008). Proline content was determined in wheat plants after their treatment with *C. procera* and *M. philaeana* leaf extracts both in wheat growing in Petri dishes (*in vitro*) or in pots (*in vivo*) (Table 3). Both *C. procera* and *M. philaeana* leaf extracts stimulated plants to increase the quantity of proline in Petri dishes plants. Higher proline content in wheat seedlings was associated with the highest leaf extract concentration of *C. procera* and *M. philaeana* (Table 3).

Discussion

Studies on the effect of different concentrations of *C. Procera* and *M. philaeana* aqueous leaf extracts indicated that the reduction in seed germination either in wheat or in radish was correlated with the dose of the extract. *Euphorbia hierosolymitana* extracts reduced the seed germination of *T. durum* and the reduction increased with increasing the concentration of the extracts (Abu-Romman and Shibli, 2010). Our findings are consistent with the previous results (El-Khatib and Abd-Elaah, 1998; Al-Zahrani and Al-Robai, 2007; Tanveer *et al.*, 2010; Chandra and Mali, 2012; Pukclai and Kato-Noguchi, 2012). Radish was more sensitive to *C. procera* and *M. philaeana* extracts than wheat. In our experiment wheat and radish seeds were more sensitive to *M. philaeana* extract than *C. procera* extract. Canola seeds were very sensitive to flower extract of *C. procera*. Some plant species or their residues selectively inhibit the development and growth of other species. This differential sensitivity can be observed in the field, green house and in laboratory experiments using residues, extracts or purified allelochemicals (Djurdjevic *et al.*, 2004; Vrchotová *et al.*, 2011; Chandra and Mali, 2012; Pukclai and Kato-Noguchi, 2012). The reduction of shoot and root length of wheat on exposure to different concentrations of *C. procera* extracts is in agreement with the results of Al-Zahrani and Al-Robai (2007). In our results the root length of wheat and radish was reduced dramatically after treatment with higher concentrations of

Table 1: Effect of *C. procera* and *M. philaena* leaf extracts on the germination and seedling growth of *T. aestivum* and *R. sativus*

Plant species →	Concentration	<i>T. aestivum</i>						<i>R. sativus</i>					
		Seed germination (%)		Days after germination		Shoot length (cm)	Root length (cm)	Seed germination (%)		Days after germination		Shoot length (cm)	Root length (cm)
Weed species ↓		3 rd	4 th	5 th	7 th	7 th day	7 th day	3 rd	4 th	5 th	7 th	7 th day	7 th day
<i>C. procera</i>	Control	100±0	100±0	100±0	100±0	9.7±1.3	5.3±1.2	97±5	100±0	100±0	100±0	4.5±2.1	5.7±2.2
	2%	60±14*	75±7**	75±7	75±7**	4.4±1.8***	3.5±0.7***	80±14	90±0***	90±0***	95±7	5.6±1.3	4.1±2.2
	5%	36±5***	66±20*	70±17	80±17*	3.8±2.0	2.5±0.8**	50±26*	70±10**	77±15	77±16	3.7±1.45	2.7±1.3*
	10%	26±5***	43±5***	43±5	47±11***	2.4±1.8***	1.9±1.2***	10±10***	13±11***	13±11**	33±15*	0.1±0.3***	0.0±0.1***
<i>M. philaena</i>	Control	100±0	100±0	100±0	100±0	3.3±0.9	4.4±0.8	100±0	100±0	100±0	100±0	1.6±0.9	4.0±0.8
	2%	93±0	93±0	93±0	93±0	1.9±0.5*	1.2±0.3***	66±20*	66±20*	66±20*	66±20*	1.18±0.6*	0.8±0.40*
	5%	30±0***	30±0***	30±0***	30±0***	1.7±0.6**	0.7±0.4*	40±20**	40±20**	40±20**	40±20**	0.6±0.3*	0.7±0.3*
	7.5%	36±20**	36±20**	36±20**	36±20**	1.4±0.2***	0.6±0.4*	16±5***	16±5***	16±5***	16±5***	0.0±0.0**	0.1±0.3*

* = significant at $p < 0.05$, ** = highly significant at $p < 0.01$, *** = very highly significant at $p < 0.001$ and value after \pm is the standard deviation

Table 2: Effect of the flower extract of *C. procera* on the germination and seedling growth of *T. aestivum* and *B. napus*

Plant species →	Concentration	<i>T. aestivum</i>						<i>B. napus</i>					
		Seed germination (%)		Days after germination		Shoot length (cm)	Root length (cm)	Seed germination (%)		Days after germination		Shoot length (cm)	Root length (cm)
Weed species ↓		3 rd	4 th	5 th	7 th	10 th day	10 th day	3 rd	4 th	5 th	7 th	10 th day	10 th day
<i>C. procera</i>	Control	100±0	100±0	100±0	100±0	7.83±0.03	9.15±0.39	57±41	63±30	80±26	93±11	2.56±0.48	5.32±2.33
	2.5%	100±0	100±0	100±0	100±0	7.3±0.22*	3.2±0.59**	15±7	45±7	70±14	90±0	3.92±0.17*	3.0±2.1
	5%	85±7*	85±7*	85±7*	95±7	4.18±0.33***	4.52±1.0**	0	5±7	20±0	55±7*	1.65±0.34	1.56±0.58
	7.5%	70±14*	80±14	90±14	90±14	3.33±0.74**	3.33±0.88***	5±7	10±7	15±7*	25±7**	0**	0.065±0
	10%	70±28*	85±7*	90±0*	100±0	2.55±0.14***	2.87±0.42***	0	0	0*	0**	0.1±0.13**	0.12±0.16

* = significant at $p < 0.05$, ** = highly significant at $p < 0.01$, *** = very highly significant at $p < 0.001$ and value after \pm is the standard deviation

Table 3: Effect of the extract of *C. procera* and *M. philaena* on dry weight and proline content in *T. aestivum*

Plant extract →	Petri dishes bioassay			Pots bioassay		
	<i>C. procera</i>	<i>M. philaena</i>	<i>C. procera</i>	<i>C. procera</i>	<i>M. philaena</i>	<i>C. procera</i>
Extract concentration ↓	Proline (mg g ⁻¹)	Proline (mg g ⁻¹)	Dry weight (g)	Proline (mg g ⁻¹)	Dry weight (g)	Proline (mg g ⁻¹)
Control	26.6 ± 1.0	26.6 ± 1.0	0.020 ± 0.005	24.9 ± 6.7	0.020 ± 0.005	24.9 ± 6.7
2%	42.1 ± 0.6***	25.8 ± 10.1	0.020 ± 0.01	30.1 ± 0.5	0.027 ± 0.01	27.2 ± 5.7
5%	64.4 ± 6.1***	50.3 ± 3.5***	0.03 ± 0.01	24.5 ± 6.5	0.013 ± 0.01	25.2 ± 11.9
7.5%	-	40.6 ± 16.1	0.021 ± 0.009	46.0 ± 12.9*	0.023 ± 0.005	20.9 ± 9.2
10%	-	-	0.023 ± 0.004	26.0 ± 2.6	0.017 ± 0.005	19.2 ± 7.1

* = significant at $p < 0.05$, *** = very highly significant at $p < 0.001$ and - = not determined (insufficient materials) and value after \pm is standard deviation

both *C. procera* and *M. philaena* extracts. Root length of both wheat and radish is more sensitive to higher concentrations of the extracts than shoot length and *M. philaena* extract was more suppressive than *C. procera* extract. This may be attributed to the higher amounts of glucosinolates and its hydrolyzed by-products (isothioyanates) present in *M. philaena* leaves, and known to have very strong allelopathic potential. The sensitivity of the root and shoot length may vary from plant to another due to the nature of the allelochemicals secreted by the donor plant and similar results have been reported by El-Khatib and Abd-Elaah (1998). Flowers extract of *C. procera* decreased significantly the shoot and root lengths of wheat at each concentration used, where as the only shoot system of canola was significantly reduced at higher concentrations of the flower extract of *C. procera*. Different parts from the plant show different effects (Tanveer *et al.*, 2010; Chandra and Mali, 2012) and this may be attributed to the profile of allelochemicals present in different organs of the donor plant (Ghafar *et al.*, 2001; Cheema *et al.*, 2007;

Sisodia and Siddiqui, 2010).

C. procera and *M. philaena* extracts had no significant effect on the dry weight of wheat. This may be attributed to the stress effect of these extracts on wheat plants that may stimulate plants to defend themselves by increasing the concentration of some metabolites such as amino acids, proteins, phenolics and carbohydrates or increasing the cell wall lignifications. Our results are in accordance with those of Das and his co-workers (Das *et al.*, 2012), which showed higher content of proline and phenolic compounds in *Cicer arietinum* after treatment with higher concentrations from the aqueous leaf leachate of different tree species in laboratory conditions. Leaf leachate of *Eucalyptus globules* increased the proline and phenolic compounds in rice, sorghum and gramblack (Djanajuiraman *et al.*, 2005). Proline content in pots experiment plants fluctuated but in all cases there was no significant difference between proline content in wheat after treatment with *C. procera* or *M. philaena* comparing to the control plants. The irregular pattern of proline profiling in control and

treated plants of the *in vivo* experiment may be attributed to the delay in treating wheat with the two extracts used. We treated these plants at 7-days old seedling stage.

In conclusion, the magnitude of reduction of seed germination, shoot and root length after treatment with *C. procera* and *M. philaeana* extracts followed the order of: *B. napus* > *R. sativus* > *T. aestivum*. *M. philaeana* leaf extract was stronger than that of *C. procera* in suppressing both seed germination and seedlings growth. Both *C. procera* and *M. philaeana* had inhibitory effect on germination rate and seedling length of the tested species. Presence of these weed species in should be field is likely to suppress its germination. It is imperative that fields must be cleared of these weeds as well as their residues before wheat, radish and canola are sown in such fields. The allelopathic nature of *M. philaeana* can be exploited to search for new metabolites that can be used for biological control of other plants and organisms.

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