Global Journal of Molecular Sciences 2 (1): 01-07, 2007
ISSN 1990-9241

Protein Expressions of Some Cultivated and Weed Plants in Response to Invasive Plant Mulching

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Abstract: Allelochemical stress produced by three invasive species, *viz*., *Heliotropium curassavicum, Bassia indica* and *Chenopodium ambrosioides* was investigated on the protein expression of two crop plants; *Lycopersicon esculentum* and *Beta vulgaris* and two weeds; *Melilotus indicus* and *Sonchus oleraceus* in greenhouse mulching experiment. The number of expressed proteins was generally increased in mulching test plants while, in rare cases, this number decreased at high mulch levels. The study demonstrated the negative effect of different mulch treatments on the intensity of protein bands in the test plants. 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 most test plants.

Key words: Allelopathic stress · protein expressions · SDS-PAGE · UPGMA dendrogram

INTRODUCTION

Plants produce diverse secondary allelochemical metabolites that are released into the environment. Some of the allelochemicals have a biological activity on other plants and microorganisms. Like many other stress factors, allelochemicals have several molecular targets and some of their physiological processes or modes of action were described [1, 2]. Allelochemical compounds are known to affect many different cellular processes in target organisms, including disruption of membrane permeability [3], ion uptake [4], inhibition of electron transport in both photosynthesis and respiratory chain [5-7], alternation of some enzymatic activities [8-10], inhibition of cell division [11-13] and protein expression [14-16]. A plant invader is a species which, most usually transported inadvertently or intentionally by man, colonizes and spreads into new territories some distance from its home territory [17]. Many studies were carried out to show the negative effect of invasive plants on the germination and growth of native plants [18-22]. The present study aims to investigate the possible allelopathic effects of the amended soil by the three invasive plants; *Heliotropium curassavicum*, *Bassia indica* and *Chenopodium ambrosioides* on the protein expression of two cultivated plants; *Lycopersicon esculentum* and *Beta vulgaris* and two weeds; *Melilotus indicus* and *Sonchus oleraceus*.

MATERIALS AND METHODS

Plant material of *Heliotropium curassavicum* L., *Bassia indica* (Wight) A.J.Scott. and *Chenopodium ambrosioides* L. were collected in the flowering stage from Ziaan county, Gamasa, Egypt. Plant shoots were air-dried and grounded into powder. In an open greenhouse experiment, plastic pots (18 cm diameter and 25 cm depth) were used. Soil samples brought from the field study site, air-dried and passed through 2-mm sieve to separate litter and gravel. The air-dried sieved soil was filled into pots (8000 gram soil / pot). Seeds of target species; *Lycopersicon esculentum* and *Beta vulgaris* were obtained from Agricultural Research Center in Giza, Egypt, while seeds of *Sonchus oleraceus* and *Melilotus indicus* were collected from naturally growing populations in the study area. Twenty-five seeds were sown in every pot at depth of 1cm. Ground powder of each invasive plant with three application rates; 0 (control), 10 (low concentration) or 50 (high concentration) gram powder per pot, were evenly mulched on the soil surface. Five replicates per treatment were used. The prevailing climatic condition during the experimental period includes temperature which ranged between a minimum of 18.8°C in January to a maximum value of 34.7°C in July. Relative humidity ranged between minimum of 45% in May to a maximum value of 61% in December.

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Plant samples were taken for the purpose of protein analysis in the flowering growth stage. The advantage of using plant material for the protein analysis purposes at the flowering stage is to subject the plants to maximum period of allelochemicals and the plants are subjected to diversity of factors that prevail in the case of mature plants [23]. Plant material was washed by distilled water and kept at -70°C until use. Cytoplasmic proteins were extracted and purified from the test species for SDS-PAGE analysis based on [24]. Green leaves of each treated and control plants were frozen in a liquid nitrogen and grind for about 30 second in a mortar with 3 ml 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°C [25]. The analysis was carried out in Agriculture Genetic Engineering Research Institute (AGERI) and in Genetic Engineering lab, Chemistry department, Faculty of Science, Cairo University. The gels were run in a mini-protein gel (Bio-Rad).

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).

RESULTS

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 mulch treatment.

In *Lycopersicon esculentum* (Fig. 1a), four proteins were expressed in control plants as well as on using both low and high mulch treatments of *H. curassavicum*, this number increased to a maximum of six proteins on using low mulch treatment of *B. indica* while five proteins were expressed on using high and low mulch treatments of *B. indica* and *C. ambrosioides*, respectively. The number of expressed proteins recorded its minimum; three proteins, on using high mulch treatment of *C. ambrosioides* (Table 1).

The effect of mulch treatment on the intensity of protein band was significant. The presence and increased mulch treatment has reduced the intensity of expressed proteins in treated plants as compared to control. The protein band of molecular weight 52 kDa recorded maximum value of 39.1 % in control plants as compared to all mulch treatments which recorded relatively lower values reached its minimum (25.1 %) on using high mulch treatment of *C. ambrosioides* (Table 1).

According to the UPGMA dendrogram (Fig. 2a), control plants of *Lycopersicon esculentum* (LO1) is considered the farthest among the different treatments; the major cluster grouped low mulch treatment of *H. curassavicum* (LHW3) and low mulch treatment of *B. indica* (LAW4) as the closest treatments recording degree of similarity 92.07%, with high mulch treatment of *H. curassavicum* (LHG2) in the same subgroup by a degree of similarity 85.36%, whereas the same dendrogram grouped high mulch treatment of *B. indica* (LAG5) and high mulch treatment of *C. ambrosioides* (LCG6) separately as a closer group of 76.07 % degree of similarity.

For *Melilotus indicus* (Fig.1b), the presented data in Table 1, gave similar results to that of the above mentioned cultivated plants, considering the expression of new proteins in plants cultivated under low mulch treatment and less expressed proteins in plants cultivated under high mulch treatments as compared to that of control. In addition, intensity of the protein bands of treated plants generally decreased as compared to control plants but unlike to this trend the high concentration of mulch treatment may increase the protein intensity as compared to low mulch concentration, that is true in case of plants treated by mulch treatments of either *B. indica* or *C. ambrosioides* where the intensity increased from 28.8 to 45.3% and from 24.1 to 28.3%; respectively, for the protein of 52 kDa molecular weight.

The dendrogram in (Fig. 2b) consider control plants of *Melilotus indicus* (MO7) as the farthest among the different treatments; the major cluster grouped plants treated with high mulch treatment of *C. ambrosioides* (MCG2) and that of high mulch treatment of *B. indica* (MAG4) as the closest treatments recording degree of similarity of 96.76%. In addition, plants treated by low mulch concentration of *H. curassavicum* (MHW5) and of *C. ambrosioides* (MCW1) in one cluster group with a degree of similarity of 96.07%.

In *Beta vulgaris* (Fig. 1c), six proteins were expressed in control plants having molecular weights of 258, 229, 200, 52, 26 and 9 kDa and band intensity of 2.5, 2.7, 4.4, 51.5, 30.3 and 16.7%, respectively (Table 1). Only one newly expressed protein was recorded in treated plants by low mulch treatment of *H. curassavicum*, while the

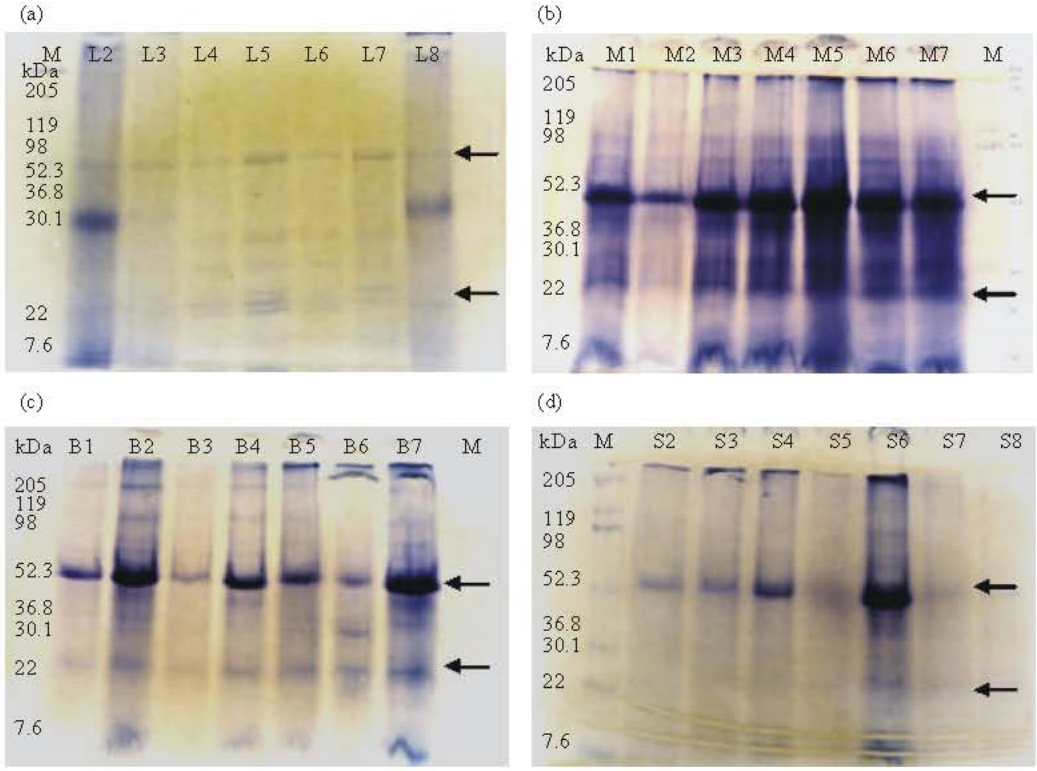

Fig. 1: SDS-PAGE of cytoplasmic shoot proteins from *Lycopersicon esculentum* (a), Lane 1 (M) correspond marker, lane 2 correspond control, lanes 3 and 4 correspond low and high treatment of *Heliotropium curassavicum* powder, lanes 5 and 6 correspond low and high treatment of *Bassia indica* powder and lanes 7 and 8 correspond low and high treatment of *Chenopodium ambrosioides* powder, cytoplasmic shoot proteins from *Melilotus indicus* (b), Lanes 1 and 2 correspond high and low treatment of *Chenopodium ambrosioides* powder, lanes 3 and 4 correspond high and low treatment of *Bassia indica* powder, lanes 5 and 6 correspond high and low treatment of *Heliotropium curassavicum* powder, lane 7 correspond control and lane 8 (M) correspond marker, cytoplasmic shoot proteins from *Beta vulgaris* (c), Lanes 1 and 2 correspond high and low treatment of *Chenopodium ambrosioides* powder, lanes 3 and 4 correspond high and low treatment of *Bassia indica* powder, lanes 5 and 6 correspond high and low treatment of *Heliotropium curassavicum* powder, lane 7 correspond control and lane 8 (M) correspond marker and cytoplasmic shoot proteins from *Sonchus oleraceus* (d), Lane 1 (M) correspond marker, lanes 2 and 3 correspond low and high treatment of *Heliotropium curassavicum* powder, lanes 4 and 5 correspond low and high treatment of *Bassia indica* powder, lane 6 correspond control and lanes 7 and 8 correspond low and high treatment of *Chenopodium ambrosioides* powder. Molecular masses (kDa) are indicated

expressed proteins reduced in treated plants by other mulch treatments especially those of high mulch concentration. Intensity of protein bands of the treated plants is greatly reduced as compared to control plants. The protein of molecular weight 26 kDa has intensity of 30.3% in control plants and this value significantly reduced in case of all treated plants recording minimum value of 9.5% in plants cultivated under high mulch treatment of *B. indica*. In addition, the more the concentration of mulch treatment the less the intensity of protein bands and the less number of expressed proteins. The relationships among different expressed proteins and mulch treatments and that of control plants illustrated in (Fig. 2c). Low mulch treated plants by *H. curassavicum* (BHW6) was found to be the farthest among the different treatments, while plants cultivated under low mulch treatments of both *C. ambrosioides* (BCW2) and *B. indica* (BAW4) were clustered in one group with a degree of similarity amounting to 94.38 % with control plants (BO7) in the same subgroup by a degree of similarity reached 86.61 %. Furthermore, treated plants by high mulch of both *C. ambrosioides* (BCG1) and *H. curassavicum* (BHG5) were grouped in a separate group of 82.09 % degree of similarity.

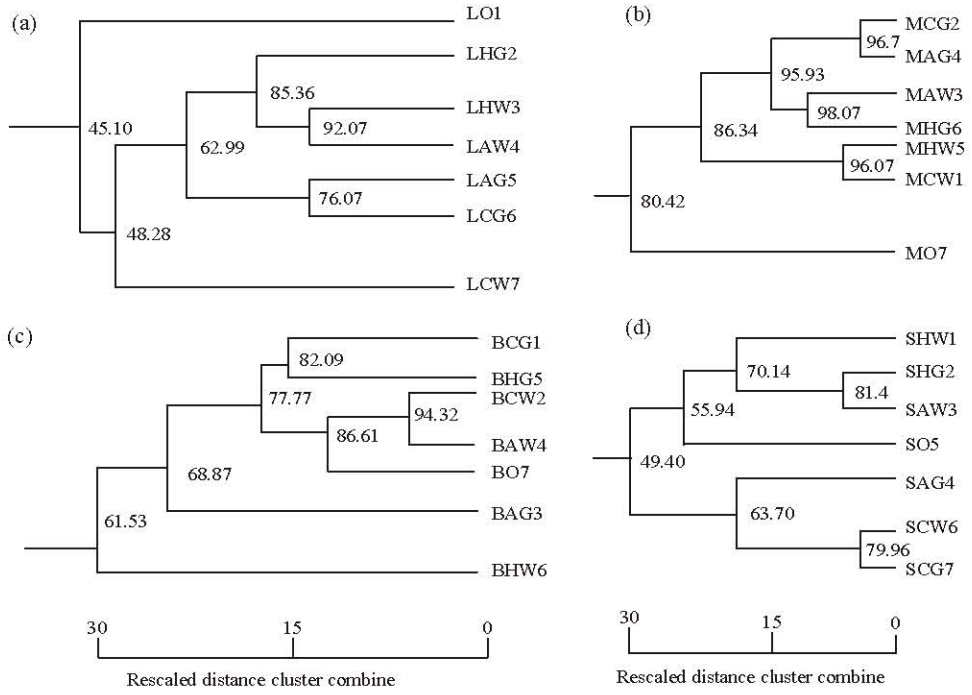

Fig. 2: Dendrogram depicting the relationships (% similarities) among different test species treatments on the basis of the hierarchical cluster analysis performed by SPSS software using the unweighted pair-group arithmetic mean method (UPGMA). L = *Lycopersicon esculentum*, M = *Melilotus indicus*, B = *Beta vulgaris*, S = *Sonchus oleraceus*, O = control, H = *Heliotropium curassavicum*, A = *Bassia indica*, C = *Chenopodium ambrosioides*, W = low mulch treatment (10 gram per pot) and G = high mulch treatment (50 gram per pot). Lane number indicated at the right of each dendrogram

Table 1: Molecular weight (kDa) and % amount (Gel documentation) of different protein specimens extracted from different test plants

	Treatment													
	Control		HW		HG		AW		AG		CW		CG	
Test plant	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%
LYES	249	24.8	101	52.3	204	32.2	127	25.3	214	18.6	244	13.9	105	51.2
	52	39.1	52	34.0	52	25.7	52	35.0	105	41.7	82	48.8	52	25.1
	26	34.5	26	10.2	26	13.3	45	11.0	52	26.4	52	36.1	9	3.7
	8	1.6	8	3.5	9	42.2	35	15.7	26	4.8	26	8.0		
							26	6.4	8	10.0	9	1.2		
							18	10.0						
BEVU	258	2.5	251	2.8	243	26.3	234	6.1	185	14.7	225	9.1	231	7.8
	229	2.7	232	3.9	52	40.7	105	18.6	52	42.2	165	7.8	159	17.3
	200	4.4	198	2.5	26	12.5	52	49.7	26	9.5	99	9.2	52	39.4
	52	51.5	52	44.9	9	20.3	26	10.9	6	36.1	52	50.7	26	11.4
	26	30.3	41	13.5			7	21.6			26	15.0	6	35.5
	9	16.7	26	22.1							8	18.3		
			7	2.4										

Table 1: Continued

	Treatment													
	Control		HW		HG		AW		AG		CW		CG	
Test plant	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%	kDa	%
MEIN	226	3.7	226	8.7	226	4.2	216	4.1	226	8.8	224	7.4	222	6.4
	85	18.2	52	32.7	84	18.4	146	5.5	52	45.3	94	14.1	94	12.5
	52	46.4	26	22.7	52	30.9	78	15.6	26	25.3	78	8.6	68	7.4
	36	14.5	8	22.3	26	30.5	52	28.8	8	20.6	52	24.1	52	28.3
	26	12.4	7	17.2	4	14.3	26	22.5			26	23.1	26	24.4
			5	3.1			18	2.7			8	21.4	9	22.4
							8	20.7						
SOOL	233	6.6	233	4.4	233	2.5	233	3.4	52	54.8	211	16.2	205	25.5
	100	17.1	181	6.4	52	41.2	156	18.9	14	32.7	85	12.1	56	17.9
	52	74.2	52	47	16	9.5	52	66.4	13	2.6	52	34.9	52	15.3
	26	15.4	18	3.2	15	3.4	26	13.6	11	3.4	26	14.9	26	8.0
	14	3.8	17	4.6	12	4.1	15	9.7	9	6.7	14	15.2	15	12.5
	11	5.8	13	4.8	10	6.4	13	2.1			13	3.4	13	2.4
	9	4.9	11	2.9			11	4.1			11	3.1	11	2.6
			7	7.3			9	7			9	7.2	10	8.4

LYES = *Lycopersicon esculentum,* BEVU = *Beta vulgaris,* MEIN = *Melilotus indicus* and SOOL = *Sonchus oleraceus* plants. H = *Heliotropium curassavicum*, A = *Bassia indica*, C = *Chenopodium ambrosioides*, W = low mulch treatment (10 gram per pot) and G = high mulch treatment (50 gram per pot)

Considering *Sonchus oleraceus* (Fig. 1d), the plant obey the general trend of other test plants, the application of low mulch treatments induce expression of new proteins while application of high mulch treatment may leads to suppression of most of these proteins as compared to that of control plants. Furthermore, intensity of protein bands of treated plants significantly decreased as compared to that of control plants.

According to the dendrogram in (Fig. 2d), the degree of similarity between plants treated with low mulch treatment of *C. ambrosioides* (SCW6) and that treated with high mulch treatment of the same invasive plant was found to be 79.96% and clustered in one group with plants treated with high mulch treatment of *B. indica* (SAG4) in the same subgroup by a degree of similarity amounting to 63.7% while control plants (SO5) clustered in another group.

DISCUSSION

The mulch treatments of *H. curassavicum*, *B. indica* or *C. ambrosioides* differentially affected the protein expression of the 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 [14], 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 [26], thiol proteases [27], proteinase inhibitors [28], osmotin [29] and polyamine [30, 31]. In addition, the expression of reduced glutathione may also increases which plays a protective role by increasing stress tolerance, in particular that of allelochemicals [32]. On the contrary, protein expression in the test plants of the present study may have reduced especially at high mulch levels as compared to controls. This reduction might be a manifestation of cell damaged caused by allelochemical stress [14, 16].

The current study illustrates the effect of different mulch treatments on the protein expression of test plants as compared to that of controls. 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 [33] who pointed out that the methionine incorporation into proteins was reduced by allelochemicals and findings of [34] who recorded that the protein pattern of *L. esculentum* was severely inhibited by all allelopathic plants and finally in accordance with findings of [35] who demonstrated that allelochemicals produced by *Chenopodium murale* decreased the protein contents of *L. esculentum*, *M. indicus* and other test plants. Furthermore, it is notable that as mulch treatment increases the intensity of protein band decreases. In this regard, many authors [17, 35- 40] 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. The changes observed in protein expression may be due to a biochemical alteration at the cellular level of the tested cultivated and weed plants.

In conclusion, the present study demonstrated that, allelochemicals produced by the studied invasive plant powder was significantly interfered with the protein expression of the studied 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. Furthermore, the changes observed in protein expression may be due to certain biochemical alteration at the cellular level of the tested cultivated and weed plants.

REFERENCES

1. Einhellig, F.A., 1995. Mechanism of action of allelochemicals in allelopathy. Allelopathy, 582: 96-116.
2. Reigosa, M.J., A. Sánchez-Moreiras and L. González, 1999. Ecophysiological approach in allelopathy. Plant Sci., 18: 577-608.
3. Galindo, J.C.G., A. Hernández, F.E. Dayan, M.R. Téllez, F.A. Macias, R.N. Paul and S.O. Duke, 1999. Dehydrozaluzanin C, a natural sesquiterpenolide, causes rapid plasma membrane leakage. Phytochemistry, 52: 805- 813.
4. Lehman, M.E. and U. Blum, 1999. Evaluation of ferulic acid uptake as a measurement of allelochemical dose: Effective concentration. J. Chem. Ecol., 25: 2585- 2600.
5. Calera, M.R., R. Mata, A.L. Anaya and B. Lotina-Hennsen, 1995. 5-O-B-D- galactopyranosy 1-7-methoxy-`3`4 4 dihydroxi-4-fenilcumarin, an inhibitor of photophosphorylation in spinach chloroplasts. Photosynth. Res., 45: 105- 110.
6. Penñuelas, J., M. Ribas-Carbo and L. Giles, 1996. Effects of allelochemicals on plant respiration and oxygen isotope fractionation by the alternative oxidase. J. Chem. Ecol., 22: 801- 805.
7. Abrahim, D., W.L. Braguini, A.M. Kelmer-Bracht and E.L. Ishii-Iwamoto, 2000. Effects of four monoterpenes on germination, primary root growth and mitochondrial respiration of maize. J. Chem. Ecol., 26: 611- 624.
8. Cruz-Ortega, R., A.L. Anaya, M. Gavilanes-Ruiz, S. Sánchez Nieto and M. Jiménez-Estrada, 1990. Effect of diacetyl piquerol on the H^+-ATPase activity of microsomes from Ipomoea purpurea. J. Chem. Ecol., 16: 2253- 2261.
9. Silva, M.G., R.A. Costa, M.L.L. Ferrarese and O. Ferrarese-Filho, 1996. Effects of phenolics compounds on soybean urease activity. Arq. Biol. Tecnol., 39: 677- 683.
10. Politycka, B., 1998. Phenolics and activities of phenylalanine ammonia-lyase, phenol-β-glucosyltransferase and β-glucosidase in cucumber roots as affected by phenolic allelochemicals. Act. Physiol. Plant., 20: 405-410.
11. Cruz-Ortega, R., A.L. Anaya and L. Ramos, 1988. Effects of allelopathic compounds of corn pollen on respiration and cell division of watermelon. J. Chem. Ecol., 14: 71-86.
12. Hegazy, A.K., K.S. Mansour and N.F. Abdel-Hady, 1990. Allelopathic and autotoxic effects of *Anastatica hierochuntica* L., J. Chem. Ecol., 16: 2183-2193.
13. Anaya, A.L., 1999. Allelopathy as a tool in the management of biotic resources in agroecosystems. Crit Rev. Pl. Sci., 18: 697- 739.
14. Cruz-Ortega, R., G. Ayala-Cordero and A.L. Anaya, 2002. Allelochemical stress produced by the aqueous leachate of *Callicarpa acuminate*: effects on roots of bean, maize and tomato. Physiol. Plant., 116: 20- 27.
15. Baerson, S.R., A. Sanchez-Moreiras, N. Pedrol-Bonjoch, M. Schulz, I.A. Kagan, A.K. Agarwal, M.J. Reigosa and S.O. Duke, 2005. Detoxification and transcriptome response in Arabidopsis seedlings exposed to the allelochemical benzoxazolin-2(3H)-one. J. biol. Chem., 280: 21867- 21881.

16. Rehman, A., F. Asmat, S. Ali, B. Saleem, M.J. Oureshi, Z. Ata and M. Rahman, 2005. Phenology and quality parameters of rice as affected by sorghum allelochemicals. Ind. J. Biol. Sci., 2: 520- 524.
17. Hegazy, A.K., M. Diekmann and G. Ayad, 1999. Impact of plant invasions on ecosystems and native gene pools. In: Hegazy, A.K. (Ed.). Environment 2000 and Beyond. Horus for computer and printing, Cairo, pp: 275-310.
18. Fox, M.D. and B.J. Fox, 1986. The susceptibility of natural communities to invasions. In: Groves, R.H. and Burdon, J.J. (eds.), *Ecol. Biol.invas.*: an Australian perspective. Australian Academy of Science, Canberra, pp: 57-66.
19. Rejmanek, M., 1989. Invasibility of plant communities. In: J.A. Drake, H.A. Mooney, F. di Castri, R.H. Groves, F.J. Kruger and M. Williamson, (eds.), Biological invasions: a global perspective (Scope 37). Wiley, Chichester, pp: 369-388.
20. Woods, K.D., 1993. Effects of invasion by Lonicera tatarica L. on herbs and tree seedlings in four New England forests. Amer. Mid. Nat., 130: 62-74.
21. Pysek, P. and A. Pysek, 1995. Invasion by *Heracleum mantegazzianum* in different habitats in the Czech Republic. J. Veg. Sci., 6: 711- 718.
22. Tilman, D., 1997. Community invasibility, recruitment limitation and grassland biodiversity. Ecol., 78: 81- 92.
23. de-Vogel, E.F., 1980. *Seedling of dicotyledons*. Wageningen: Centre for Agricultural Publishing and Documentation (PUDOC).
24. Nelson, T., M.H. Harpster, S.P. Mayfield and W.C. Taylor, 1984. Light regulated gene-expression during maize leaf development. J. Cell. Biol., 98: 558-564.
25. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat., 227: 680- 685.
26. Heikkila, J.J., J.E.T. Papp, G.A. Schultz, J.D. Bewley, 1984. Induction of heat shock protein messenger RNA in maize mesocotyls by water stress, abscisic acid and wounding. Plant Physiology, 76: 270- 274.
27. Williams, J., M. Bulman, A. Huttly, A. Phillips and S. Neill, 1994. Characterization of a cDNA from *Arabidopsis thaliana* encoding a potential thiol protease whose expression is induced independently by wilting and abscisic acid. Plant Molec. Biol., 25: 259- 270.
28. Reviron, M., P.N. Bartanian, M. Sallantin, J.C. Huet, J.C. Pernollet and D. de Vienne, 1992. Characterization of a novel protein induced by rapid or progressive drought and salinity in *Brassica napus* leaves. Plant Physiology, 100: 1486- 1493.
29. Cruz-Ortega, R., J.C. Cushman and J.D. Ownby, 1997. cDNA clones encoding 1,3-β-glucanase and fimbrin-like cytoskeletal protein are induced by aluminum toxicity in wheat roots. Plant Physiology, 114: 1453- 1460.
30. Turano, F.J. and G.F. Kramer, 1993. Effect of metabolic intermediates on the accumulation of polyamines in detached soybean leaves. Phytochemistry, 34: 959- 968.
31. Botella, M.A., F. del Amor, A. Amoros, M. Serrano, V. Martinez and A. Cerda, 2000. Polyamine, ethylene and other physico-chemical parameters in tomato (*Lycopersicon esculentum*) fruits as affected by salinity. Physiol. Plant., 109: 428- 434.
32. De Kok, L.J., F.A. Oosterhuis, 1983. Effects of frost-hardening and salinity on glutathione and sulfhydryl levels and on glutathione reductase activity in spinach leaves. Physiol. Plant., 58: 47- 51.
33. Baziramakenga, R., G.D. Leroux, R.R. Simard and P. Nadeau, 1997. Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. Canad. J. Bot., 75: 445- 450.
34. Romero, R.T., A.L. Anaya and C.R. Ortega, 2002. Screening for effects of phytochemical variability on cytoplasmic protein synthesis pattern of crop plants. J. Chem. Ecol., 28: 617- 629.
35. El-Khatib, A.A. and G.A. Abd-Elaah, 1998. Allelopathic potential of *Zilla spinosa* on growth of associate flowering plants and some rhizosphere fungi. Biol. Plant., 41: 461-467.
36. Einhellig, F.A. and J.A. Rasmussen, 1993. Effect of root exudates sorgoleone on photosynthesis. J. Chem. Ecol., 19: 369-375.
37. Hejl, A.M., F.A. Einhellig and J.A. Rasmussen, 1993. Effect of juglone on growth, photosynthesis and respiration. J. Chem. Ecol., 19: 559- 568.
38. Inderjit and Dakshini, K.M.M., 1995. On laboratory bioassay in allelopathy. Bot. Rev., 61: 28-44.
39. El-Khatib, A.A., A.K. Hegazy and H.K. Galal, 2004. Does allelopathy have a role in the ecology of *Chenopodium murale*?. Ann. Bot. Fenn., 41: 37- 45.
40. Hegazy, A.K., W.M. Amer and A.A. Kheder, 2001. Allelopathic effect of *Nymphaea lotus* L., on growth and yield of cultivated rice around Lake Manzala (Nile Delta). Hydrobiolgia, 464: 133-142.