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**Herbicidal Activity of *Asphodelus
microcarpus* against Selected Weed Species
(*Chenopodium album*) of Wheat
(*Triticum aestivum*)**

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Herbicidal Activity of *Asphodelus microcarpus* against Selected Weed Species (*Chenopodium album*) of Wheat (*Triticum aestivum*)

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Abstract

The objective of the present study was to evaluate the bio-herbicidal potential of *Asphodelus microcarpus* L. (AM) on *Chenopodium album* L. (CA); a major wheat pest (*Triticum aestivum* L., TA). This research was conducted to study the allelopathic effects of AM on some growth parameters and photosynthetic pigments of CA and TA in both mono and mixed cultures. Moreover, some metabolites were determined in TA. The results showed a reduction in the plant growth in both CA and TA, with considerably stronger allelopathic effects on the growth of CA, as compared with that of TA, in the presence of different AM concentrations. Photosynthetic pigments in CA were also significantly decreased. The organ length of CA under control was lower in mixed cultures than in monocultures. This may be an indication of the allelopathic potential of TA on CA. The results also indicated that, proline and amino acids in TA were accumulated significantly under the allelopathic effect of AM. In this respect, TA has more tolerance and resistance to the allelopathic treatments when compared with CA. This study suggests the suppressive potential of allelopathy against selected weed species, and offers promises for their usefulness as a tool of weed management.

Keywords: *Allelopathy, Asphodelus microcarpus, Chenopodium album, Triticum aestivum*

Introduction

AM is a natural herbaceous perennial plant belonging to the Liliaceae family which grows in the Mediterranean region and contains biologically active secondary metabolites against different diseases such as skin diseases, leukemia and malaria (Ghoneim et al., 2013). In agricultural production, the most dependable source of weed control for over 50 years has been the synthetic herbicides. This happens because herbicides are effective, easy to apply, relatively cheap and they reduce the need for mechanical means of weed control since mechanical means enhance soil erosion (Gianessi and Reigner, 2007). However, the overuse of synthetic herbicides may affect the environment, human health and food (Mansour et al., 2014). Therefore, there is a great demand for compounds with selective toxicity that can be readily degraded by the plant or the soil microorganisms. In addition, plants, microorganisms, other soil organisms and insects can produce allelochemicals which provide new strategies for maintaining and increasing agricultural production in the future (Sodaeizadeh and Hosseini, 2012). Much more work has been done on plant derived compounds as environmentally safe alternatives to herbicides for the weed control (Sodaeizadeh et al., 2010). In this regard, the use of crops which have allelopathic properties can reduce the dependency on synthetic herbicides and increase crop yields (Khanh et al., 2005). The allelopathy is expressed through the release of chemicals by a plant which has been suggested to be one of the possible alternatives for achieving sustainable weed management (Abu-Romman et al., 2010; Moore et al., 2015). The use of allelopathy for controlling weeds could be either through the direct utilizing of natural allelopathic interactions or by using allelochemicals as natural herbicides (Singh et al., 2009). Allelochemicals may affect plants indirectly through the alternation of soil properties, nutritional status and population or activity of microorganisms and nematodes (Nekonam et al., 2013). Mycorrhizal fungi form a symbiosis with over 80% of the vascular plant species and are also considered keystone species in temperate ecosystems because of their influence on plant nutrient supply and the overall ecosystem functioning (Gianinazzi, et al., 2010; Cameron, 2010). The use of allelopathic and medicinal plants has been suggested as a viable option for alternative weed management under sustainable agriculture (Nekonam et al., 2014).

The aim of this study was to investigate the allelopathic potential of AM on some growth parameters and photosynthetic pigments of CA and TA at mono and mixed cultures in the green-house. Some metabolites were also carried out of TA. There are hopes that the study will provide information on the possibilities of using AM as bio-herbicides.

Materials and Methods

Sample Collection and Plant Analysis

AM tuberous root samples were collected from non-saline habitats throughout the month of March 2014 in 17 locations in the western Mediterranean region of Egypt. Samples were washed with tap and bi-distilled water and air-dried, then cut into pieces and grounded to powder. The crude powders were stored at room temperature. CA seeds and TA 'Sakha 94' grains were grown in plastic pots (15 cm in diameter, 20 cm in length) filled with 1 kg of soil (1:2 clay to sand) completely mixed (w/w) with 1, 2 and 4% of crude powder of AM, in addition to control treatment (without AM). Soil samples were air-dried, sieved to remove gravel and plant debris and finally sterilized (80°C for 24h) to remove any microorganisms and weed seeds. 0.1 g of CA seeds and ten grains of TA were sown in each pot. All pots were kept at 80% water holding capacity for the soil by the addition of tap water. The experiment was carried out as a monoculture for each of species and as a mixed culture between CA and TA. The experiment was performed under greenhouse conditions with day temperatures ranging from 20-22°C and night temperatures from 14-16°C. The pots were arranged in a completely random block design with three replicates. The plants were harvested after 35 days and carefully freed from the soil with gentle motions, then washed with distilled water.

Growth Parameters Estimation (Organs Length and Plant Biomass)

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. Homologous individuals were selected from each treatment, dissected into shoots and roots and then weighed separately to determine fresh weight. The samples were dried in an oven at 60°C to determine the dry weight for each treatment of CA and TA.

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.

Determination of Soluble Metabolic Compounds in Plant Extract

Fresh samples from TA 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. Inhibition % was calculated as: $[(\text{control} - \text{treatment})/\text{control}] \times 100$.

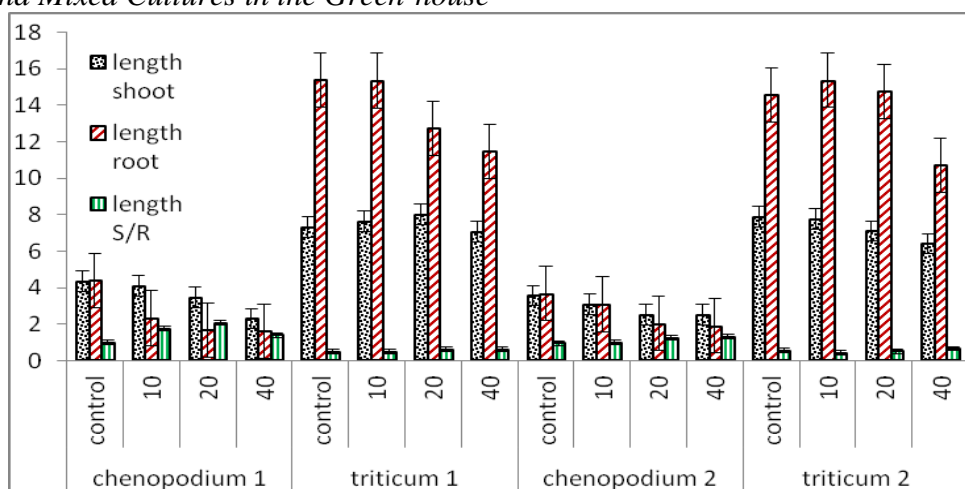
Results

The significant effect of AM on growth parameters and photosynthetic pigments in CA and TA in addition to some metabolites in TA is illustrated in Table 1. In mono and mixed culture the organs length of CA was significantly reduced gradually in response to different concentrations of AM, while the reduction in mixed cultures was lower than in monoculture (Figure 1). Under the control treatment the organ length of CA in mixed cultures were lower than in monoculture. The greatest reduction in both shoot (47 and 63%) and root (29 and 48.4%) of CA for mono and mixed cultures respectively were recorded at the highest treatment (4%) relative to control. The notable reduction in the length of shoot (3, 34.1%) and root (19 and 26.7%) of TA in both mono and mixed cultures respectively was recorded only at 4% relative to control. SL/RL ratio of CA and TA in mono and mixed cultures increased at 4% (Figure 1).

Table 1. Statistical Analysis for Different Parameters of CA and TA at Different Concentrations of AM (F: F test, *Statistically Significant at $p \leq 0.05$ and LSD= Least Significant Difference at $p=0.05$)

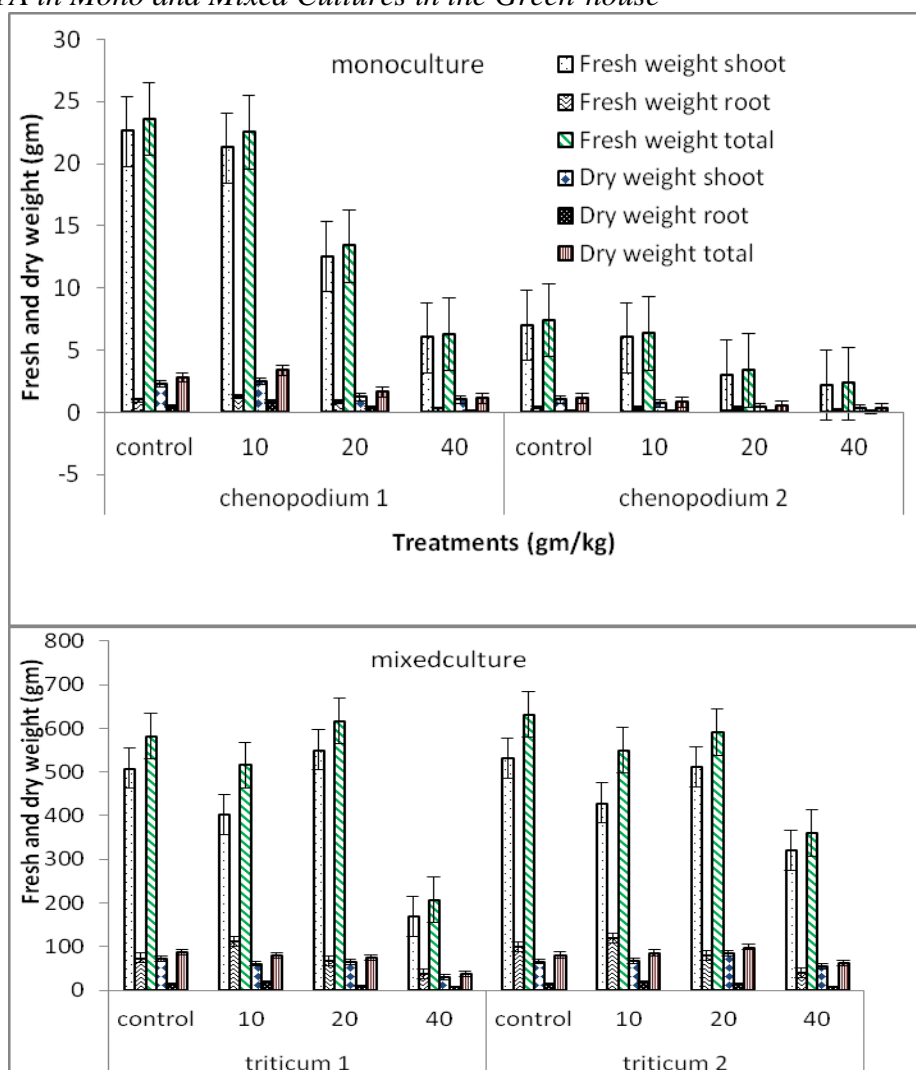
Culture		Organ	Test	Length	Fresh	Dry	Protein	Proline	Amino acids
Mono	CA	Shoot	F	545.63*	5652.212*	1538.322*	-	-	-
			LSD	0.153	0.403	0.075			
		Root	F	10386.56*	36.952*	483.909*	-	-	-
			LSD	0.050*	0.249	0.056			
	TA	Shoot	F	118.39*	7600.215*	124.364*	1513	12.474*	4.781
			LSD	0.156	7.739	6.445	0.142	0.058	2.455
		Root	F	168.97*	280.148*	53.444*	-	-	-
			LSD	0.620	7.223	2.949			
Mixed	CA	Shoot	F	2123.04*	2861.718*	175.870*	-	-	-
			LSD	0.042*	0.170	0.096			
		Root	F	106.147*	87.378*	99.000*	-	-	-
			LSD	0.355	0.054	0.017*			
	TA	Shoot	F	186.633*	2234.465*	48.752*	16.44*	1.733	6.706*
			LSD	0.196	7.985	7.019	0.124	0.141	1.846
		Root	F	366.266*	7.223	153.500*	-	-	-
			LSD	0.421	356.889*	1.445			

Figure 1. Allelopathic Effect of Different Concentrations (1, 2, 4%) of AM Crude Powder on Shoot Length and Root Depth (cm) of CA and TA in Mono and Mixed Cultures in the Green-house



The effect of AM on the shoots and roots fresh weight of CA and TA is illustrated in Figure 2. The data in mono and mixed cultures demonstrated that the organs fresh weight of CA reduced remarkably in response to the increase of AM concentrations, While the remarkable reduction in both shoot (66.5, 48.7%) and root (43.6 and 60%) of TA which was only recorded under 4% in mono and mixed cultures respectively. The highest reduction percentages in CA for both shoot (74 and 72%) and root (68.6, 72.5%) in mono and mixed cultures respectively were recorded at 4% relative to control. The total fresh weight of CA in mono and mixed cultures was reduced gradually and recorded the highest reduction (73.4, 68.78%) at 4%. While the reduction in TA (11.2 and 6.5 %) in both mono and mixed cultures were slightly at 2% as compared to control.

Figure 2. Allelopathic Effect of Different Concentration (1, 2, 4%) of AM Crude Powder on Fresh and Dry Weight for Shoot and Root (mg/plant) of CA and TA in Mono and Mixed Cultures in the Green-house



Generally, the dry weights of shoots and roots of CA and TA showed a gradual decrease when the concentration of AM was increased (Figure 2). In both mono and mixed cultures the shoot and root dry weight of CA was significantly reduced in response to that of AM except at 1% in monoculture. The highest reduction in CA shoot (56.52, 80%) and root (67 and 78.6%) in both mono and mixed cultures respectively were recorded at 4% relative to control. On the other hand, the highest reduction in the TA dry weight of shoot (59, 42.6%) and root (16.7 and 50%) were recorded under 4% treatment in both mono and mixed cultures respectively relative to control. The total dry weight of CA in monoculture was significantly reduced in response to AM. The greatest reduction % (60.7 and 68.4%) of total dry weight of CA were recorded at 4% for both mono and mixed cultures respectively as compared to control. The total dry weight of TA in monoculture was reduced gradually in response

to AM concentrations and recorded the highest reduction percentages (56.3%) at 4%. In mixed culture the reduction in the total dry weight of TA (22.8%) was only recorded at 4%.

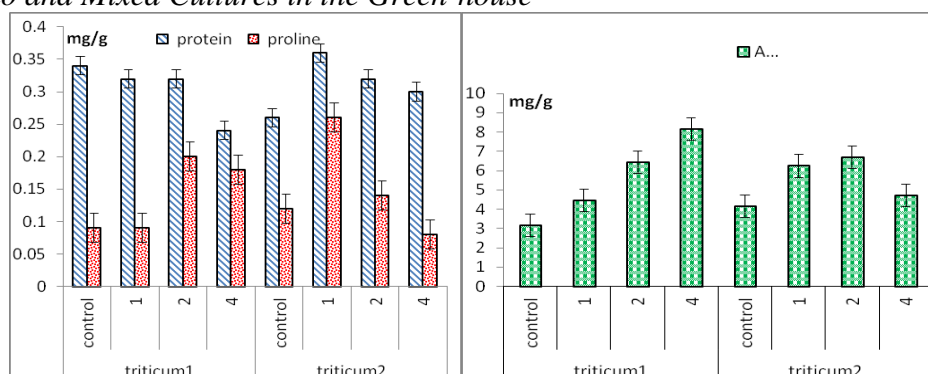
Generally, chlorophyll pigment fractions of CA significantly reduced gradually under mono and mixed culture (Table 2). In monoculture the highest reduction for pigment fractions (chl.a, chl.b, carot.) and the total pigments were 36.5, 38.1, 34.5 and 36.5% which were recorded respectively at 2% relative to control. In mixed culture chl.a, chl.b, carot. and the total pigments of CA only reduced at 2% by 11.6, 15.0, 13.9 and 12.0% respectively relative to control. On the other hand, chl.a, chl.b, carot. and total pigments of TA in monoculture reduced slightly (4, 4, 2.6 and 2.7 %), only at 4% respectively while in the mixed culture the pigment fractions and the total pigments were stimulated in response to different concentrations of AM. On the other hand, chl.a, chl.b, carot. and the total pigments of TA in monoculture were reduced slightly (4, 4, 2.6 and 2.7 %) respectively only at 4% while in mixed culture the pigment fractions and the total pigments were stimulated in response to different concentrations of AM. The chl.a/b in both CA and TA in mono and mixed cultures under the effect of AM exhibited an unclear trend with a different concentration of AM (Table 2).

Table 2. Allelopathic Effect of Different Concentrations of AM Crude Powder (w/w) Mixed with Soil on Photosynthetic Pigments (mg/g f.w) in Leaves of CA and TA in Mono and Mixed Cultures in the Green-house. F: F Test

Culture		Treatment	Photosynthetic pigments (mg/g)				
		(%)	Chl.a	Chl.b	Carot.	total	Chl.a/b
Mono	CA	control	1.685	0.586	0.403	2.674	2.873
		1	1.155	0.411	0.279	1.845	2.81
		2	1.07	0.363	0.264	1.697	2.952
		4	-	-	-	-	-
	F		24.749*	48.586*	47.362*	-	-
	LSD 5%		0.266	0.065	0.043*	-	-
	TA	control	2.008	0.69	0.462	3.16	2.911
		1	2.228	0.766	0.505	3.499	2.909
		2	2.226	0.766	0.463	3.455	2.905
		4	1.964	0.662	0.45	3.076	2.966
	F		43.709*	17.975*	5.497*	-	-
	LSD 5%		0.093	0.051	0.045*	-	-
Mixed	CA	control	1.269	0.46	0.311	2.04	0.311
		1	1.38	0.512	0.323	2.215	0.323
		2	1.122	0.4	0.273	1.795	0.273
		4	-	-	-	-	-
	F		248.651*	416.052*	208.034*	-	-
	LSD 5%		0.028*	0.010*	0.006*	-	-
	TA	control	1.954	0.672	0.451	3.077	2.908
		1	2.085	0.698	0.477	3.26	2.985
		2	2.544	0.861	0.553	3.958	2.957
		4	2.161	0.722	0.501	3.384	2.995
	F		124.161*	86.049*	88.718*	-	-
	LSD 5%		0.076	0.032*	0.015*	-	-

In monoculture soluble protein of TA reduced significantly in response to AM, while in mixed culture it increased significantly (Figure 3). The highest reduction of soluble protein (41.7%) was recorded under the highest concentration. In contrast, the greatest increase (38.5%) of soluble protein was recorded at 1% in the mixed culture relative to control. Generally, proline in TA increased in response to different concentrations at both mono and mixed cultures (Figure 3). In monoculture, the highest accumulation of proline (122.2%) was recorded at 2%, while in mixed culture the highest accumulation percentages (116.7%) were recorded at 1% treatment relative to control. In mono and mixed culture treatments, the soluble amino acids content in TA increased in response to different concentration of AM (Figure 3). The maximum increase in soluble amino acids in monoculture (157.7%) was recorded at 4% relative to control while in mixed culture it attained the highest accumulation (61.6%) under 2%.

Figure 3. Allelopathic Effect of Different Concentration of AM Crude Powder on Soluble Amino Acids, Protein and Proline (mg/g fr.w) in Shoot of TA in Mono and Mixed Cultures in the Green-house



Discussion

Allelopathy is an interference mechanism in which living or dead plants release allelochemicals exerting an effect on the associated plants, and can play an important role in natural and managed ecosystems (Inderjit and Duke, 2003). Weeds are one of the major constraints to plant yield worldwide, and herbicide use has risen significantly over the recent decades. In fact, several studies have reported the use of allelopathic higher plants for managing paddy weeds under field conditions (EL-Darier and EL-Bakkosh, 2012; Nekonam et al., 2014). The use of allelochemicals as natural herbicides against weeds besides enhancing production potential of economically important crops can provide alternative or a complementary tactics for sustainable integrated weed management (Aslani, et al., 2013; Nekonam et al., 2014).

In the present study the growth parameters of CA and TA were significantly reduced with the increase of AM concentrations. The reduction in SL and RL of CA and TA may be due to a reduction in cell division and cell elongation due to the presence of allelochemicals (Javaid and Anjum, 2006). RL was more sensitive to the allelopathic effect of AM than SL. This matched the findings of Jafariehyazdi and Javidfar (2011) when they studied the allelopathic effect of *Brassica* spp. on sunflowers. This is because of the fact that the root had direct contact with the soil and it absorbed many allelochemicals (Batish et al., 2002; Sodaiezadeh et al., 2010). The reduction in root length may indicate that the cell elongation was also affected, as the allelopathic agents have been found to block gibberellins and IAA functions (Tomaszewski and Thimann, 1966). If the data of the SL/RL ratio in both CA and TA under the different concentrations of AM were compared, it becomes evident that this ratio in CA was higher than in TA. It was obvious that the allelopathic treatment of AM has a stressful effect on root length of CA more than TA.

The shoot fresh weight of CA and TA species reduced significantly in response to the increase of AM concentrations in both mono and mixed

cultures. Generally, the total fresh weight of CA and TA that decreased was highly significant in response to the increase of AM concentrations in both cultures. Tesio et al. (2012) reported that fresh weight in pea was significantly inhibited when *Helianthus tuberosus* residues were present in the pot for a long period, while stimulation was recorded during shorter periods (Han et al., 2013). The stimulation may likely be due to enhanced water retention due to the organic material, even if the positive effect disappeared with an increasing presence of residues, showed by contrast inhibition on the pea. The shoot, root and total dry weight of CA and TA reduced significantly in response to the allelopathic effect of AM. This reduction that may be attributed to the presence of allelochemicals released from AM or due to auto toxicity is known for example in *Triticum aestivum* (Wu et al., 2012). The results were agreed, with those reported, that two foliar sprays of *Sorghum* extracts inhibited weed biomass (Javaid et al., 2006). Tesio et al. (2012) reported that the biomass of *Digitaria sanguinalis* was strongly depressed if *Helianthus tuberosus* residues were added to the substrate. Recently, Sangeetha and Baskar (2015) show that various plant species are now achieving importance as an agent of weed control for having special types of allelochemicals. These allelochemicals are capable of suppressing germination and growth of several weeds. Some of which are herbicide resistance.

The contents of photosynthetic pigment was reduced only in CA in response to the increase of allelopathic effect of AM under both mono and mixed cultures. The reduction in the chlorophyll content in response to allelochemicals has been reported in a number of plants (Khaliq et al., 2013; Nekonam et al., 2014). The result of the pigment fractions of CA reduced was highly significant in response to the increase of AM. The total chlorophyll pigments in CA were remarkably affected with AM concentrations under both cultures. The decrease in the photosynthetic pigment contents of CA may be related to the inhibitory effect of the released allelochemical substances from AM on the synthesis of the pigments and/or the structure of chloroplasts (Singh et al., 2009). The chl.a/b ratio of CA and TA increased significantly under the highest concentration of AM.

The allelopathy of AM at different concentrations has an inhibition effect on soluble protein of TA in monoculture and stimulation in mixed culture relative to control. Under this study, the decline in protein may be related to the stimulation of protein degradation which is influenced by the impairment of various metabolic activities under the allelopathy of AM. 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 mean of osmolytes to prevent water loss. The accumulation of compatible solutes may help to maintain the relatively high water content necessary for growth and cellular functions (Karimi et al., 2012). Free proline in the present study showed a highly significant accumulation in the TA under the allelopathic effect of AM in both cultures. Allelopathic stress generally induced a marked accumulation of free proline in TA except at 4% in mixed culture. These results were confirmed by Hatata and El-Darier (2009) who

reported that the accumulation of proline acts as a osmoprotectant or osmoregulator which in turn exerts a positive role in the allelochemicals stress. Alia et al. (1997) reported that proline is an important part of structural proteins and enzymes and participates in the repair processes; it is also supposed to participate in the reconstruction of chlorophyll, activate the Krebs cycle and constitute an energy source. The allelopathic effect of AM caused significant accumulation in the soluble amino acids of TA, but greater accumulation was seen in the monoculture than in the mixed culture. The creation of amino acids in TA was more sensitive to the allelopathy of AM in mixed cultures than those in monoculture.

Conclusions

It can be concluded that AM can play an important role in the formation of its natural habitats, as it accumulates many of the secondary products that enable the plant to compete with other species. It was also indicated that TA has more tolerance and resistance to the different allelopathic treatment when compared with CA especially in monoculture. Finally, the study indicated the allelopathic potential of donor species against selected weed species, and offered a promise for using these species as bio-herbicides.

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