



Weed management in wheat crop through bio-herbicidal activity of two natural medicinal plants

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Received : 28.12.2022 ; Revised : 02.02.2023 ; Accepted : 12.02.2023

DOI: <https://doi.org/10.22271/09746315.2023.v19.i1.1673>

ABSTRACT

Several studies reported that some natural medicinal plants have allelochemical potentials which show an important role in the weed control. Therefore, this study focused on the role of two natural medicinal species (*Artemisia monosperma* Del. And *Silybum marianum* (L.) Gaertn) in *Chenopodium album* L. management; a major wheat pest (*Triticum aestivum* L.) Crop in both, mono and mixed cultures. The experiment was performed under greenhouse conditions, growth parameters and chemical analyses were determined for both *Chenopodium album* and wheat. Data showed a remarkable reduction in the organs length and leaf area with considerably stronger effects on *Chenopodium album*. Water content was significantly reduced in case of *Chenopodium album*, while it was increased in *Triticum aestivum* in mono culture and was not affected in mixed culture as compared to control. Specific root length was increased in wheat and decreased in weed in response to the allelopathic effect of both medicinal species. This result indicated that the root area of crop increased on the expense of the weed root. Photosynthetic pigments in *Chenopodium album* were remarkably affected more than in the wheat. Free proline accumulated in *Chenopodium album* at most treatments while it was slightly decreased in wheat in response to *Artemisia monosperma* but increased in *Silybum marianum* treatments as compared to control. This investigation concluded that the two medicinal plants have allelopathic compounds with strong potential on weed growth but very slightly on crop. Specifically, *Artemisia monosperma* has allelopathic potential more than *Silybum marianum* and offers promises for their usefulness as a tool of weed management.

Keywords: Allelochemicals, *Artemisia monosperma*, *Chenopodium album*, medicinal plants, *Silybum marianum*, *Triticum aestivum*

Allelopathy is an important mechanism of plant competition by releasing the allelochemical compounds into environment to prevent or stimulate other plants growth and development which depend on the plant species (Bakhshayeshan-Agdam *et al.*, 2015). Allelochemicals are secondary metabolites which are not edible and can be used as growth regulators and bio-herbicides (Anwar *et al.*, 2021). In agricultural production, the most dependable source of weed control for over 50 years has been the synthetic herbicides. However, the overuse of synthetic herbicides may disturb the environment, human health and food (Mansour *et al.*, 2014). Therefore, much more effort has been done on plant derived compounds as ecologically safe substitutes to herbicides for the weed control (Sodaeizadeh *et al.*, 2010). The use of allelopathy for weed control could be either through the direct applying of natural allelopathic interactions or by using allelochemicals as natural herbicides (Singh *et al.*, 2009; Migahid *et al.*, 2015). Allelochemicals may affect plants indirectly via alternation of soil properties, nutritional status and population or activity of microorganisms and

nematodes (Nekonam *et al.*, 2013). The use of allelopathic and medicinal plants has been recommended as a viable option for alternative weed management under sustainable agriculture (Zeng *et al.*, 2009). The allelopathic compounds are more biodegradable and less damaging to the environment than synthetic chemical herbicides, hence, they are attractive alternatives to present herbicides which have caused development of herbicide resistance in weeds (Scognamiglio *et al.*, 2013).

Artemisia monosperma Del. (AM) is a perennial fragrant plant belonging to the Asteraceae family which grows wild in the desert plains and wadis, both inland and in the Mediterranean coastal region, often not too far from the coast in northern Sinai (Badr *et al.*, 2012). AM is one of the common medicinal species of *Artemisia*. It is extensively used in folk medicines. It contains bioactive compounds such as sterols, flavonoids, saponins and tannins that might be concerned as allelochemicals agents (Sadek *et al.*, 2016). *Silybum marianum* (L.) Gaertn. (SM) is an annual to biennial herbaceous plant belonging to the Asteraceae

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How to cite : Elghobashy, R., El-Darier, S., Elnouby, A. and Migahid, M. 2023. Weed management in wheat crop through bio-herbicidal activity of two natural medicinal plants. *J. Crop and Weed*, 19 (1): 145-157.

family, commonly known as milk thistle (Gresta *et al.*, 2007). SM is native to the Mediterranean basin and is now widespread all over the world. It is spread on roadsides, waste ground, fields of cereal crops and it prefers high fertility soils (Karkanis *et al.*, 2011).

The aim of this study was to investigate the herbicidal activity of AM and SM crude powder on some growth parameters and some metabolic changes of *Chenopodium album* (CA) and *Triticum aestivum* (TA) at mono and mixed cultures in the green-house. There are hopes that the study will provide information on the possibilities of using one or more of the donor species as bio-herbicides.

MATERIALS AND METHODS

Sample collection

Samples of shoots of AM were collected from ten different locations in the habitat of non-saline depression at El Omayed during spring 2020; SM was collected from nine different canal banks around Alexandria city during fall season 2020. The seeds of CA were collected from some agricultural fields around Alexandria city during spring 2019, while the grains of TA 'Sakha 94' were obtained from Egyptian ministry of Agriculture. Samples of fresh shoots of the wild species were air-dried then cut into small pieces and ground to powder. The seeds of CA and the grains of TA were surface sterilized for 5 min in a diluted solution of sodium hypochlorite (5%), washed thoroughly with running water and then washed with distilled water for at least 5 times.

Green-house experiment

CA seeds and TA grains were grown in plastic pots (15 cm in diameter, 20 cm in length with holes at the bottom) filled with 1 kg of soil (1:2 clay to sand) completely mixed (w/w) with 1, 2 and 4% of crude powder of the two wild species, in addition to control treatment (without donor species). Soil samples were collected from control site, air-dried, sieved to remove gravel and plant debris, finally sterilized (80°C for 24h) to remove any microorganisms and weed seeds. 0.1 g CA seeds and ten grains of TA were sown in each pot. All pots were kept at 80% water holding capacity (WHC) for the soil type by addition of tap water. The experiment was carried out as monoculture for each of the studied species and mixed culture between CA and TA treatments. The experiment was performed under greenhouse conditions. Pots were arranged in a complete randomized block design with three replications. The plants were harvested after 35 days and carefully freed from soil with gentle motion in tap water, then washed with distilled water.

Growth parameters and water content estimation

Determination of growth parameters

Homologous individuals were selected from each treatment, dissected into shoots and roots and then weighed separately to determine fresh weight. The samples were then dried in an oven at 60°C till constant weight to determine the dry weight for each treatment of the two target species. Fifteen plant individuals per treatment were used for the determination of the shoot and root length of CA and TA. The shoot/root ratio (SL/RL) was calculated in each treatment. Specific root length (SRL) was calculated by root length relative to its dry weight of the two wheat cultivars (Ostonen *et al.*, 2007). Leaf width and length was measured to the nearest cm then leaf area (LA) was estimated using the following equation (Cain and Castro, 1959): $LA = 0.667 * L * W$ Where L is the leaf length, W is the leaf width, and 0.667 is a correction factor used to convert the rectangular product of leaf length and width into the area of the leaf.

Determination of water content

The water content was calculated using the dry and fresh weights for shoot system of the study species applying following equation:

$$\% \text{WC} = (\text{Fresh wt} - \text{Dry wt}) * 100 / \text{Fresh wt.}$$

Chemical analyses

Estimation of the photosynthetic pigments

The photosynthetic pigments chlorophyll a and b (Chl.a, Chl.b) and the carotenoids (Carot.) were extracted and determined according to Inskeep and Bloom (1985). Pigment fractions and the total pigment content were calculated as mg/g fresh weight. The chlorophyll a/b ratio was calculated for the fresh sample.

Determination of soluble metabolic compounds in plant extract

Fresh samples were weighed and ground with distilled water. The extract was prepared according to Migahid and El Khazan (2002). Soluble proteins (Bradford, 1976), proline (Bates *et al.*, 1973) and amino acids (Ya and Tunekazu, 1966) were determined in the extract.

Statistical analysis

The data was subjected to a one-way analysis of variance (Zar, 1984). Pairwise comparisons of means were performed using least significant differences (LSD) at probability 0.05.

RESULTS AND DISCUSSION

Plant biomass

The effect of the two donors on shoots and roots fresh weight of two recipient species CA and TA is illustrated in Fig. 1. The use of allelochemicals as natural herbicides against weeds besides enhancing production potential of economically important crop can offer an alternative or a complementary approach for sustainable combined weed management (Anjum and Bajwa, 2007, Naeem *et al.*, 2022). Data in monoculture treatment demonstrated that shoot fresh weight of CA was significantly reduced in response to the increase of AM and SM concentrations as compared to control. Root fresh weight of CA was significantly reduced in response to the increase in concentrations of AM. In contrast, it was significantly increased in response to the increase in concentrations of SM. Total fresh weight of CA significantly reduced in response to the two donor. In monoculture treatment shoot fresh weight of TA significantly reduced in response to the increase in concentration of the two donor species. No variation was observed in root fresh weight of TA in monoculture treatment in response to AM. On the other hand, it was significantly increased in response to increase in the concentrations of SM. In mixed culture treatment shoot fresh weight of CA was significantly reduced in response to the increase in concentrations of selected donor species. Root fresh weight of CA was not affected in response to AM except in 2% concentration where it was increased significantly as compared to control. In contrast, root fresh weight of CA was significantly decreased in response to SM. The total fresh weight of TA in mixed culture treatment was significantly decreased in response to the selected donor species. Generally, the data exhibited that dry weight of shoots and roots of CA and TA shows a gradual decrease with increasing the concentration of the two donor species as compared to control (Fig. 2).

Similar results were attained by Tesio *et al.* (2012) who informed that fresh weight in pea was significantly inhibited when *Helianthus tuberosus* residues were existing in the pot for a long period while stimulation was noted during shorter periods. The stimulation may be due to improved water retention due to the organic material, even if the positive effect disappeared with an increasing presence of residues, showing by contrast inhibition on pea. This reduction may be attributed to the presence of allelochemicals released from the donor species. Baziramakenga *et al.* (1994) found that allelochemicals reduced the number of lateral roots, root and shoot dry biomass of soybean. Likewise, Mahmood and Cheema (2004) found that sorghum mulch significantly reduced the density and the dry biomass of one of the world's worst weed *Cyperus rotundus*. Khanh *et al.* (2006) reported that the aqueous extracts

of *Passiflora edulis* strongly suppressed germination, growth and seedling dry weight of lettuce, radish and two major paddy weeds *Echinochloa crusgalli* and *Monochoria vaginalis*.

Shoot and root length

Data demonstrated that organs length of CA in mono and mixed culture was significantly reduced in response to the increase of AM and SM concentrations as compared to control. On the other hand, there is non significant effect in shoot (SH) and root length (RL) of TA in response to increase in the effect of the two species concentrations, except in mono culture under the low and moderate concentrations (1% and 2%) of AM. RL was increased with 37.46% and 25 % respectively relative to control (Figure 3; Plate 1). These results are in agreement with the previous investigations (Al-Hawas and Azooz, 2018) who reported that leaf aqueous extracts of AM reduced shoot and root length. Abu-Romman *et al.* (2010) recorded that allelochemicals in *Euphorbia hierosolymitana* made significant reduction in root and shoot lengths of *Triticum durum*. The decrease in shoot and root growth might be due to a reduction in cell division and cell elongation due to the presence of allelochemicals in the donor species crude powder (Javaid and Anjum, 2006). Furthermore, Einhellig (1996) reported that allelochemicals decreased elongation, expansion and division of cells which are growth prerequisite. In addition, Wu *et al.* (1998) reported that the phenolic acids found in extracts of *Buchloe dactyloides* clipping showed various degree of seedling growth inhibition especially on root growth. Similarly, parthenin inhibited the root and shoot lengths of *Avena fatua* and *Bidens pilosa* (Batish *et al.*, 2002). Root length (RL) was more sensitive to the allelopathic effects of crude powder of the two donor species than SL. This result is similar to the previous finding that parthenin exerted more effect on root than on shoot of *Avena fatua* and *Bidens pilosa* (Batish *et al.*, 2002). Such an observation that parthenin is a potent root inhibitor has also been made earlier by Kamara *et al.* (1999). Root length was significantly reduced, this is because of the fact that root had direct contact with soil and absorb many allelochemicals. Similar findings were also reported by Sodaieizadeh *et al.* (2010). The reduction in root length may indicate that cell elongation was also affected as the allelopathic agents have been found to block gibberellins and IAA (Indole acetic acid) functions (Tomaszewski and Thimann, 1966). Root growth of TA was less sensitive to AM than CA. Variant response to the allelopathic substance could be related to the species-specific growth regulatory effect of allelochemicals, and, could be concentration dependent (Einhellig, 1996).

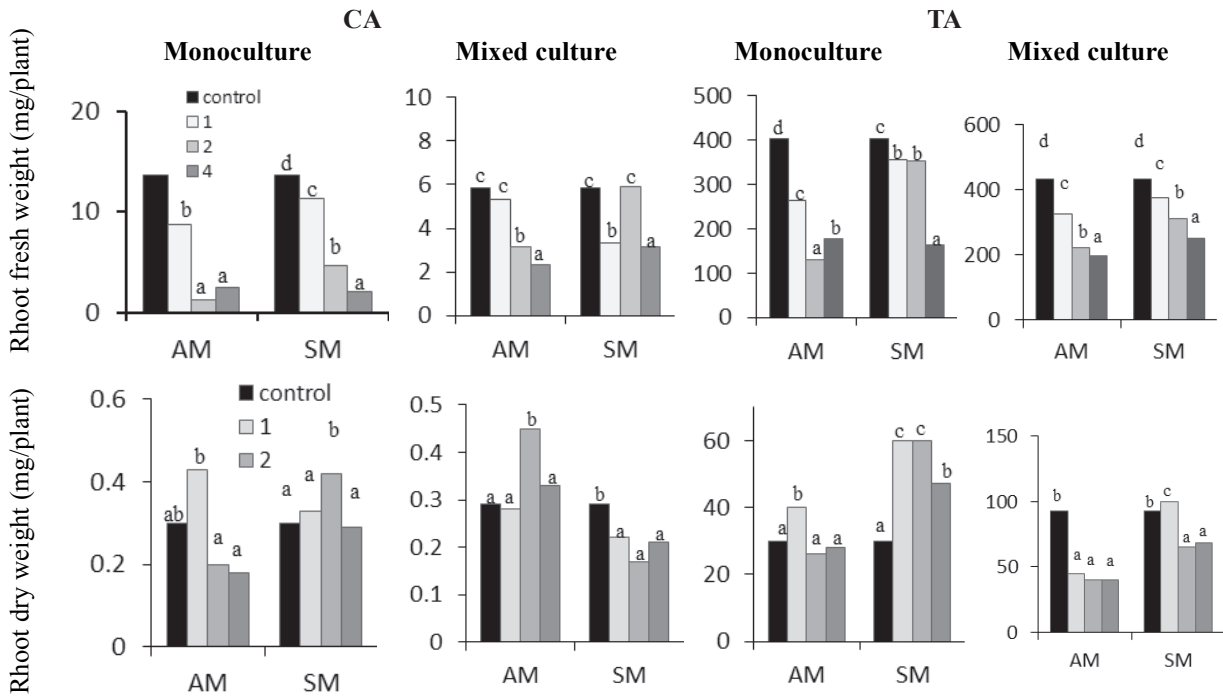


Fig. 1 : Allelopathic effect of different concentrations of the two wild species aqueous extract AM and SM on shoot and root fresh weight (mg/plant) of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures in green-house experiment.

Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test.

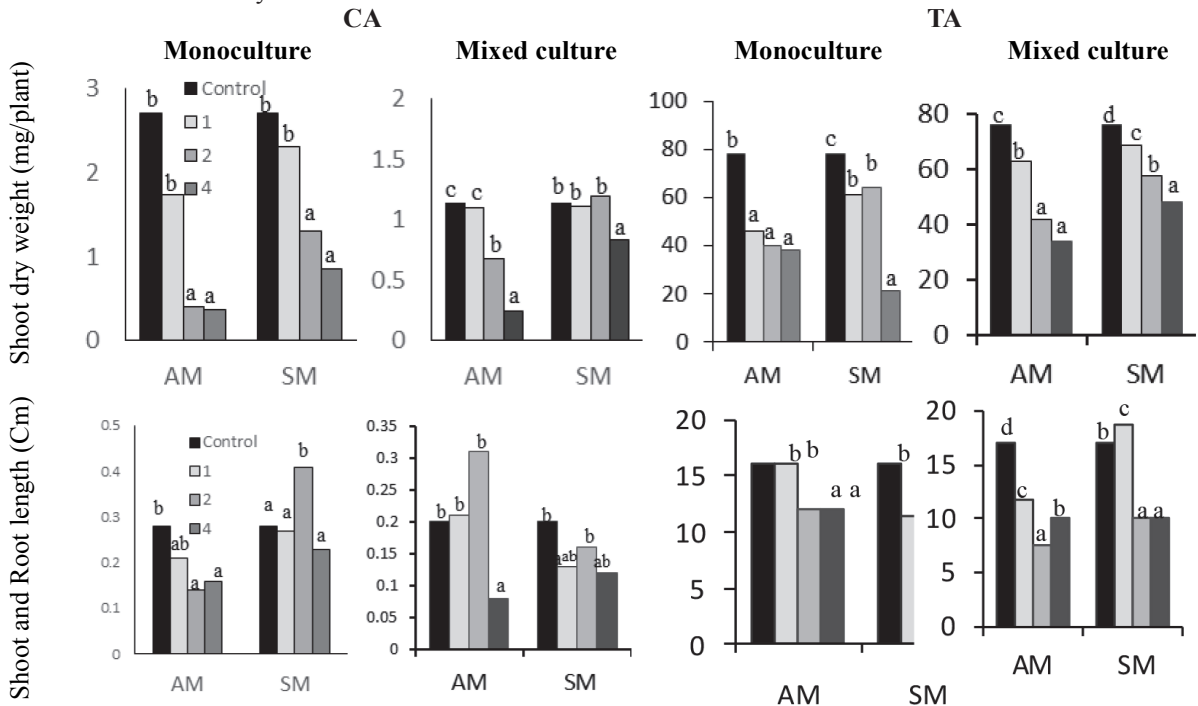


Fig. 2: Allelopathic effect of different concentration of the two wild species aqueous extract AM and SM on shoot and root dry weight (mg/plant) of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures in green-house experiment.

Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test.

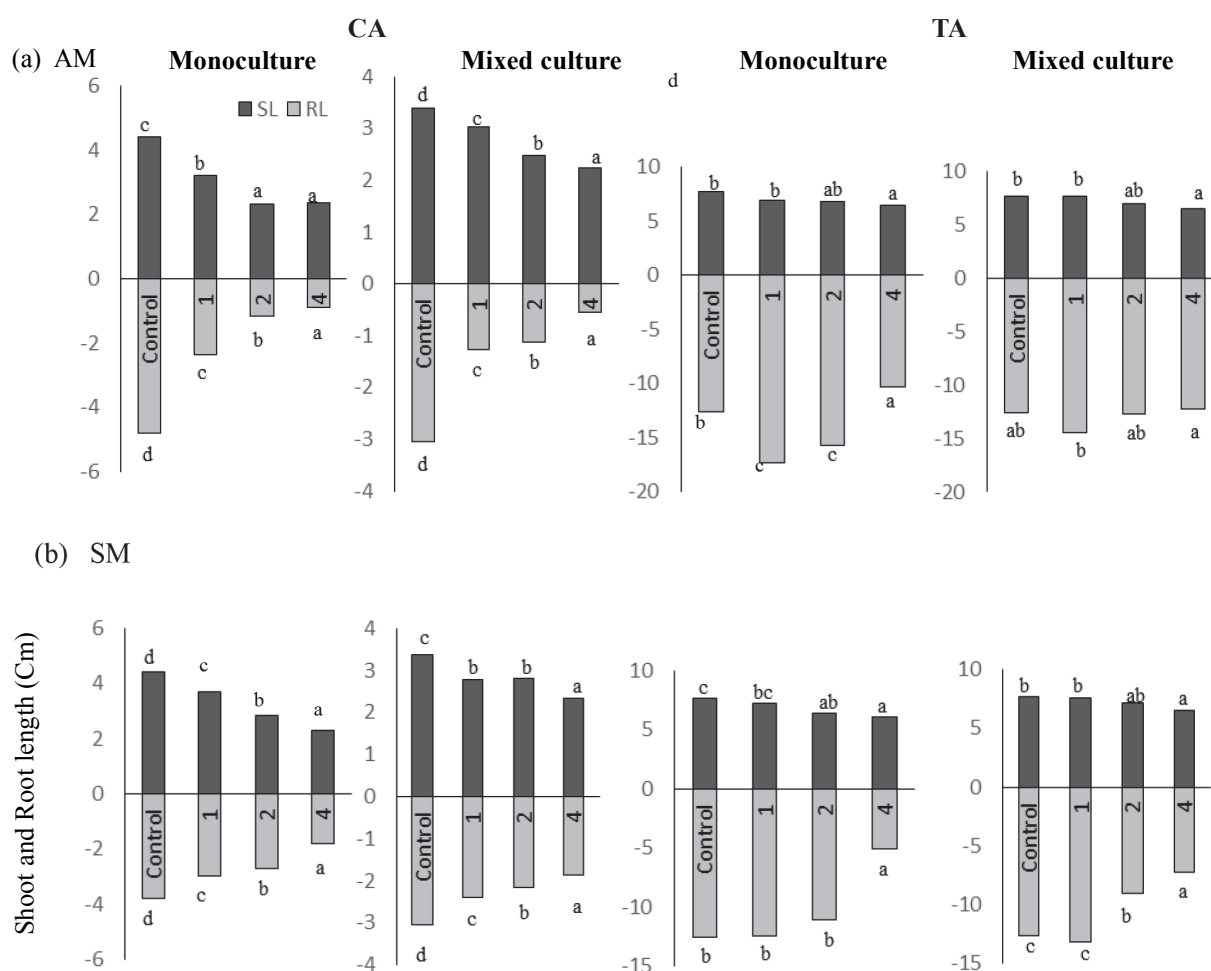


Fig. 3: Allelopathic effect of different concentrations of the crude powder AM (a) and SM (b) on shoot length (SL) and root length (RL) of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures.

Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test.

SL/RL ratio of CA and TA in monoculture treatments was gradually increased with increasing the effect of SM, while this ratio in response to AM was increased in CA and decreased in TA. The mixed culture treatment exhibited different responses on SL/RL ratio of CA and TA under the two wild species. The SL/RL ratio of CA was increased by considerable values in response to the effect of AM but with a slight increase in response to SM. In mixed culture SL/RL ratio of TA was reduced in response to the different concentrations of AM. On the other hand, as a response to increasing of SM concentrations, SL/RL ratio of TA in mixed culture was increased gradually.

As a result of the reduction in RL, SL/RL ratio of CA and TA was increased in response to the allelopathic effect of SM, while it was decreased in TA under the allelopathic effect of AM in both mono and mixed

culture treatments. It was obvious that, allelopathic treatments of these donor species have a stressful effect on root length of CA more than TA.

Specific Root Length (SRL)

There was significant increase in SRL in case of TA in both mono and mixed culture treatments in response to AM low concentrations while there was no significant difference in response to SM, on the other hand, CA exhibited a significant reduction in SRL under low concentrations of AM and SM except in case of mixed culture under SM treatments which exhibited non-significant variation as compared to control (Fig. 4). In the current study, the most interesting aspect is the relation between the root length and dry weight presented as specific root length (SRL), which was increased in TA in response to the allelopathic effect of AM. This may indicate that the plant was increased in

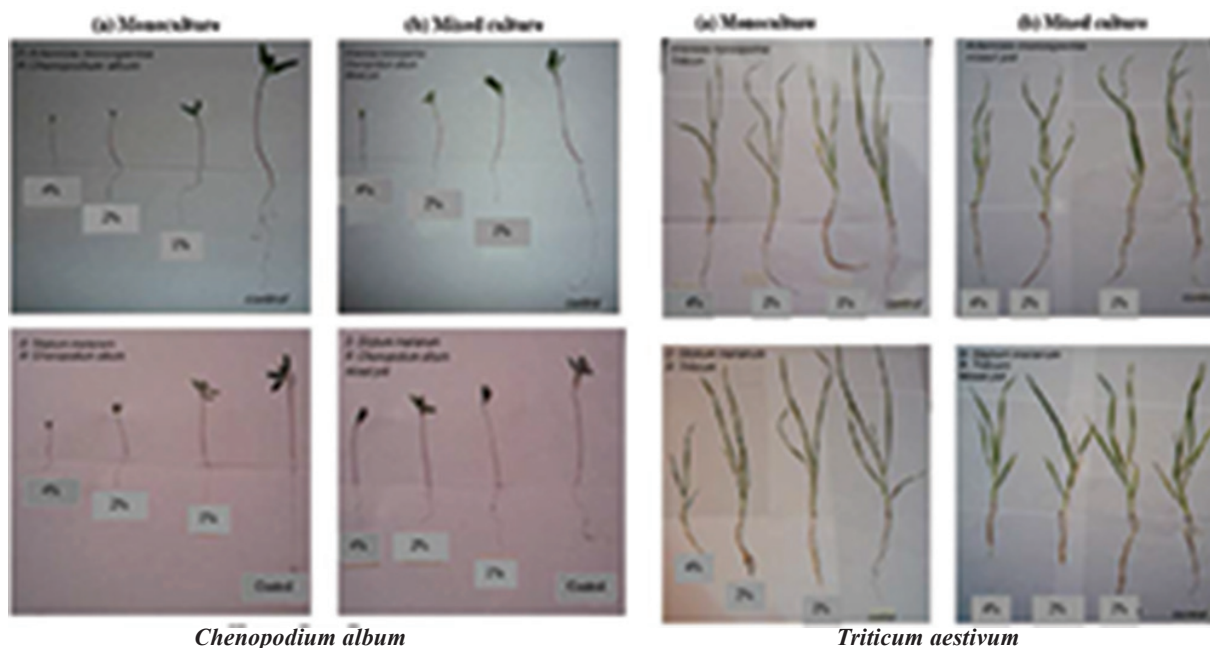


Plate 1: *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) 35-day old after subjected to different concentrations of crude powder of the two donor species AM and SM

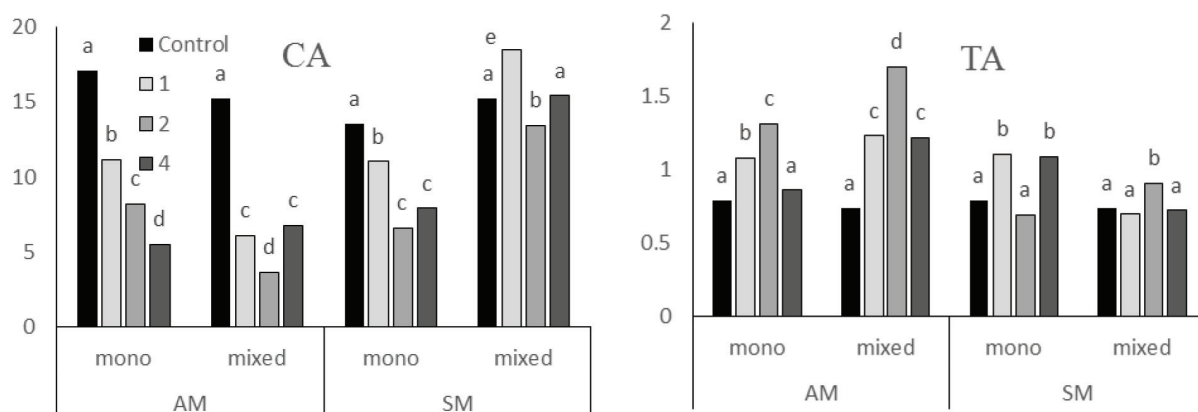


Fig. 4: Allelopathic effect of different concentrations of the crude powder AM (a) and SM (b) on Specific Root Length of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures.

Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test.

root surface area. Comas *et al.* (2013) explained that the short adventitious roots with large SRL allow the plant to easily increase hydraulic conductivity by increasing the root surface area in contact with soil water and the soil volume that can be exploited for water absorption.

Leaf Area (LA)

Generally, the data in Fig. 5 indicating that LA of CA and TA showed a gradual decrease with increasing the concentration of the two wild species as compared with control. LA of CA in monoculture treatment was

reduced significantly in response to AM and SM. In mixed culture treatment, AM had significant inhibitory effect on LA of CA, while the effect of SM on LA was non-significant. The greatest reduction percentage (75%) of LA of CA was recorded at the highest level of AM as compared with control. LA of TA in mono and mixed culture was significantly reduced by increasing concentration of the two wild species. The highest reduction percentage (59.7% and 57.6%) for LA of TA in mono and mixed were attained at 4% of AM relative to control. The reduction percentage in LA of CA was higher than in TA under all treatments of the two wild

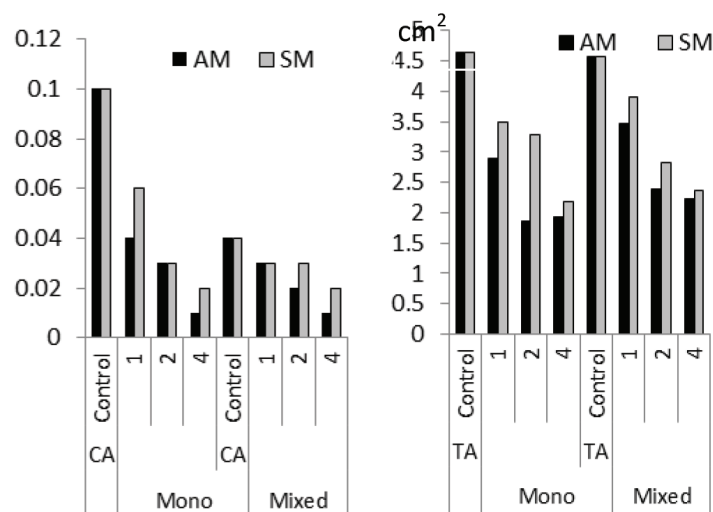


Fig. 5: Allelopathic effect of different concentrations of the crude powder AM and SM on leaf area (cm²) of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) mono and mixed cultures.

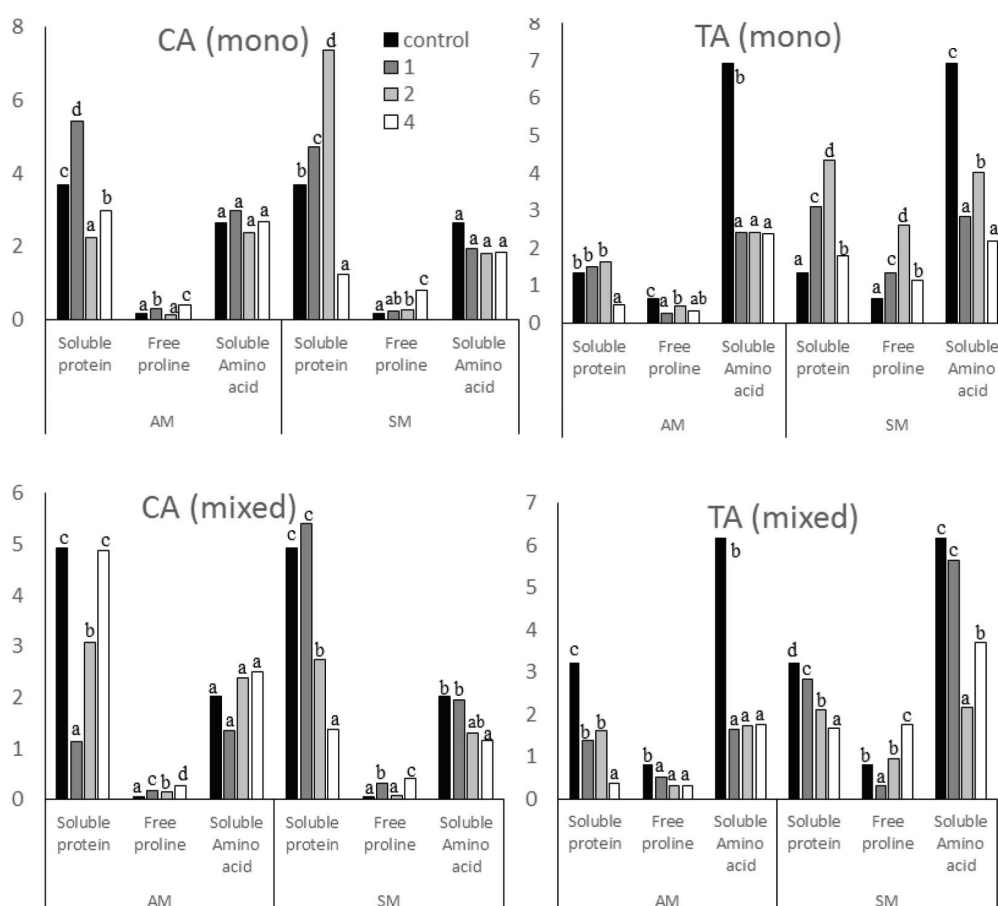


Fig. 6: Allelopathic effect of different concentrations of the crude powder AM and SM on soluble protein, free proline and soluble amino acids (mg/g f.w) in shoots of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures.

Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test

Table 1: Allelopathic effect of different concentrations of the two donor species crude powder species (AM and SM) (w/w) mixed with soil on water content (%) of shoot system of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures in green-house experiment

Mixed (CA-TA)	TA	LSD	-	0.25
		F-values	1.93	116.26**
		4	82.83 ^a	80.81 ^a
		2	81.08 ^a	81.60 ^b
		1	80.73 ^a	81.65 ^b
	control	82.51 ^a	81.51 ^b	
	CA	LSD	1.1	2.13
		F-values	316.29**	143.42**
		4	89.32 ^c	73.48 ^b
		2	78.62 ^a	79.93 ^c
1		79.25 ^a	66.67 ^a	
control	80.55 ^b	80.55 ^c		
Mono	TA	LSD	1.95	2.43
		F-values	141.97**	21.78**
		4	78.65 ^b	87.27 ^b
		2	82.23 ^c	81.92 ^a
		1	82.58 ^c	82.80 ^a
	control	80.65 ^c	80.65 ^a	
	CA	LSD	12.16	13.04
		F-values	6.95*	11.44*
		4	65.60 ^a	57.00 ^a
		2	66.67 ^a	71.90 ^b
1		80.00 ^{ab}	79.65 ^b	
control	80.15 ^{ab}	80.15 ^b		
Culture	Treatment (%)	AM	SM	

*Significant difference at P<0.05

**Significant difference at P<0.01

species in both cultures. Similar findings were obtained by Javaid and Anjum (2006) who suggested that the reductions in LA may be attributed to the reduction rate of cell division and cell elongation due to the presence of allelochemicals in the donor species crude powder. Several studies had shown that compounds of plant origin, such as allelochemicals, affected mitotic activity of the growing roots (Rizvi *et al.*, 1992; Einhellig, 1996). Such an inhibitory effect on mitotic activity may directly decrease plant growth, and so, mitotic activity can be used to evaluate root growth resulting from cell division of meristematic cells and cell expansion in the elongation zone of roots (Dayan *et al.*, 2000).

Water content

The effect of crude powder for the two donor species (AM and SM) on water content (%) in shoot system of the two recipient species CA and TA is presented in Table 1. In monoculture treatment the water content of CA was significantly decreased in response to the

increase in concentrations of AM and SM. On the other hand, CA attained with the highest reduction percentage (28.9%) for water content in CA at the highest treatment of SM as compared to control. The water content of TA in monoculture was significantly reduced in response to AM. On the other hand, the water content of TA in monoculture was gradually increased with highly significant relative to control treatment by 8.21% in response to SM. This highest percentage of increase was in the highest concentration treatment. In mixed culture treatment the water content of CA was significantly reduced in response to the effect of the two donor species, except under the highest treatment of AM the water content was increased with 10.9% as compared to control. There is no significant response in water content of TA in mixed culture treatment in response to AM and SM

Total photosynthetic pigments and chlorophyll a/b ratio

Table 2: Allelopathic effect of different concentrations of the crude powder AM and SM on total pigments content (mg/g f.w) and chlorophyll a/b ratio in leaves of *Chenopodium album* (CA) (weed species) and *Triticum aestivum* (TA) (crop species) in mono and mixed cultures Chl.a: Chlorophyll a Chl.b: Chlorophyll b

Treatment	AM	SM	Total Pigments		Chl. a/b		
			CA	TA	CA	TA	
Mixed	TA	LSD		0.24		0.22	
		F-values		5.76*		6.12*	
		4	3.14	3.08 ^{ab}	3.48	2.99a	
		2	2.86	3.17 ^{ab}	3.43	3.05ab	
		1	2.9	2.86 ^a	3.32	3.33b	
		control	3.97	3.28 ^b	3.97	3.28b	
	CA	LSD		0.07		0.95	
		F-values		41.73**		0.23	
		4	1.09	2.91 ^c	2.8	2.59a	
		2	1.99	3.02 ^d	3.4	2.52a	
		1	2.9	2.84 ^b	3.26	2.56a	
		control	4.2	2.72 ^a	4.2	2.72a	
	Mono	TA	LSD		0.26		0.24
			F-values		0.9		3.54
			4	3.17	3.07 ^a	2.66	2.88a
			2	3.43	3.05 ^a	2.98	2.86a
1			3.5	3.07 ^a	3.22	3.17a	
control		4.18	2.90 ^a	4.18	2.90a		
CA		LSD		0.22		0.04	
		F-values		5.98*		46.4**	
		4	1.61	3.09 ^b	2.19	2.65a	
		2	2.84	3.07 ^b	3.68	2.62a	
	1	4.92	2.97 ^b	4.13	2.62a		
control	4.9	2.75 ^a	4.9	2.75b			

*Significant difference at P<0.05 **Significant difference at P<0.01

The content of total pigments and chlorophyll a/b ratio (chl.a/b) in both CA and TA under the effect of crude powder of the two species are represented in Table 2. In monoculture treatment total content of photosynthetic pigments of CA and TA was decreased in response to the increase of wild species concentration. In mixed culture treatment the content of total pigments in fresh leaves of CA was decreased in response to the increase in concentrations of AM and SM. It may be noted that the reduction in the content of total pigments in CA with AM concentration was greater than that in SM. Also, in mixed culture, the content of total pigments in TA was reduced in response to the increase in concentration of the two wild species. The total chlorophyll pigments in CA were remarkably affected with AM and SM concentrations more than in the TA under both mono and mixed culture treatments. In monoculture treatment chl.a/b ratio of CA was increased gradually with significant level in response to the increase of AM concentration, while it was decreased

significantly to SM. In monoculture treatment chl.a/b ratio of TA was slightly increased with non significant level in response to the increase of AM concentration, and decreased in response SM of concentrations. In mixed culture treatment chl.a/b ratio of CA was significantly increased in response to AM concentration. On the other hand, it was decreased non-significantly at SM treatment as compared with control. Results of the present study revealed that the total contents of photosynthetic pigments were reduced in response to the increase of allelopathic effect of the donor species. The reduction in chlorophyll content in response to allelochemicals has been described in a numerous plants (Dayan *et al.*, 1999). The decrease in photosynthetic pigments of the two recipient species may be related to the inhibitory effect of the released allelochemical substances from the donor species on the synthesis of the pigments and/or the structure of chloroplasts. Similar results were obtained by Batish *et.al.* (2002). Abu-Romman *et.al.* (2010) found a decrease in total

chlorophyll content of wheat seedlings under allelochemical stress of *Euphorbia hierosolymitana*. Bagavathy and Xavier (2007) also reported the reduction in total chlorophyll in sorghum plants when treated with *Eucalyptus* leaf extract. However, the reduction of chlorophyll under leachate treatments could be attributed to the inhibition of chlorophyll biosynthesis and/or the encouragement of chlorophyll degradation (Yang *et al.*, 2004). Singh *et al.* (2009) described that the effect of *Nicotiana plumbaginifolia* aqueous leachate decreased the amount of photosynthetic pigments in *Zea mays* seedlings. Moreover, total photosynthetic pigments of *Lactuca sativa* leaf were declined when treated with *Artemisia judaica* shoot extract (Zeng *et al.*, 2009). Furthermore, Batish *et al.* (2002) concluded that this reduction was attributed to the modification in the integrity of chloroplast and thylakoid membranes in response to allelochemicals.

The results of the present study indicated that chl. a/b ratio of CA was significantly increased under the allelopathic effect of AM and decreased in response to SM in both culture treatments. Furthermore, chl.a/b ratio of TA was slightly increased in response to AM and decreased in response to SM in monoculture, while in mixed culture the ratio of TA was reduced in response to AM and SM. The variation in the reduction of photosynthetic pigments especially chlorophyll b of the two recipient species may be related to the inhibitory effect of the released allelochemical substances from the donor species on the synthesis of the pigments. This result was similar to the previous investigation obtained by Singh *et al.* (2009) who reported that higher degradation rate of chlorophyll b or controlled conversion of chlorophyll a to chlorophyll b in leaves of maize seedlings treated with higher concentration of *Nicotiana* leachate resulted in increase of chlorophyll a/b ratio. This can be explained by the fact that the first step in chlorophyll b degradation involves its conversion to chlorophyll a (Fang *et al.*, 1998).

Soluble protein

Generally, in monoculture the soluble protein of CA was reduced in low concentrations of AM. Contrary, it was significantly increased under the effect of SM. In case of TA soluble protein was slightly increased in response to increase concentration of AM and was slightly increased in response to SM. In mixed culture treatment the data of soluble protein of CA has no obvious trend in response to increased concentration of AM and SM. Besides, in mixed culture treatment soluble protein of TA was significantly reduced in response to increase concentration of AM and SM, it is notable that the soluble protein in TA was increased in response to SM at monoculture treatment but decreased with high

percentage in mixed culture treatment relative to control (Fig. 6). The reduction in soluble protein may be attributed to the presence of allelochemicals in the donor species. Similar observations have been recorded by Einhellig (1996) who found that protein synthesis was reserved in *Lactuca sativa* when treated with cinnamic acid. These results were confirmed by Abu-Romman *et al.* (2010) on *Triticum durum*. Under this study, the decline in soluble protein may be related to the stimulation of protein degradation which was influenced by impairment of various metabolic activities under the allelopathic effect of donor species crude powder. Hoque *et al.* (2007) reported that the degradation of the protein to amino acids, such as proline, might be an adaptation mechanism against the allelochemical stress and/or a means of osmolytes to avoid water loss. In addition, allelochemicals are known to generate reactive oxygen species (ROS) which caused oxidative modification/degradation of proteins (Lara-Nunez *et al.*, 2006).

Free proline

Generally, free proline in CA was slightly increased in response to the effect of AM in monoculture but it accumulated in mixed culture as compared with control, while free proline in TA was reduced at both monoculture and mixed culture treatments. On the other hand, free proline in CA and TA was increased under the effect of SM concentrations. In monoculture, free proline in CA was significantly increased in response to the increase of AM, and SM concentrations. In addition, in monoculture, free proline in TA was significantly reduced in response to the effect of AM. On the other hand, free proline in TA significantly increased in response to SM. TA attained the highest accumulation percentage (307.8%) of free proline at 2% SM relative to control. In mixed culture free proline of CA was significantly increased in response to AM and SM, on the contrary, in TA. It was reduced significantly in response to AM. On the other hand, free proline in TA was significantly accumulated in response to SM, except under low concentration (1%) (Fig. 6). These results were confirmed by Hatata and El-Darier (2009) who reported that the accumulation of proline and soluble carbohydrates act as osmoprotectants or osmoregulator which in turn exert a positive role in the allelochemicals stress. Exposing plants to any stress lead to a series of reactions which generates numerous free radicals which may be reflected by altered levels of major anions and accumulation of proline (Ramon *et al.*, 2003). Alia *et al.* (1993) reported that proline is important part of structural proteins and enzymes and participates in repair processes, it is also supposed to participate in the reconstruction of chlorophyll, activates the Krebs cycle and constitutes an energy source. Proline

is the most commonly studied as a biochemical indicator of stress (Claussen, 2005). Proline has been shown to protect plants against damages caused by free radicals by scavenging the radicals and stabilizing of macromolecules (Matysik *et al.*, 2002). In addition, proline acts as a source of energy, carbon, and nitrogen, which enhances tissue recovery, and the relief of stress effects (Singh *et al.*, 1973).

Soluble amino acids

Generally, in mono and mixed culture treatments, the amount of soluble amino acids was increased in CA and decreased in TA in response to AM, while soluble amino acids in both CA and TA was decreased in response to SM concentrations, but CA attained the opposite trend where the highest percentage of soluble amino acids was higher in mixed culture than in monoculture treatment relative to control (Fig. 6). During adaptation to various types of environmental stress, plants accumulate cellular solutes (Patakas *et al.*, 2002). The cellular solutes include quaternary amino acid derivatives such as proline, glycine, betaine, alanine betaine, and proline betaine (Nuccio *et al.*, 1999). This accumulation may be as a response to osmotic adjustment for water stress caused by the allelopathic effect on the crops (Bohnert *et al.*, 1995). The previous compounds have been used by many scientists such as Stewart and Larcher (1980), Rozema *et al.* (1985) to evaluate the sensitivity or tolerance degree against stress.

ACKNOWLEDGEMENT

The authors would like to thank members of the Plant Department Faculty of Science, Alexandria University for their cooperation to use the department's labs.

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