International Research Journal of Biotechnology (ISSN: 2141-5153) Vol. 4(3) pp. 47-60, March, 2013
Available online http://www.interesjournals.org/IRJOB

Full Length Research Paper

Allelopathic potentiality of two *Heliotropium* species on germination and protein expression of some plants

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Accepted March 18, 2013

In this study we compare the allelopathic potentiality of two invasive species; *Heliotropium curassavicum* and *H. bacciferum* on germination, seedling growth and protein expression of *Calotropis procera, Faba sativa* and *Lycopersicon esculentum*. The germination of *C.procera*, *F.sativa* and *L.esculentum* controls was 100%, and inhibition of this germination increased with increasing the extract concentrations. Minimum germination values were recorded at the highest extract concentration (T_3) and amounting to 20, 52 and 76% for *C.procera*, *F.sativa* and *L.esculentum* plants treated with *H. curassavicum* and were 52, 72 and 60% for the same treatment and test plants but treated with *H. bacciferum* extract. Values of plumule lengths were generally higher than that of radicle in all test species using either of the two invasive species extract. Radicle-plumule ratios for all test species and using any of the two extracts were less than unity except control *F.sativa* plants which recorded 1.14. Dry matter allocation toward plumule is generally higher than that of radicle in all test plant treatments except for one case; *L.esculentum* control which recorded allocation amounting to 44.83% for plumule and 55.17% for radicle. Expression of proteins in treated plants of the present study was significantly increased or decreased at the level of number and intensity of protein bands as compared to control plants, depending on the type and concentration of extract treatment. According to the unweighted pair-group arithmetic mean method (UPGMA) dendrogram, the expressed proteins of control plants were the farthest among the different treatments of the three test plants.

Keywords: Allelopathy, *Heliotropium curassavicum*, *Heliotropium bacciferum*, germination, seedling growth, dry matter allocation, protein expression, SDS-PAGE, UPGMA dendrogram.

INTRODUCTION

Allelopathy can describe any direct or indirect effect of plant chemical compounds on another plant or microbe (Rice, 1984; Weir et al., 2004; Taiz and Zeiger 2010). Moreover, allelochemicals affect cell division, production of plant hormones, membrane permeability, germination of pollen grains, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, protein synthesis, nitrogen fixation, specific enzyme activities and development of conductive (El-Khatib et al. 2004; Florentine et al. 2005; Rafael et al. 2005; Jamali et al. 2006; Hegazy et al. 2007; Farrag 2007; Zeng et al., 2008; Inderjit et al. 2008; Pisula and Meiners 2010; Kim and Lee 2011; Djurdjević et al. 2012; Mansour 2013). Allelochemicals include phenolic acids, coumarins, terpenoids and flavonoides. These compounds are released from the plants as vapour, as leachings from the foliage, as exudates from the roots, or in the course of breakdown or decomposition of dead plant residues. Allelopathy is existent in the natural and agricultural ecosystems. It is a mechanism by which weeds affect crop growth and yield. Allelopathy is possibly a significant factor in maintaining the present balance among the various plant species (Rao 1983; Florentine et al. 2006; Kim and Lee 2011).

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There is increasing evidence that many plant invaders interfere with native plants through allelopathy. This allelopathic interference may be a key mechanism of plant invasiveness (Bousquet-Mélou et al*.,* 2005; Dorning and Cipollini, 2006; Callaway and Maron, 2006; Farrag 2007; Inderjit et al., 2008; Thorpe et al., 2009; Pisula and Meiners, 2010; Kim and Lee, 2011; Djurdjević et al. 2012). Invasive plants excel in their new ranges because they produce novel metabolites to which native species possess little resistance (Ni et al., 2010; Zaplata et al., 2011).

In recent decades many researchers, such as Einhellig (2002), Yang et al. (2002), Setia et al. (2007), Farrag (2007), Jabeen et al. (2011)Raoof and Siddiqui (2012) have reported the effect of various allelochemicals of different plants on physiological and biochemical processes but reports regarding effects of invasive plant allelochemicals on germination, seedling growth and protein expression of some native species are still scanty. In Saudi Arabia, *Heliotropium curassavicum* L. and *Heliotropium bacciferum* (Boraginaceae) have become two of the most common polycarpic invasive weeds infesting many Wadis and newly reclaimed fields at many areas of Taif regions (Farrag 2012). So, in the present work an attempt has been made to evaluate and compare the allelopathic potentiality of the two common invasive weeds on the germination, seedling growth and protein expression of two important economic crops; *Faba sativa* (Fabaceae) and *Lycopersicon esculentum* (Solanaceae), and one common toxic weed; *Calotropis procera* (Asclepiadaceae).

MATERIALS AND METHODS

Seeds, plant materials, treatments, preparing of seeds and experimental conditions

The seeds of the two crops; *L.esculentum* and *F.sativa* were obtained commercially from Panda store –Taif, while seeds of the weed; *C.procera* was collected from naturally growing populations at Wadi Al-Argy, Seesed, about 5 km east of Taif (21^0 $17^/$ N and 40^0 $29^/$ E and altitude of 1595m). *H.curassavicum* and *H.bacciferum* plant materials were collected from naturally growing populations at Wadi Al-Argy. Extracts were prepared by soaking weighed amounts of air dry plant shoots; 1.5, 3 and 6 gram per 100 ml distilled water at room temperature for 24 hour. These concentrations are equivalent to 0.006, 0.0012 and 0.06% (w/v) and referred as T_1, T_2 and T_3; respectively. The extracts were filtered through filter paper (Whatman # 1) and 10 ml of the filtrate were added to every Petri dish (9 cm diameter) containing one layer of filter paper. Distilled water was used as control. Intact seeds, which were homogenous and identical in size and colour were chosen then sterilized with 70 % ethanol for 5 min. Ten seeds of *L.esculentum*, and five seeds of *F.sativa* and *C.procera* were placed in each dish. Five replications of each treatment were used and incubated in dark growth chamber at 25 ^oC for 15 days. This temperature was chosen after preliminary seed germination experiments of the test species at different temperature levels.

Growth measurements

Daily readings of the germinated seeds were recorded during the experimental period and final measurements of plumule and radicle lengths were recorded for three replicates of each treatment. Percent germination and percent germination inhibition were calculated for each replicate as follow:

% germination= (no. of germinated seeds/ total no. of seeds) x 100

% germination inhibition= (% germination in control- % germination in treatment/ % germination in control) x 100

Dry matter allocation

The whole plant seedlings divided into two separate organs; plumule and radicle, each of which then oven dried at 75 ^0C until constant weight. Dry phytomass was recorded for each plant organ. Five replicates were used for every measurement.

Protein analysis

Seedlings were taken for the purpose of protein analysis and was washed by distilled water several times and kept at -70 Co until use. Cytoplasm proteins were extracted and purified from the test species for SDS-PAGE analysis based on Nelson *et al*., 1984. Seedlings of each treated and control plants were frozen in a liquid nitrogen and grind for about 30 second in a mortar with 3 ml of buffer D/g of tissue. Filter through muslin, and centrifuge for 15 minutes in a microfuge then dilute to about 2 mg protein / ml, ensuring that the final protein solution contains about 2% (w/v) SDS, 0.002% (w/v) bromophenol blue, and at least 6% (w/v) sucrose. Aliquots then separated by SDS-PAGE on 10% non-denaturing polyacrylamide gels and electrophoresed at 40 V for 6 hours at 4 ^oC (Laemmli, 1970). The analysis was carried out in Agriculture Genetic Engineering Research Institute (AGERI) and in Genetic Engineering lab. The gels were run in a mini-protein gel (Bio-Rad).

Table 1. Percent germination and % germination inhibition for *C. procera*, *F.sativa* and *L. esclentum* under aqueous extract of *H.curassavicum and H.bacciferum*. Values are given as Mean±S.D. asterisks are significantly different than control. *P<0.05, **P<0.01, n.s. = non significant

Target species	Treatment	% ± (SD) of			
		H.curassavicum		*H.bacciferum*	
		% germination	% ger.inhibition	% germination	% ger.inhibition
C. procera	control	100± (3.2)a	0	100± (3.2)a	0
	T_1	72 ± (2.5)ab	28	100± (1.9)a	0
	T_2	52± (1.2)b	48	60 ± (1.5)b	40
	T_3	20± (0.9)c **	72	52± (1.3)b *	48
F.sativa	control	100± (2.8)a	0	100± (2.8)a	0
	T_1	92± (1.8)a	8	100± (2.3)a	0
	T_2	86± (1.1)b	14	92± (1.9)a	8
	T_3	52± (1.7)b *	48	72± (1.1)a n.s.	28
L.esculentum	control	100± (3.7)a	0	100± (3.7)a	0
	T_1	96± (2.0)a	4	93± (1.6)a	7
	T_2	90± (2.2)a	10	86± (1.4)b	14
	T_3	76± (1.8)a n.s.	24	60 ± (0.7)b *	40

A dendrogram depicting the degree of relationships among different test species treatments were produced on the basis of the hierarchical cluster analysis performed by SPSS software using the unweighted pair-group arithmetic mean method (UPGMA).

Statistical analysis

Data were analyzed by ANOVA test to determine the significant differences among the mean values at P< 0.05 and P < 0.01 probability levels using a "general linear model" procedure of the Statistical Analysis System (SAS) program (SAS Institute, 1985). The correlation between RGR and other growth parameters was undertaken by using SPSS program version 10.

RESULTS

Effect of allelochemicals on germination

Effect of allelochemicals produced from the two *Heliotropium* extracts on the germination of the three test plants varied. According to the given data in (Table 1), *H.curassavicum* extract had more inhibitory effect than *H.bacciferum*. The germination of *C.procera*, *F.sativa* and *L.esculentum* controls was 100%, and inhibition of this germination increased with increasing the extract concentrations. Minimum germination values were recorded at (T_3) and amounting to 20, 52 and 76% for *C.procera*, *F.sativa* and *L.esculentum* plants treated with *H. curassavicum* and were 52, 72 and 60% for the same treatment (T_3) and test plants but treated with *H. bacciferum* extract. In addition, as extract concentration increase, germination inhibition for all test species were significantly increase as compared with control (Table 1).

Plumule and radicle lengths

The response of the studied species; *C.procera, F.sativa* and *L.esculentum*, towards the extract treatment using *H.curassavi*cum or *H.bacciferum*, showed particular differences (Figure 1). Values of plumule were generally higher than that of radicle in all test species using either of the two types of extract treatment. Increase the extract concentration; significantly ($p<0.05$) increase the inhibitory effect on both plumule and radicle as compared to control. For example the plumule and radicle lengths for control *C.procera* were 8.03 and 9.1cm; respectively, and these values greatly inhibited to 3.2 and 5.7cm; respectively, for T_1 plants treated with *H.curassavicum*. Minimum values for the same test plant and using T_3 extract, recorded 1.2 and only 0.5cm for plumule and radicle (Figure 2). Considering *C.procera*, Figure 2 showed more inhibitory effect of *H.curassavicum* on the plumule and radicle lengths than that in case of *H.bacciferum* extract. Plumule lengths were 5.7, 2.1 and 1.2cm for T_1, T_2 and T_3 of *C.procera* treated plants with *H.curassavicum* extracts, while the same test plant when treated with *H.bacciferum* gave plumule values

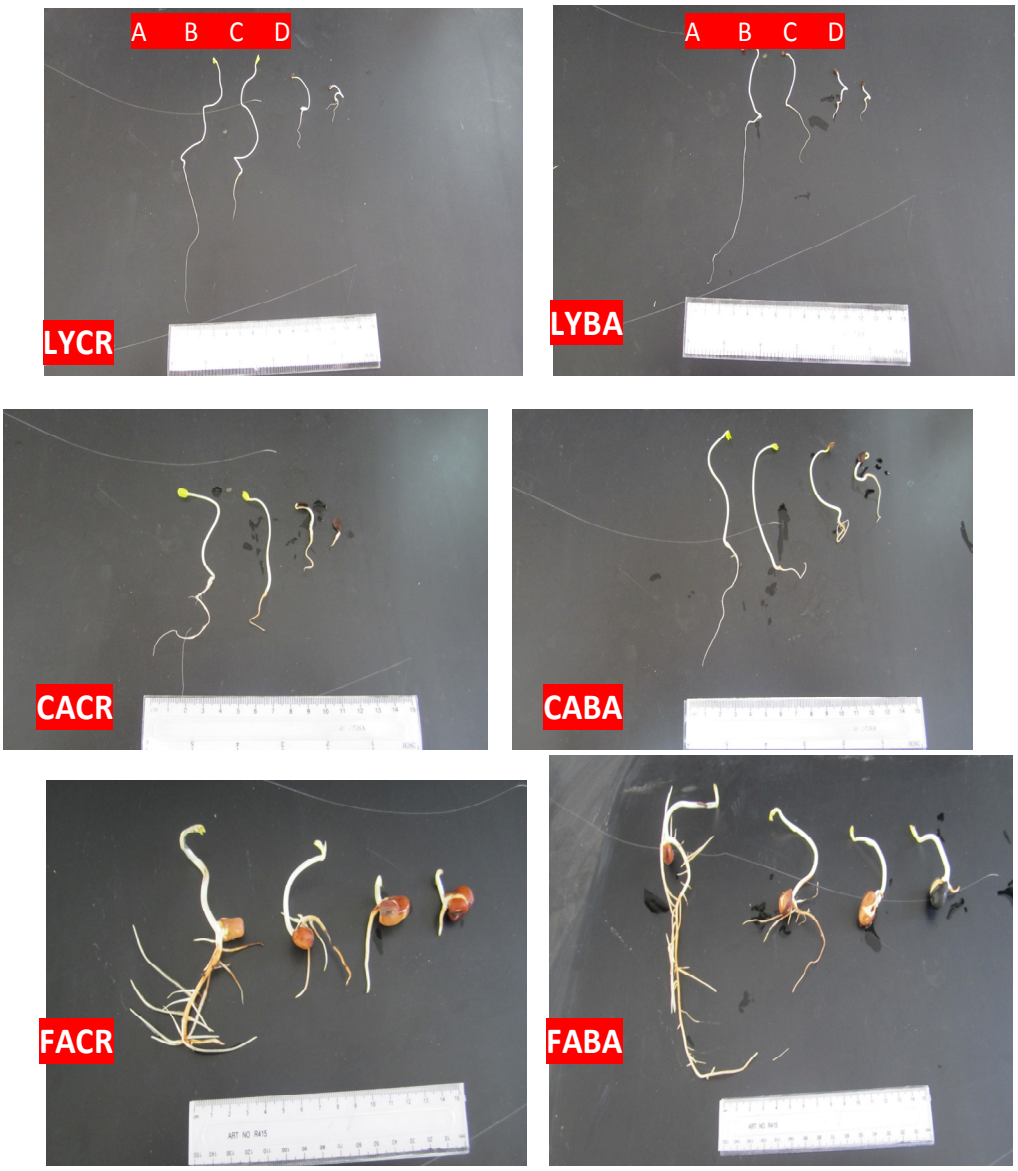

Figure 1. Allelopathic potential of *H.crussavicum* on seedling growth of *L.esculentum* (LYCR), *C.procera* (CACR) and *F.sativa* (FACR), and allelopathic potential of *H.bacciferum* on seedling growth of *L.esculentum* (LYBA), *C.procera* (CABA) and *F.sativa* (FABA). A = Control, B = 1.5, C = 3, D = 6 gram / 100ml dist. Water

amounting to 4.6, 4.2 and 2.3cm; respectively. Radicle length on the other hand obey the same trend, it recorded 1.8 and 0.5cm for T_2 and T_3 plants treated by *H.curassavicum*, and recorded 3.13 and 2.2cm for the same extract concentrations but using *H.bacciferum*. *F.sativa*, follow the same above mentioned trend. Length of radicle significantly varied ($p<0.05$) recording, 10.1, 5.4, 3.1 and 1.6cm for control, T_1, T_2 and T_3; respectively, for *F.sativa* plants treated with *H.curassavicum* extracts. The recorded values for the same test plant and concentrations but using extracts of *H.bacciferum* were 8.85, 6.13, 3.4 and 2.93cm; respectively. Opposite trend was followed in case of the third test plant (*L.esculentum*), here the obtained results showed more inhibitory effect of *H. bacciferum* extract on the plumule and radicle length than the *H. curassavicum* extract. For instance, radicle lengths were 5.86, 3.73 and 2.03cm for T_1, T_2 and T_3; respectively, for *L.esculentum* plants treated with *H.curassavicum* extracts. The recorded values for the same test plant and concentrations but using extracts of *H.bacciferum* were 2.83, 2.3 and 1.13cm; respectively (Figure 2).

Radicle-plumule ratios for all test species and using any of the two extracts were less than unity except control *F.sativa* plants which recorded 1.14 (Figure 3).

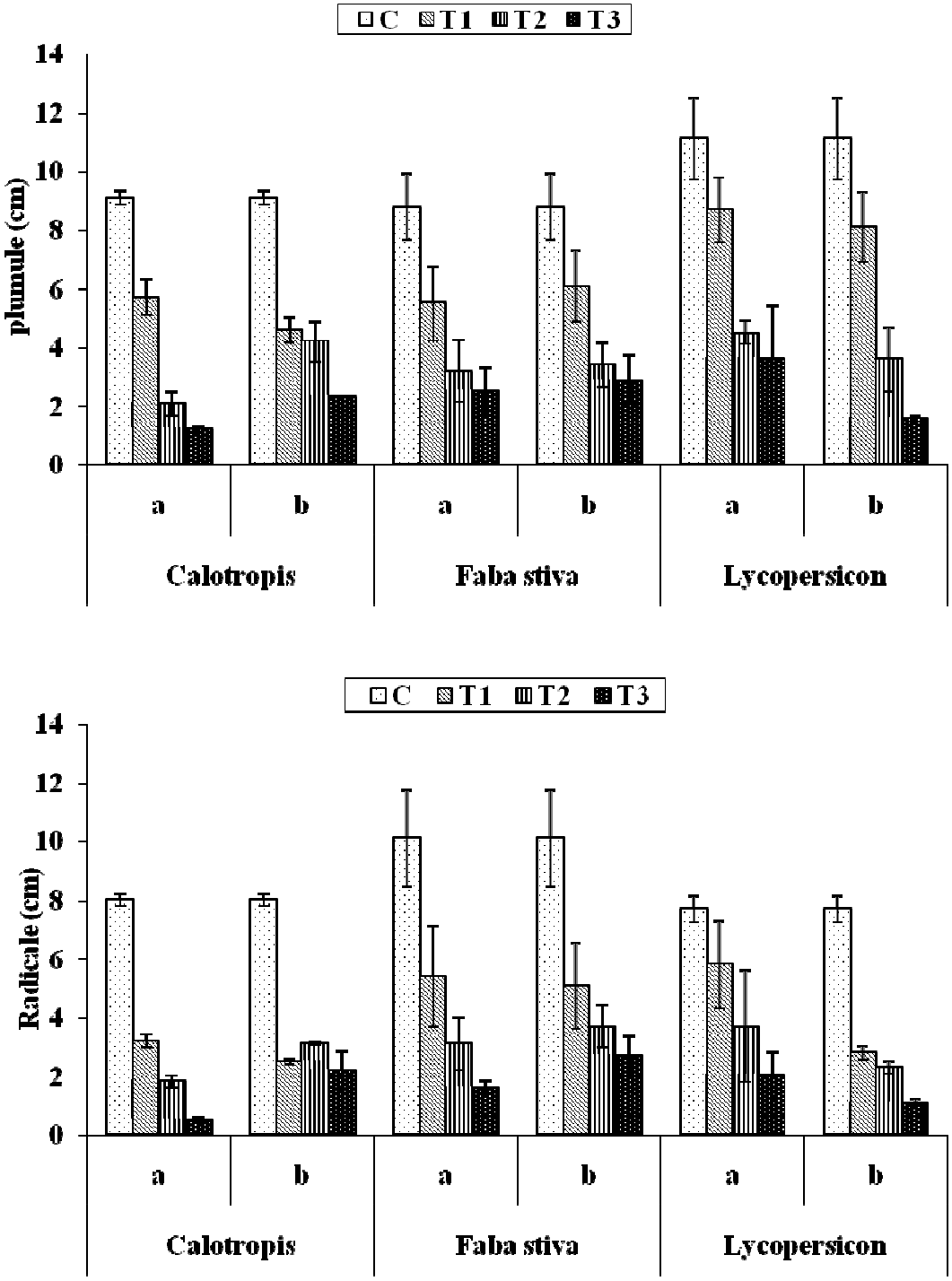

Figure 2. Mean and standard deviation of mean radicle and plumule lengths of *C.procera, F.sativa* and *L.esculentum*, growing under aqueous extract of (a) *H.curassavicum* and (b) *H.bacciferum*. Vertical bar around the mean is the standard deviation.

Dry matter allocation

Allocation of dry matter between the two plant organs of each seedling; plumule and radicle, for the three test species; *C. procera*, *F.sativa* and *L.esculentum*, is illustrated in Figures 4.

Considering *C.procera* seedlings treated by *H.curassavicum*, the allocation to plumule was higher

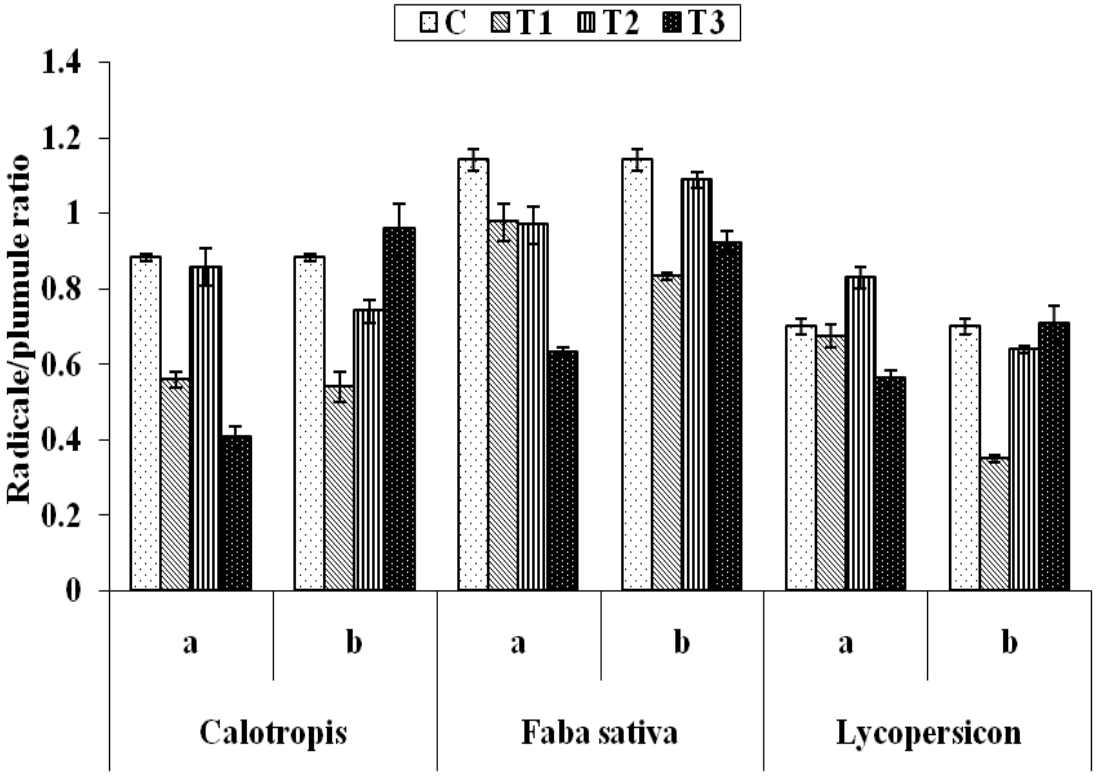

Figure 3. Mean and standard deviation of Radicle/plumule ratio of *C.procera*, *F.sativa* and *L.esculentum*, growing under aqueous extract of (a) *H.curassavicum* and (b) *H.bacciferum*. Vertical bar around the mean is the standard deviation

than that of radicle in all plant treatments. The dry matter allocation ranged between 80 - 88.31% in plumule, and between 11.69 - 20% in radicle. In addition, dry matter allocation for seedling treated with *H.bacciferum* extracts was ranged between 86.36- 92.11% for plumule and between 7.9- 13.64% for radicle. Dry matter allocation of *F.sativa* seedlings gave somewhat nearly equal ratio between plumule and radicle, even if plumule allocation still higher than that of radicle. For instance, allocation was 53.85 and 46.15% for plumule and radicle T_1 seedlings treated by *H.curassavicum*. The allocation towards either plumule or radicle was 50% in case of *F.sativa* seedlings treated by T_3 *H.bacciferum* (Figure 4). Dry matter allocations through the plumule and radicle in case of *L.esculentum*, sometimes follow the same trend as that of *C. procera* seedlings and other times as that of *F.sativa* seedlings. For example, allocation was 72.73 and 27.28% for plumule and radicle T_1 seedlings treated by *H. curassavicum*. On the other hand, allocation toward plumule is generally higher than that of plumule in all test plant treatments except for one case; *L.esculentum* control which recorded allocation amounting to 44.83% for plumule and 55.17% for radicle. It is to be mentioned here that there were significant differences in most cases between the dry matter allocation results of different treatments and controls.

Protein expression

Expression of proteins in treated plants of the present study was significantly increased or decreased at the level of number and intensity of protein bands as compared to control plants, depending on the type and concentration of extract treatment.

In *C. procera* (Figure 5 a), eight proteins were expressed in control plants while seven proteins were expressed in all treated seedlings except T_1 treated plants by *H. curassavicum* which express only six proteins amounting to the following molecular weights; 387.143, 190.770, 124.088, 60.716, 57.295 and 53.606 K Da (Table 2). In other words, treatment of *C.procera* seedlings by either *H. curassavicum* or *H. bacciferum* extracts caused decrease in the number of expressed proteins and this decrement reached its maximum in the former case of extract (*H. curassavicum*). The R.F. values for the different bands of each lane were given in Table 2. The values of R.F. generally ranged between 0.134 and 0.778 for control samples and ranged between 0.185 and 0.776 in treated plants.

Considering protein expression of *F. sativa*, five proteins were expressed in seedlings of control plants and the three treatments of both *H.curassavicum* and *H. bacciferum* (Figure 5 b and Table 3). Five proteins were

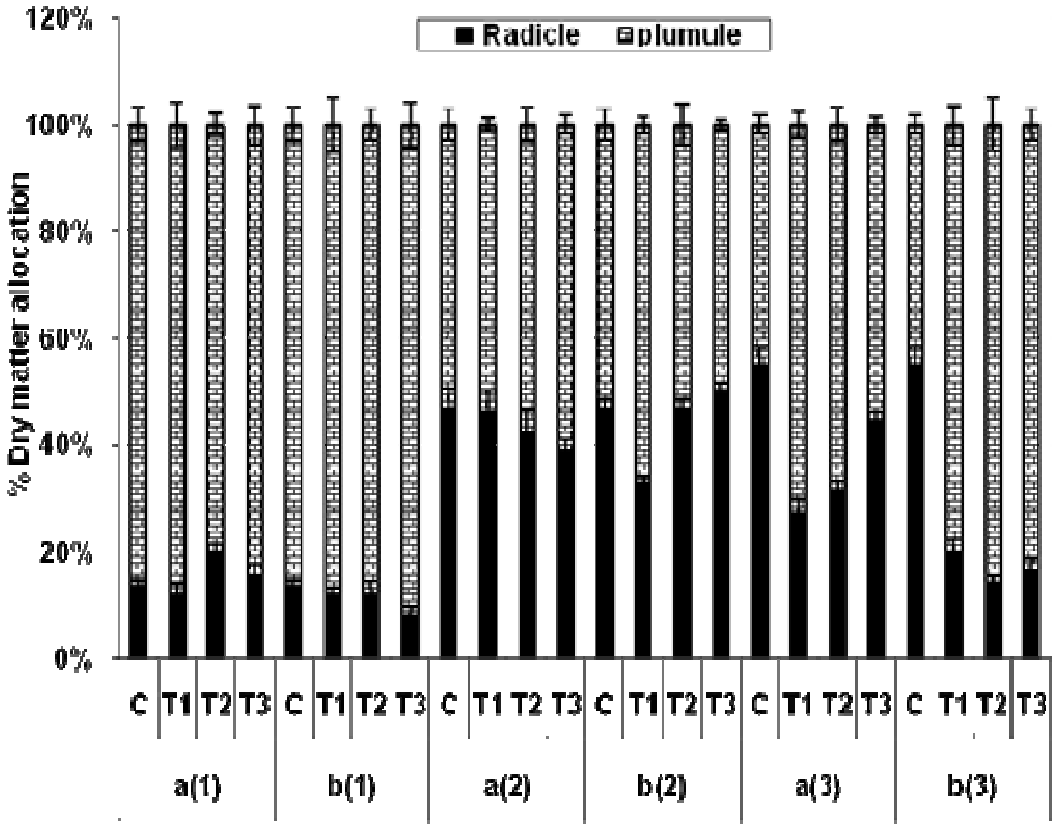

Figure 4. Dry matter allocation of (1)= *C.procera*, (2)= *F.sativa* and (3)= *L.esculentum*, subjected to the aqueous extract of (a)= *H.curassavicum* and (b)= *H.bacciferum*. Vertical bar around the mean is the standard deviation

expressed for control plants with the following molecular weights; 61.565, 56.699, 52.225, 33.697 and 31.802 KDa. In addition, the intensity of protein bands varied according to type of *Heliotropium* extract; for example, the molecular weights for T_1 treated seedlings with *H. curassavicum* were 61.171, 56.351, 52.515, 34.537 and 31.802 KDa and these weights slightly increased to 61.565, 56.351, 51.938, 35.626 and 32.710 K Da for seedlings of T_1 treated plants by *H. bacciferum*. The values of R.F. generally ranged between 0.379 and 0.698 for control samples and ranged between 0.379 and 0.690 in treated plants (Table 3).

Protein expression for *L. esculentum* seedlings was generally increased at the level of number and intensity of protein bands as extract concentration increase (Figure 5 c). Six protein bands were only expressed in control seedlings recording the following molecular weights; 67.603, 55, 50.868, 42.251, 38.701 and 31.310 K Da. The number of expressed proteins then increased to seven bands in all treatments of *H. bacciferum* and T_2 and T_3 of *H. bacciferum*. The values of R.F. generally ranged between 0.071 and 0.762 for control samples and ranged between 0.285 and 0.792 in treated plants (Table 4).

UPGMA- dendrogram

According to the UPGMA dendrogram (Figure 6 a), control plants of *C. procera* (CCO) is considered the farthest among the different treatments; the major cluster grouped high extract treatment of *H. curassavicum* (CRT3) and high mulch treatment of *H. bacciferum* (CbT3) as the closest treatments recording degree of similarity 95.38 %, with medium extract treatment of *H. curassavicum* (CRT2) in the same subgroup by a degree of similarity 76.63%, whereas the same dendrogram grouped high mulch treatment of *H. bacciferum* (CbT3) and low extract treatment of *H. bacciferum* (CbT1) separately as a closer group of 81.13 % degree of similarity.

For *F. sativa*, the dendrogram in (Figure 6 b) there were two major sub groups, the first consider control plants of *F. sativa* (FCO) as the farthest among the different treatments; the major cluster grouped seedlings treated with low (FRT1) and medium (FRT2) extract of *H. curassavicum* together with degree of similarity 75.6% with high (FCT3) extract concentration of *H. bacciferum*. The other major subgroup, consider seedlings of the medium (FbT2) and high (FbT3) extract concentrations of

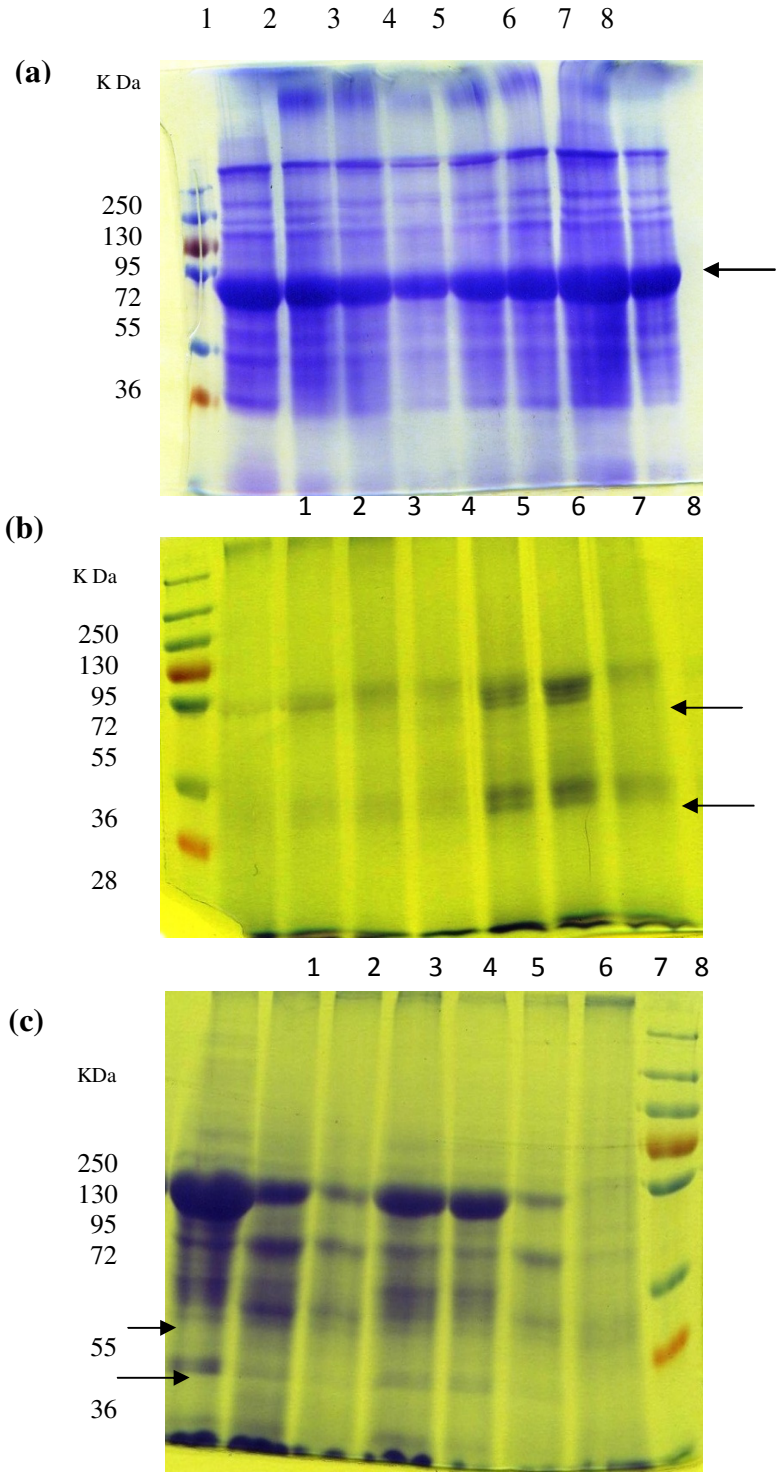

Figure 5. SDS-PAGE of cytoplasmic seedling proteins from *C. procera* seedlings **(a)** treated with T_1, T_2 and T_3 of *H. curassavicum* extracts (lanes 3-5) and treated with T_1, T_2 and T_3 of *H. bacciferum* (lanes 6-8), T_3 repeated sample (lane 9). Lane 2, corresponds to *C.procera* control, lane 1 correspond to marker, cytoplasmic proteins from *F. sativa* seedlings **(b)** treated with T_1, T_2 and T_3 of *H. curassavicum* extracts (lanes 3-5) and treated with T_1, T_2 and T_3 of *H. bacciferum* (lanes 6-8). Lane 2, corresponds to *F.sativa* control, lane 1 correspond to marker, cytoplasmic proteins from *L. esculentum* seedlings **(c)** treated with T_3, T_2 and T_1 of *H. curassavicum* extracts (lanes 4-6) and treated with T_3, T_2 and T_1 of *H. bacciferum* (lanes 1-3). Lane 7, corresponds to *L. esculentum* control, lane 8 correspond to marker. Molecular masses (KDa) are indicated.

Table 2. Molecular weight (kDa) and R.F. of different protein specimens extracted from *C.procera* seedlings, T_1= 0.006, T_2= 0.0012 and T_3= 0.06% (w/v)

				H.curassavicum			*H.bacciferum*		
Treatment →	M	Control	T1	T2	T3	T1	T2	T3	
M.W.(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	250.000	490.000	387.143	384.286	384.286	384.286	387.143	395.714	401.429
Band2	130.000	381.429	190.770	203.392	209.863	206.616	206.616	209.863	206.616
Band3	95.000	190.770	124.088	151.444	144.322	146.635	151.444	156.484	153.936
Band4	72.000	125.565	60.716	123.349	121.873	122.611	124.088	124.088	124.088
Band5	55.000	110.855	57.295	60.716	60.069	60.069	60.380	60.716	60.380
Band6	36.000	61.080	53.606	57.369	57.369	57.390	57.427	57.473	57.511
Band7		57.346		53.801	53.801	53.801	53.990	53.801	54.173
Band8		53.801							

				H.curassavicum			*H.bacciferum*		
Treatment →	M	control	T1	T2	T3	T1	T2	T3	
R.F.Values	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	0.264	0.134	0.190	0.191	0.191	0.191	0.190	0.185	0.182
Band2	0.330	0.193	0.292	0.286	0.283	0.285	0.285	0.283	0.285
Band3	0.404	0.292	0.342	0.314	0.319	0.317	0.314	0.311	0.313
Band4	0.460	0.339	0.515	0.344	0.347	0.345	0.342	0.342	0.342
Band5	0.659	0.370	0.614	0.515	0.521	0.521	0.518	0.515	0.518
Band6	0.762	0.512	0.672	0.610	0.610	0.608	0.605	0.600	0.596
Band7		0.611	0.782	0.670	0.670	0.670	0.669	0.670	0.667
Band8		0.670		0.782	0.781	0.779	0.778	0.776	0.776
Band9		0.778							

H. bacciferum as the closest treatments recording degree of similarity of 85.6 %. In addition, plants treated by low mulch concentration of *H. bacciferum* (FbT1) in one cluster group with a degree of similarity of 45.6%.

UPGMA dendrogram for *L. esculentum* (Figure 6 c) demonstrated the relationships among different expressed proteins of extract treatments and that of control plants. Low extract treated plants by *H. curassavicum* (LRT1) was found to be the farthest among the different treatments, while control seedlings (LCO) and the rest of treated plants were clustered in one group with a degree of similarity amounting to 65.57 %. The low treated (LbT1) seedlings and medium treated (LbT2) seedlings by *H. bacciferum*, form subgroup by a degree of similarity reached 77.8 %. Furthermore, treated plants by high extract of both *H.*curassavicum (LRT3) and H. bacciferum (LbT3) were grouped in a separate group of 92.68 % degree of similarity.

DISCUSSION

Seed germination is widely used parameter in allelopathic bioassays (Rice 1984). Allelochemicals reduced the germination and seedling growth of various weeds and crops as has been reported earlier (Hegazy et al., 2007; Singh et al., 2002; Mushtaq et al., 2010; Mubeen et al., 2011; Raoof and Siddiqui, 2012; Badmus and Afolayan, 2012). This is in accordance with the results of the present study which confirm that allelochemicals produced from the two invasive *Heliotropium* species causes a reduction in germination and seedling growth of all test species. Allelopathic activity of the two *Heliotropium* species may attribute to some alkaloids e.g. esters of the nonhepatotoxic saturated necine, trachelanthamidine (Birecka et al. 1983) and pyrolizidine alkaloid from *Heliotropium* roots, and to alkaloids related to acetyl indicine (Pestchanker et al. 1986) which are C-9 esters of retronecine and exihibit 1, 2-unsaturation and esterification at C-9 in the necine moiety. The structures of these alkaloids were found to be 9-(3 □-Isovaleryl) viridifloryl retronecine, 7-Acetyl-9-(3 □-isovaleryl) viridifloryl retronecine and 9-(3 □-Acetyl) viridifloryl retronecine. Curassanecine is a saturated amino alcohol pyrolizidine alkaloid isolated from *H. curassavicum* and its first total synthesis was achieved from N-acetylpyrolidine (Farrag, 2007). The root/shoot ratio of control plants of the present study test species were

Table 3. Molecular weight (kDa) and R.F. of different protein specimens extracted from *F.sativa* seedlings, T_1= 0.006, T_2= 0.0012 and T_3= 0.06% (w/v)

			H.curassavicum			***H.bacciferum***		
Treatment	M	control	T1	T2	T3	T1	T2	T3
M.W.(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
Band1	250.000	61.565	61.171	60.781	61.171	61.565	61.565	61.565
Band2	130.000	56.699	56.351	56.699	56.699	56.351	56.699	57.409
Band3	95.000	52.225	52.515	52.810	52.515	51.938	51.377	51.377
Band4	72.000	33.697	34.537	34.842	35.338	35.626	36.000	36.092
Band5	55.000	31.802	31.802	32.372	32.372	32.710	32.485	32.598
Band6	36.000							
Band7	28.000							
			H.curassavicum			***H.bacciferum***		
	M	control	T1	T2	T3	T1	T2	T3
R.F.Values	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
Band1	0.093	0.379	0.381	0.383	0.381	0.379	0.379	0.379
Band2	0.178	0.402	0.404	0.402	0.402	0.404	0.402	0.399
Band3	0.254	0.427	0.425	0.423	0.425	0.429	0.432	0.432
Band4	0.335	0.668	0.654	0.649	0.640	0.635	0.628	0.626
Band5	0.411	0.698	0.698	0.690	0.690	0.684	0.688	0.686
Band6	0.628							
Band7	0.755							

generally reduced under the effect of using different allelochemicals and this reduction reached its maximum in the high extract treatment (T_3) and that can be interpreted as explained by Nilsson (1994) who suggested that the decrease in root/shoot ratio as a response to nutrient deficiency appears to be applied for plants subjected to allelopathic interactions.

Dry matter allocation toward plumule in the current work is generally higher than that of radicle in all test plant treatments except for one case (*L.esculentum*). This is in according with many authors (Einhellig and Rasmussen 1993; Hejl et al. 1993; Inderjit and Dakshini 1995; El-khatib and Abd-Elaah 1998; Hegazy et al. 1999, 2001 and 2007, and Raoof and Siddiqui 2012) who have reported the inhibitory effects of allelochemicals on the chlorophyll content and net photosynthetic rate of the test species which intern affect the phytomass production opportunity of the test species.

The extract treatments of *H.curassavicum* and *H.bacciferum* differentially affected on the protein expression of the three test plants. The new proteins have been expressed in treated test plants as compared to controls. The expression of these new proteins could be explained on the basis that to neutralize the effect of allelochemicals produced by invasive plant powders on the treated test plants. This is in accord with Cruz-Ortega et al. (2002), who mentioned that plants appear to respond to allelochemical stress by increasing the expression of specific proteins. Moreover, some environmental stresses induce expression of proteins not specially related to a particular stress, but as a reaction to cell damage. These include some classes of heat shock proteins (Heikkila et al. 1984), thiol proteases (Williams et al. 1994), proteinase inhibitors (Reviron et al. 1992) osmotin (Cruz-Ortega et al. 1997), polyamine (Turano and Kramer 1993; Botella et al. 2000) and anti-oxidative enzymes (Freitas et al., 2007). In addition, the expression of reduced glutathione may also increases which plays a protective role by increasing stress tolerance, in particular that of allelochemicals (Hegazy et al. 2007, and Raoof and Siddiqui 2012). On the contrary, protein expression in the test plants of the present study may have reduced especially at high extract levels as compared to controls. This reduction might be a manifestation of cell damaged caused by allelochemical stress (Cruz-Ortega et al. 2002; Rehman, et al. 2005; Hegazy et al., 2007). In addition, the present work demonstrated that these allelochemicals significantly interfered with the protein expression of the test plants. This interference took place either by induction or repression of the protein expression. The induction or repression of protein expression could take place either on transcriptional or translational level. These allelochemicals could play an important role in inhibiting enzymes involved in these two processes. This is in accordance with findings of Baziramakeng et al. (1997) who pointed out that the methionine incorporation into proteins was reduced by allelochemicals, and findings of Romero et al. (2002) who recorded that the protein pattern of *L.esculentum* was severely inhibited by all allelopathic plants, and in accordance with findings of

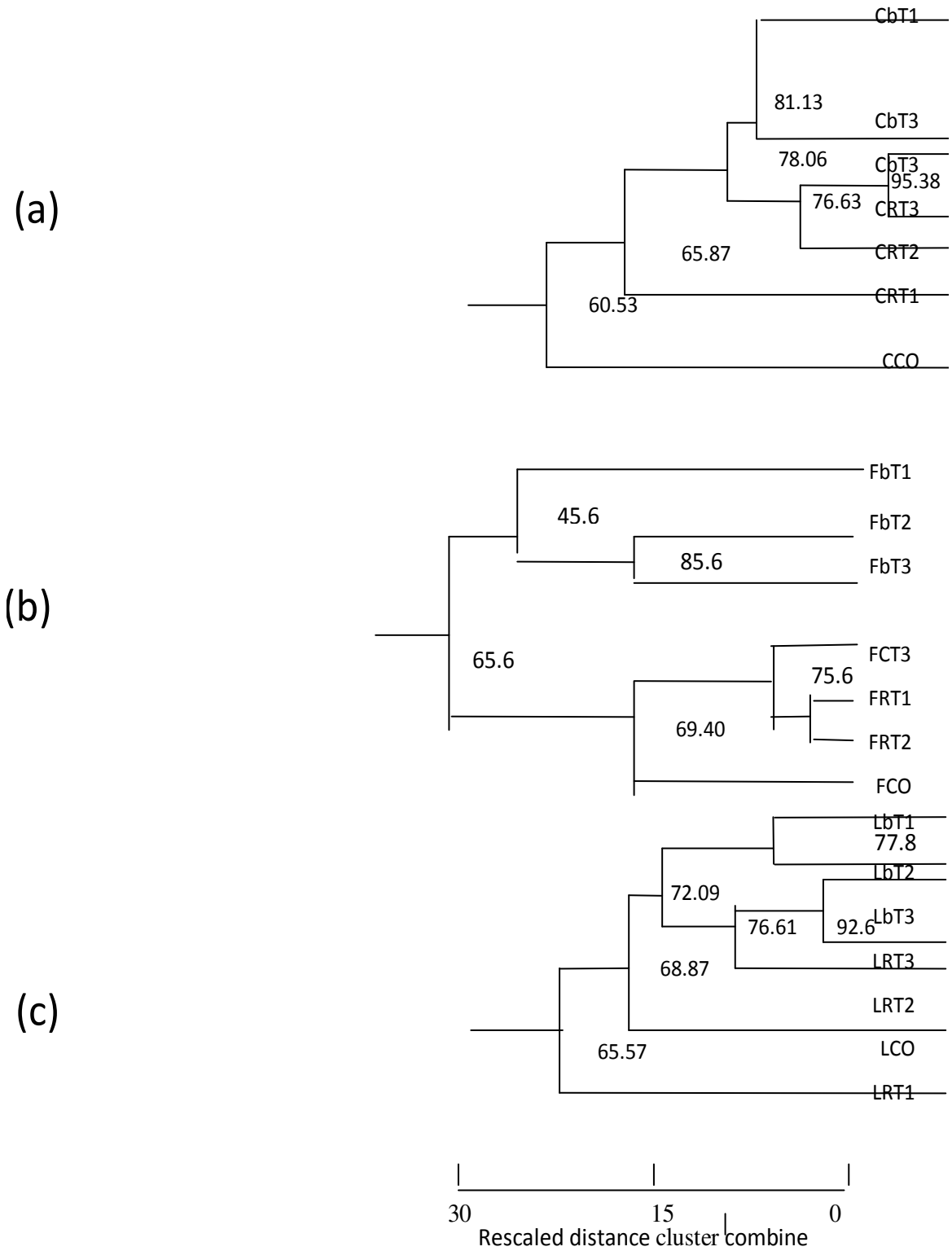

Figure 6. Dendrogram depicting the relationships (% similarities) among different test species treatments on the basis of the hierarchical cluster analysis performed by SPSS software using the unweighted pair-group arithmetic mean method (UPGMA). C = C. procera, F = F.*sativa* and L = L.*esculentum*, CO = control, R = H.*curassavicum*, b = H.

El-Khatib et al. (2004) who demonstrated that allelochemicals produced by *Chenopodium murale* decreased the protein contents of *L. esculentum* and other test plants. In accordance with findings of the present study, Hegazy et al., (2007) reported that *H.curassavicum* affect on protein expression of

Table 4. Molecular weight (kDa) and R.F. of different protein specimens extracted from *L.esculentum* seedlings, T_1= 0.006, T_2= 0.0012 and T_3= 0.06% (w/v)

				H.curassavicum		***H.bacciferum***		
Treatment	M	control	T1	T2	T3	T1	T2	T3
M.W.(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
Band1	250.000	67.603	71.264	72.513	71.632	71.632	71.264	74.654
Band2	130.000	55.000	56.762	57.066	57.997	58.312	58.312	59.274
Band3	95.000	50.868	47.294	47.483	47.675	48.879	49.089	49.956
Band4	72.000	42.251	38.701	39.318	39.228	39.050	39.501	39.876
Band5	55.000	38.701	34.896	34.896	35.069	35.069	35.069	35.155
Band6	36.000	31.310	30.926	31.119	31.023	31.119	31.215	31.787
Band7	28.000			26.419	26.605	26.512	26.689	26.698
				H.curassavicum		*H.bacciferum*		
	M	control	T1	T2	T3	T1	T2	T3
R.F.Values	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8
Band1	0.071	0.314	0.297	0.292	0.295	0.295	0.297	0.285
Band2	0.167	0.380	0.370	0.368	0.363	0.361	0.361	0.356
Band3	0.229	0.408	0.438	0.436	0.434	0.424	0.422	0.415
Band4	0.293	0.495	0.556	0.543	0.545	0.549	0.540	0.533
Band5	0.380	0.556	0.635	0.635	0.632	0.632	0.632	0.630
Band6	0.613	0.703	0.710	0.707	0.708	0.707	0.705	0.694
Band7	0.762			0.792	0.788	0.790	0.786	0.786

L.esculentum and other test species. Furthermore, it is notable that as extract treatments of the two invasive species increases, the intensity of some protein band decreases and others increases. In this regard, many authors (Einhellig and Rasmussen 1993; Hejl et al. 1993; El-khatib and Abd-Elaah 1998; Hegazy et al., 2007; Freitas et al., 2007; Raoof and Siddiqui 2012; Salama, 2012) have reported the inhibitory effects of allelochemicals on the chlorophyll content and net photosynthetic rate of the test species which subsequently affect the protein expression qualitatively and quantitatively.

UPGMA dendrogram of the present work considered expressed proteins of control plants as the farthest among the different treatments of most test plants and this ensure and illustrate the allelopathic stress of different invasive plants on the protein expression as compared to that of controls. This is in agreement with findings reported by Hegazy et al., 2007. The changes observed in protein expression may be due to a biochemical alteration at the cellular level of the tested plants.

CONCLUSION

In conclusion, the present study demonstrated that, allelochemicals produced by either *H. curassavicum* or *H. bacciferum* extracts was significantly caused inhibition in germination and seedling growth of all test species and interfered with the protein expression of the studied test plants. This interference took place either by induction or repression of the protein expression. The present study recommend the use of the two *Heliotropium* species for the biocontrol of harmful weeds like *C. procera* and in the same time alert for the inhibitory effect of these species on the growth of economic plants like *F. sativa*.

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