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RESEARCH ARTICLE

Management of A Noxious Weed; *Melilotus indicus* L.via Allelopathy of *Cotula cinerea* Del.

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Abstract

The main objective of the present study was to assess the biological activity of *Cotula cinerea* (donor species) on germination efficiency as well as some physiological parameters of *Melilotus indicus* (recipient species). Germination percentage (GP), plumule (PL) and radicle (RL) lengths as well as seedling dry weight (SDW) (mg seedlings^{-1}) was significantly inhibited by applying different concentrations of *C. cinerea* shoot extract (CCSAE). The shoot (SHL) and root (ROL) lengths of *M. indicus* was significantly affected by applying the different levels of *C. cinerea* shoot crude powder (CCSCP). Expectedly, the leaf area index (LAI) (mm^2/mm^2) had also reported a significant reduction. The total photosynthetic pigments were significantly decreased in the recipient species as affected with different CCSCP levels. Conversely, Carotenoids content exhibited a significant increase with the increase in CCSCP level. Total available carbohydrates (TAC) and total protein contents of the recipient species were significantly decreased with increasing of CCSCP level.

INTRODUCTION

Cotula cinerea Del. (Saharan camomile, Asteraceae) is a small medicinal annual plant 10-20 cm high and thrives in desert conditions (xerophytic plant**)** with an average 100 mm. rainfall a year and favors sand-loamy soils. The plant is usually found on non-saline wadi beds, on gravelly-sandy soils **(Boulos, 1983).** Geographically, it is very common in North Africa especially in the Algerian Sahara, Red Sea region, Sinai and Qattara Depression **(Ozenda, 1991)**.

The whole plant, is collected in the spring when in flower, and sold in the market in several countries. The plant is prepared as a decoction and may be mixed with other plants and taken internally and used externally as a poultice. It is used for broncho-pulmonary conditions, coughs, digestive problems (including nausea, vomiting and stomach pain), sunstroke and rheumatism. It is applied as a poultice to the forehead for its febrifugal properties **(Boulos, 1983; Batanouny, 1999).** This plant contains many active compounds such as flavonoids **(Dendougui *et al*., 2012),** essential oils and turpentines which gives a powerful and specific odor to the plants **(Markouk *et al*., 1999).**

Melilotus indicus L. (Indian melilot, Fabaceae) is an annual a noxious weed grows in crop fields, gardens, orchards and canal banks and causes great loss in crop productivity **(Boulos and El-Hadidi, 1984; Hassanein *et al*., 2000).** The dried leaves can be toxic due to the presence of coumarin. The fresh leaves are quite safe (**Duke and Ayensu (1985)** and can be taken internally to prevent blood clotting.

So far, no data has been reported in Egypt regarding its allelopathic effect and interaction with other plants. Therefore, the present study was planned to evaluate the biological interaction between *C. cinerea* (donor species) extract and crude powder from one hand and germination efficiency as well as some metabolic contents of *M. indicus* (recipient species) from the other hand.

I. MATERIALS AND METHODS

2.1 Plant Materials

Some of the fresh aerial shoots of *Cotula cinerea* Del. (donor species) and seeds of *Melilotus indicus* L. (recipient species) were harvested and collected from El-Qattara depression and wheat fields at Burg EL-Arab, respectively during May 2014. The seeds were surface cleaned and stored in paper bags at room temperature (24 ±2^{o}C) until further use.

2.2 Preparation of *Cotula cinerea* Extract for Phytochemical Screening

Samples from the fresh aerial shoots of *C. cinerea* were crushed and extracted in water for 24 h. The extracted solution was then filtered and concentrated until the water evaporates from the extract (**Benmenine *et al*., 2011).**

2.3 Preparation of Donor Species Aqueous Extracts

The collected aerial shoot samples of the donor species were air-dried, then, cut into 0.5 – 1cm pieces. Stock aqueous extract was obtained by soaking 75 g of the air-dried donor plant materials in one liter of distilled water at room temperature (24 ±2^{o}C) for 24 hours. The mixture was filtered through four layers of cheesecloth to remove the fiber debris and kept at 5 ºC in the dark until further use. Different concentrations (5, 10, 20 and 40%) were prepared from the stock solution, in addition to the control (distilled water).

2.4 Germination Bioassay Experiment

The experiment was carried out to explore the potential allelopathic effects of *C. cinerea* shoot aqueous extracts (CCSAE) on germination percentage (GP), plumule (PL) and radicle (RL) lengths, as well as seedlings dry weight (SDW) of the recipient species; *M. indicus.* Consequently, twenty seeds of each of the recipient species were arranged in 9-cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 24 - 26^{o}C and night temperature from 19-21^{o}C. Ten cm^{3} of each level of the donor species extracts were added to five replicates. Before sowing, the seeds were treated with H_2SO_4 (96%) for 2 minutes, then, washed several times with distilled water. Thereafter, the seeds were surface sterilized by soaking for two minutes in 4% sodium hypochlorite, then, rinsed four times with distilled water. Measurements of GP, PL and RL were recorded after 9 days at the end of the experiment. IP was calculated according to the following equation **(Khanh *et al*., 2005).**

$$\text{Inhibition percentage (\%)} = [1- (\text{sample extract / control})] \times 100$$

2.5 Growth Experiment

The experiment was performed to verify the effect of the different levels of *C. cinerea* shoot crude powder (CCSCP) mixed (w/w) with sandy loam soils (collected from control locations) on some growth parameters and metabolic content of *M. indicus*.

Ten seeds of the recipient species were sown in plastic pots (15 cm in diameter) with one kilogram soil thoroughly mixed (w/w) with 5 and 10% of electrically crushed crude powder of the donor species. The experiment was performed under normal laboratory conditions (24-26°C day temperature, 19-21^{o}C night temperature, 70-72% relative humidity and 14/10 h light/dark photoperiod). One treatment was run as control with zero percent of crude powder. The pots were irrigated every two days with normal tap water. After 21 days, the homogenous seedlings were carefully taken from each treatment, washed with tap water to remove the adhered soil particles then by distilled water and gently blotted with filter paper.

2.5.1 Growth parameters

The samples were separated into two parts; the first (fresh) for the determination of leaf area, shoot and root lengths as well as pigment content while the other part was dried at 70^{o}C till constant weight to determine the seedling dry weight and stored for further chemical analyses. Leaf area was measured using Digital Planimeter (Placom KP-90) to the nearest mm^{2}. Leaf area index (LAI) was calculated by dividing the total plant leaf area by its specific ground area **(El-Darier, 2002).** Shoot and root lengths were measured to the nearest cm, in addition, dry weight of shoots and roots were also estimated. The photosynthetic pigments chlorophyll a (Chl a), b (Chl b) and carotenoids (Carot) were extracted and determined using the spectrophotometric method described by **Metzner *et al*. (1965).** Formula and extinction coefficients used for determination of photosynthetic pigments were:

$\text{Chl a} = 10.3\ E_{665} - 0.918E_{647}$.

$\text{Chl b} = 19.7\ E_{647} - 3.87\ E_{665}$.

Carot = $4.2\ E_{453} – (0.0264\ Chl\ a + 0.426\ Chl\ b)$.

2.5.2 Chemical analysis

Total soluble carbohydrates and total proteins were determined for the recipient species according to **Naguib (1964)** and **Allen *et al.* (1984)** respectively. Test for sterols, tannins, flavonoids and alkaloids for the donor species was carried out according to the methods described by **Harborne (1973, 1998 and 1999** respectively). Additionally, saponins were detected by the procedures of **Lewis and Smith (1967).**

2.6 Statistical Analysis

All the data of the present study were subjected, where appropriate, to standard one-way analysis of variance (ANOVA) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company **(Zar, 1984).** Pair wise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

II. RESULTS

3.1 Germination Bioassay Experiment

The allelopathic effects of *C. cinerea* shoot aqueous extract (CCSAE) on germination percentage (GP), plumule (PL) and radicle (RL) lengths of *M. indicus* are represented in Table 1. The data demonstrated that the GP was significantly affected by applying the different concentrations of CCSAE. Commonly, GP decreased with the increase in treatment concentrations. More obvious reduction (55.55%) in GP was prominent at 40% CCSAE concentration level. Moreover, all treatment concentration levels had reduced PL. Actually; PL attained a value of about 88mm and 7 mm at control and 40% CCSAE respectively with a reduction percentage of about 92%. Similarly and compared to control, a gradual decrease in radicle length (RL) was observed along gradual CCSAE concentrations. Values of about 70 and 12 mm for RL were attained at control and 40% CCSAE respectively. The relevant reduction percentage was about 82.85%. All through, data in Tables 1 also pointed up that seedling dry weight (SDW) (mg $seedlings^{-1}$) of *M. indicus*, was significantly affected by the increase in CCSAE concentration. The reduction percentage at the maximum extract concentration (40%) was about 57.14% relative to control.

3.2 Growth Experiment

3.2.1 Soil analysis

The results of analyses for the soil applied in the pot experiment are presented in **Table 2.** Generally, the results illustrated that the applied soil is a sandy loam soil as soil texture represented by 55% sand, 32% clay and 12% silt. Accordingly, this type of soil contains considerable amount of organic matter (about 9%). A pH value of about 7.7 indicated that the soil is slightly basic. Nitrogen, phosphorus and potassium contents were about 1.1, 0.5 and 3.5 mg g^{-1}, respectively. The content of the total soluble salts in the soil (EC) was about 2.70 dsm^{-1}.

3.2.2 Qualitative phytochemical screening of the donor species

Chemical constituents of concentrated aqueous extract of *Cotula cinerea* air-dried shoots were found to be flavonoids, saponins, essential oils, tannins, steroids and terpenoids **(Table 3).** On the other hand the samples were free from alkaloids.

3.2.3 Growth parameters

The allelopathic effects of different levels of *Cotula cinerea* shoot crude powder (CCSCP) are listed in **Table 4.** Data demonstrated that shoot length (SHL) of *M. indicus* was significantly affected by applying the different levels of CCSCP. Generally, SHL decreased with the increase in treatment concentrations. At control level, SHL attained a value of about 16.6 cm which reduced to 10.6cm at 10% CCSCP level with a reduction percentage of about 36.14% relative to control. Similarly, root length (ROL) exhibited a significant reduction along gradual CCSCP levels. The control value of was about 5.6 cm declined to 2.0 cm at 10% CCSCP level with 64.28% reduction percentage relative to control. Shoot dry weight (SHDW) was significantly affected by CCSCP concentrations. The control value of SHDW was about 0.12g and gradually decreased to 0.08 g at 10% CCSCP level. On the other hand, the control value of RODW was about 0.04g which reduced to 0.01g at 10% level. The reduction percentages for the last-mentioned two parameters were 33.33 and 25% respectively. Expectedly, the leaf area index (LAI) (mm^2/mm^2) had also reported a significant reduction. Correspondingly, the value at control level was reduced to 82.81% at 10% CCSCP level.

3.2.4 Photosynthetic pigments

The total photosynthetic pigments were significantly decreased in the recipient species as affected with different CCSCP levels **(Table 5)**. Reduction percentages of about 58.15, 58.18 and 50.87 were attained for Chl a, Chl b and total chlorophyll relative to the control. Conversely, Carotenoids content exhibited a significant increase with the increase in CCSCP level.

3.2.5 Total available carbohydrates and protein contents

Generally, Total available carbohydrates and total protein contents of the recipient species were significantly decreased with increasing of CCSCP level **(Figure 1).** The reduction percentages for the two parameters were about 46.72 and 74.24% at 10% CCCP level relative to control.

Table 1. Allelopathic effect of different concentration levels of *Cotula cinerea* shoots aqueous extracts (CCSAE) on germination percentage and some growth parameters of *Melilotus indicus*.

Concentration (%)	**Germination percentage (GP) (%)**	**Growth parameter**		
		Plumule length (PL) (mm)	**Radicle length (RL) (mm)**	**Seedling dry weight (SDW) (mg seedling^{-1})**
0	90^{a}	88^{a}	70^{a}	21^{a}
5	70^{b}	62^{b}	40^{b}	12^{b}
10	70^{b}	50^{c}	32^{c}	11^{c}
20	50^{c}	32^{d}	18^{d}	11^{c}
40	40^{d}	7^{e}	12^{e}	9^{d}

Different letters within each column indicate a significant difference at P < 0.05 level of probability as evaluated by One-Way ANOVA test.

Table 2. Means ± SD of six soil samples representing some physical and chemical characteristics of the experimental soil.

Character	**Value ± SD**
EC [a]	2.71 ±0.01
pH	7.70 ±0.40
Ca [b]	12.00 ±1.30
Mg [b]	18.00 ±2.30
Cl [b]	17.00 ±2.80
CO_3 [b]	34.00 ±3.40
SO_4 [c]	2.06 ±0.03
OM [d]	8.68 ±1.02
Soil Texture (sandy loam)	

Sand [d]	55.10 ± 3.50
Clay [d]	32.10 ± 2.06
Silt [d]	12.80 ± 1.40
Available Nutrients (mgg^{-1})	
N	1.10 ± 0.001
P	0.50 ± 0.001
K	3.50 ± 0.12

a: In ds/m **b:** In meq/kg
c: In ppm **d:** In

Table 3. Qualitative phytochemical screening of *Cotula cinerea***.**

Constituent	Flavonoids	Alkaloids	Saponins	Essential oils	Tannins	Steroids	Terpenoids
Rank	+++	None	++	++	+	++	++

+ = Positive; ++ = strongly positive; +++= extremely positive

Table 4. Allelopathic effect of different levels of *Cotula cinerea* shoot crude powder (CCSCP) mixed (w/w) with sandy loam soil on some growth parameters of *Melilotus indicus* in a pot experiment carried out during the year of 2014.

Treatment (%)	Growth parameter				
	Shoot length (SHL) (cm)	**Root length (ROL) (cm)**	**Shoot dry weight (SHDW) (g)**	**Root dry weight (RODW) (g)**	**Leaf area index (LAI)** (mm^2/mm^2)
0	16.6[a]	5.6[a]	0.12[a]	0.04[a]	7.04[a]
5	13.4[b]	3.2[ab]	0.10[b]	0.01[b]	2.06[b]
10	10.6[c]	2.0[b]	0.08[b]	0.01[b]	1.21[c]

Different letters within each column indicate a significant difference at $P < 0.05$ level of probability as evaluated by One-way ANOVA test.

Table 5. Allelopathic effect of different levels of *Cotula cinerea* shoot crude powder (CCSCP) mixed (w/w) with sandy loam soil on changes in photosynthetic pigments (mg g^{-1}f.w.) in leaves of *Melilotus indicus* in a pot experiment carried out during the year of 2014.

Treatment (%)	Chlorophyll "a"	Chlorophyll"b"	Carotenoids	Total
0	24.95^{a}	5.07^{a}	1.88^{c}	31.9^{a}
5	13.22^{b}	3.11^{b}	2.67^{b}	$19.o^{b}$
10	10.44^{c}	2.12^{c}	3.11^{a}	15.67^{c}

Different letters within each column indicate a significant difference at P < 0.05 level of probability as evaluated by One-Way ANOVA test.

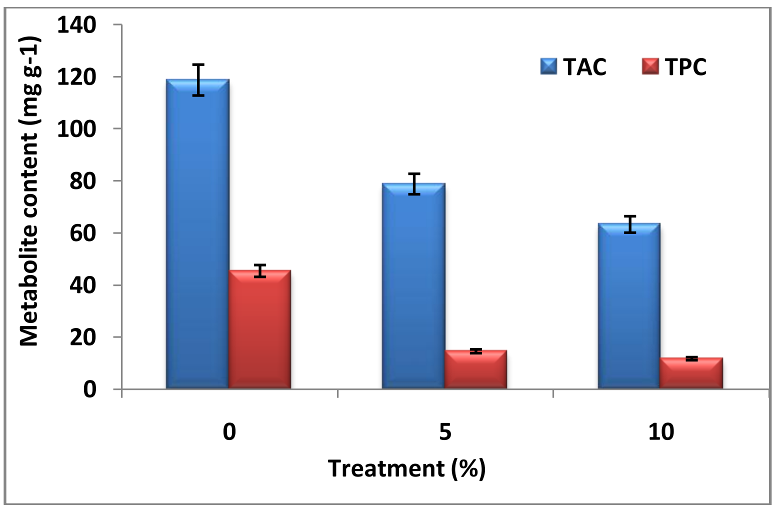

Figure 1. Allelopathic effect of different levels of *Cotula cinerea* shoots crude powder (CCSCP) mixed (w/w) with sandy loam soil on total available carbohydrates (TAC) and total protein content (TPC) (mg g^{-1}) in *Melilotus indicus* in a pot experiment carried out during the year of 2014. Bars represent standard deviation.

DISCUSSION

Economic and environmental constraints of crop production systems have stimulated interest in alternative weed management strategies **(Mortensen *et al*., 2000; Singh *et al*., 2001).** Recently, natural products released from allelopathic and medicinal plant residues may help to reduce the use of synthetic herbicides for weed management and therefore cause less pollution and safer agricultural products **(Khanh *et al*., 2007).**

Chemical constituents of aqueous extract of *Cotula cinerea* shade dried shoots were found to be flavonoids, saponins, essential oils, tannins, steroids and terpenoids. The present study investigated the action of *C. cinerea* shoots aqueous extracts (CCSAE) on some germination considerations and seedling growth of *M. indicus* under laboratory conditions. The extracts exerted inhibitory effects on seed germination, plumule and radicle lengths as well as 7-days seedling dry weight of the recipient species. To go through with this, the shoot and root lengths, shoot and root dry weight as well as leaf area index were inhibited in all treatment when compared with the control upon applying CCSCP. The inhibitory effect of the donor plant for all measured parameters was directly proportional to the increasing extract concentrations. **El-Darier (2002)** reported that the aqueous extract and crud powder of *Eucalyptus rostrata* significantly suppressed seed germination of *Vicia faba* and *Zea mays* under different concentrations.

Abou-Zeid and El-Darier (2014b) indicated that the reduction in plumule and radicle lengths for *Vicia faba* and *Zea mays* may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts of *Moringa oleifera* **(Javaid and Anjum, 2006).** The same was obtained by **Hossain *et al.* (2012)** on mungbean. Furthermore, the aqueous extract of *E. camaldulensis* at various concentration levels inhibited the germination, reduced fresh and dry weights of wheat seedlings **(Khanh *et al*., 2008)** as well as *Chenopodium album* and *Portulaca oleracea* (**Abou-Zeid and El-Darier, 2014a)**. Its effectiveness suggests that its leaves may act as a source of allelochemicals after being released into soil or after decomposition.

Allelochemicals can affect the performance of the three main processes of photosynthesis: stomatal control of CO_2 supply, thylakoid electron transport (light reaction), and the carbon reduction cycle (dark reaction) (**Zhou and Yu, 2006**). The changes in plant pigment content can be used to assess the impact of environmental stresses and were used for plant stress diagnosis in terms of chlorophyll synthesis inhibition (**Kancheva *et al*., 2014)**. Chlorophyll is a key biochemical component that is responsible for photosynthesis and is a physiological indicator of plant condition. In the present study it was found that CCSCP significantly reduces Chl a, Chl b and total chlorophyll relative to the control. Conversely, Carotenoid content exhibited a significant increase with the increase in CCSCP level. **Yang *et al*. (2004)** reported that phenolic influence both degradative and synthetic pathways of chlorophyll. Furthermore, it has been reported that the allelochemicals produced by invasive species affect the photosynthesis and plant growth by destroying the chlorophyll (**Peng *et al*., 2004**). Various studies have shown that, allelochemicals released by allelopathic plants do have negative effects on leaf chlorophyll content of neighboring plant species (**Oyerinde *et al*., 2009**). The action of allelochemicals affects large number of biochemical reactions of target species resulting in alteration of different physiological functions (**Gniazdowska and Bogatek, 2005**). The allelochemicals released to the environment by cohort plant species, have significant effects on neighboring plants by reducing the rate of photosynthesis and respiration processes and finally reduce yield (**Bogatek and Gniazdowska, 2007**). Recently, it was found that *Moringa oleifera* leaf crude powder (MOLCP) decreased the efficiency of chlorophyll fluorescence spectra, photosynthetic pigments and photosystem II photochemistry (Fv/Fm). The effect was more detected in *V. faba* compared to *Z. mays*. Chlorophyll fluorescence, as a measure of photosynthesis (Fv/Fm) provides insights into a plant's ability to tolerate environmental stresses (**Abou-Zeid and El-Darier, 2014b).**

The decrease in total available carbohydrates (TAC) of the recipient species in the present study, may be attributed to the inhibitory effect of the released allelochemical substances on the synthesis of photosynthetic pigments, and hence, on photosynthesis. **Bernat *et al*. (2004)** reported that the decrease of TAC of *Sinapis alba* under allelochemicals stress may be related to the reduction of the photosynthetic leaf area and stomatal frequency. In addition, the reduction of photosynthetic allocation may be due to the destructive effect of allelochemicals stress on plasma membrane and phloem elements (**Singh and rao, 2003**). Also, **Gniazdowska and bogatek (2005)** concluded that the consumption of photosynthesis in respiration for diverting energy to maintenance of the physiological processes, under allelochemicals stress, resulted in decrease of TAC in *Zea mays* seedlings. Thus, this suggestion, in line with the present study, may explain the decrease in dry weight and growth of *Melilotus indicus*. The decrease in total protein contents of the studied recipient species may be related to the stimulation of the protein degradation to amino acids, such as proline, might be an adaptation mechanism against the allelochemical stress and/or means of osmolaytes to prevent water loss. Therefore, the decline in total protein content may be related to reduce the growth of the recipient species. These observation are in agreement with other results in several plants species including mango (**Venkateshwarlu *et al*., 2001),** *Lycopersicon esculentum* (**lara Nunez *et al*., 2006**) and Tobacco (**Hoque *et al*., 2007**).

Based on the present study, there is possibility of using allelopathic potential of *Cotula cinerea* directly or in a structure leads for the discovery and development of environmental herbicides to control weeds. Analysis of possible allochemicals in this plant may be urgent needs to distinguish and characterize the different bioinhibitors molecules.

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