Ann. Bot. Fennici 41: 37–45 ISSN 0003-3847
Helsinki 27 February 2004

Does allelopathy have a role in the ecology of *Chenopodium murale*?

Ahmed A. El-Khatib[1], Ahmed K. Hegazy[2] & Hanaa K. Galal[1]

[1)] *Department of Botany, Faculty of Science, 82524 Sohag, Egypt*
[2)] *Department of Botany, Faculty of Science, Cairo University, Egypt*

Received 10 Jan. 2003, revised version received 3 June 2003, accepted 12 June 2003

El-Khatib, A. A., Hegazy, A. K. & Galal, H. K. 2004: Does allelopathy have a role in the ecology of *Chenopodium murale*? — *Ann. Bot. Fennici* 41: 37–45.

Bioassays on *Chenopodium murale* demonstrated that root and shoot aqueous extracts reduced the seed germination, seedling establishment, plant growth and metabolite production of four target species. Leaf area and dry matter production showed a decreasing trend in response to the different treatments. Similar effects were found for pigment, carbohydrate and protein contents. In general, inhibition percentage was a function of extract concentration and plant tissue type. Shoot treatment was more strongly inhibitory than root treatment. The target species arranged from the most affected to the least affected were *Melilotus indicus–Trifolium alexandrinum–Triticum pyramidal–Lycopersicon esculentum–Cucumis sativus*.

Key words*:* allelopathy, *Chenopodium murale*, ecology, inter-specific competition, phytotoxicity

Introduction

The importance of allelopathy in nature has attracted ecologists' attention with the main goal of using the phenomenon in interpretation in many disciplines related to plant community structure (cf. Einhellig 1995, El-Khatib 1998, 2000, Hegazy 1999). Allelopathy, or the chemical mechanism of plant interference, is characterized by a reduction in emergence or growth of some target species in the community.

Chenopodium murale (nettleleaf goosefoot) is one of the fast-growing annuals of the family Chenopodiaceae and is widespread throughout different habitat types in Egypt (Kosinova 1975, Shaltout *et al*. 1992). It was introduced from Europe and grows in moist soil. It is an abundant winter weed and is considered a pest in agro-ecosystems, roadsides etc. Galal (2000) studied the plant sociological characteristics of a *C. murale* and reported its negative association with many species, even with those with similar ecological requirements. Also, this study showed that the rhizosphere soil of *C. murale* inhibited growth of the species they tested. It can therefore be hypothesized that *C. murale* may have allelopathic activity, which might play a part in its wide geographic distribution.

The present investigation focused on using aqueous extract to study the hypothesis that water-soluble materials of *C. murale* can be released from the plant tissue and exert allelopathic effects on the neighboring species. This information will assist our understanding of structuring mechanisms in its ecology.

Materials and methods

The community of *Chenopodium murale* was surveyed from a range of environmental conditions at Sohag, Egypt, where the climate is extremely arid (El-Khatib 1997). Samples were taken from different habitat types including agro-ecosystems, roadsides and waste places.

Sampling

Sufficient amounts of above- and below-ground organs of adult *C. murale* individuals were collected to allow analysis. The samples were brought to the laboratory in paper bags and allowed to air dry, and the material was then refrigerated until used. Ripe seeds of an associate weed species (*Melilotus indicus*), crop species (*Trifolium alexandrinum*), cereal species (*Triticum pyramidal*) and two vegetable species (*Lycopersicum esculentum* and *Cucumis sativus*) were collected from the natural stands at Shandawil Agriculture Research Station, Sohag Governorate, Egypt.

Tissue extract bioassay

Each tissue type was mixed with deionized water to prepare 15% (m/v) aqueous extracts according to Wardle *et al.* (1992), El-Khatib and Abd-Elaah (1998), El-Khatib and Hegazy (1999) and El-Khatib (2000). The purified extracts were adjusted to pH 6.8 with 1M HCl (Rice 1972). A series of dilutions (8%, 10%, 12%) from the stock solution were used in the bioassay. These concentrations were prepared based on the results of a preliminary experiment to detect the lethal dose. All experiments were conducted in a growth chamber, with conditions adjusted to 75% humidity, a temperature of 22 ± 2 °C, and photon flux density of 170 μmol m^{-2} S^{-1} during 11 h photoperiod.

Bioassay tests were carried out in 9-cm Petri dishes. Five ml of the bioassay extract was added to dishes containing 25 seeds of the test species on two pieces of Whatman No. 1 filter papers. Dishes were incubated under the growth chamber conditions. One ml of deionized water was added to each dish, as needed. The percentage of germination was recorded every day for 14 days. Distilled water replaced the extracts in the control samples. Three replicates were set up for each tissue type × species × concentration combination. Speed of germination index S was calculated as described by Khandakar and Bradbeer (1983), i.e.:

$$S = (N_1/1 + N_2/2 + N_3/3 + \ldots + N_n/n) \times 100 \quad (1)$$

where N_1, N_2, N_3, …, N_n = Proportion of seeds which germinate on day 1, 2, 3, …, n following setup of the experiment.

The previous experiment was repeated with five pre-germinated seeds of each test species placed in each petri dish, and the seedling length was then measured after one week. Percentage seedling length inhibition of the test species (PLI) was calculated according to the formula of Vokou (1992):

$$\text{PLI} = [(\Delta L_C - \Delta L_R)/\Delta L_C] \times 100 \quad (2)$$

where ΔL_C = difference of mean initial seedling length from mean final seedling length of the respective control, ΔL_R = difference of mean initial seedling length from mean final length of the treated species.

Metabolite and growth characteristics bioassays

Fifteen seeds of each test species were planted in pots (9-cm diameter × 10 cm deep) filled with soil collected from an area distant from the community of *Chenopodium murale*. The pots were irrigated with the aqueous tissue extracts and kept at field capacity. Tap water was used with the control samples. Three replicates were set up for each tissue type × species × concentration combination. After 14 days, the developing seedlings were thinned to five individual plants per pot for every test species. The experiments continued for two months. The plants were then harvested, and their leaf area (Watson & Watson 1953), dry matter, pigment content (Metzner *et al.* 1965), carbohydrate content (Badour 1959) and protein content (Lowery *et al.* 1951) were determined.

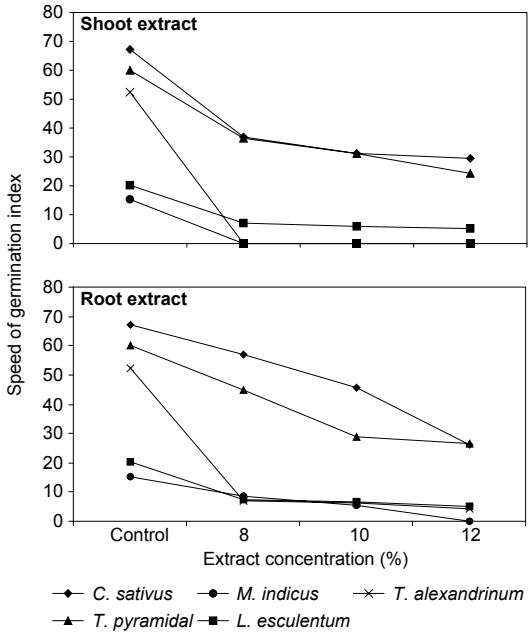

Fig. 1. Effect of extract concentration of *C. murale* on the speed of germination index of the target species.

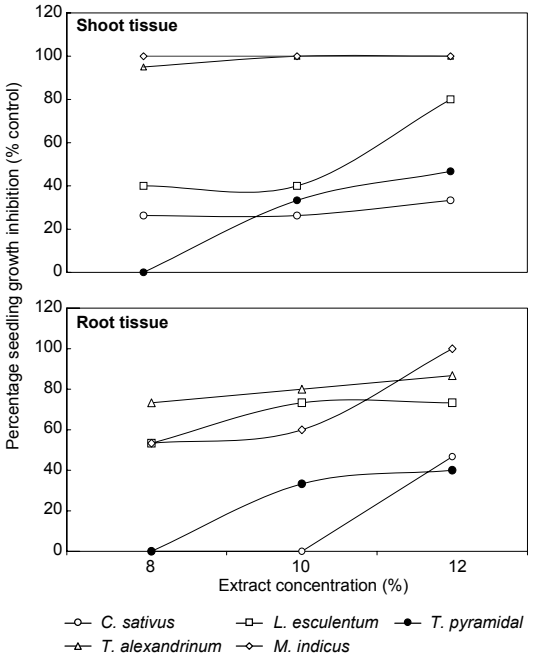

Fig. 2. Percentage seedling inhibition of the target species in response to the effect of tissue extract concentration of *C. murale*.

Statistical analysis

All data for germination and seedling growth were subjected to analysis of variance followed by least significant differences test (LSD) to determine significant differences among mean values at $P < 0.05$ and $P < 0.01$ probability level using "general linear model" procedure of the Statistical Analysis System (SAS) program (SAS Institute 1985). The growth responses of the test species to the concentrations of shoot and root aqueous extracts of *Chenopodium murale* were measured by fitting the appropriate regression model using the regression procedure of SAS program.

Results and discussion

Concerning the speed of germination, data of all target species demonstrated a significant degree of suppression and a negative response to the increasing concentration of different extracts. There were significant differences ($P < 0.01$) between the test treatments and control. Regression analysis revealed that the degree of inhibition was a function of the extract concentration. The shoot extract has more negative effects on the speed of germination than the root extract (Fig. 1). This supports the findings of El-Khatib and Abd-Elaah (1998) on *Zilla spinosa* and El-Khatib and Hegazy (1999) on wheat (*Triticum pyramidal*), who found that shoot extract was more toxic than root extract on the seed germination of the test species. This inhibition may be attributed to the alteration of enzyme activity, which affects the mobilization of storage compounds during germination (Einhellig 1995) and, therefore, may explain the situation under the field conditions, where the different test species in association with *Chenopodium murale* had low density values.

The seedling elongation inhibition of the test species was more obvious at the high shoot concentrations (Fig. 2). *Trifolium alexandrinum* and *Melilotus indicus* were more susceptible to the inhibitory effect of the extract than the other test species. In some cases, the seedling growth inhibition of the test species responded variably to the various concentrations of the extract. Statistical analysis showed significant differences

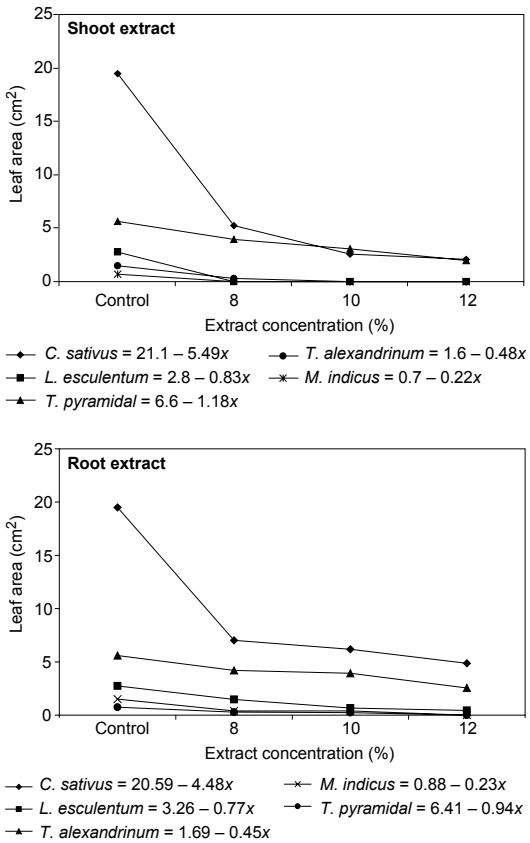

Fig. 3. Effect of aqueous tissue extract of *C. murale* on the leaf area of the different test species.

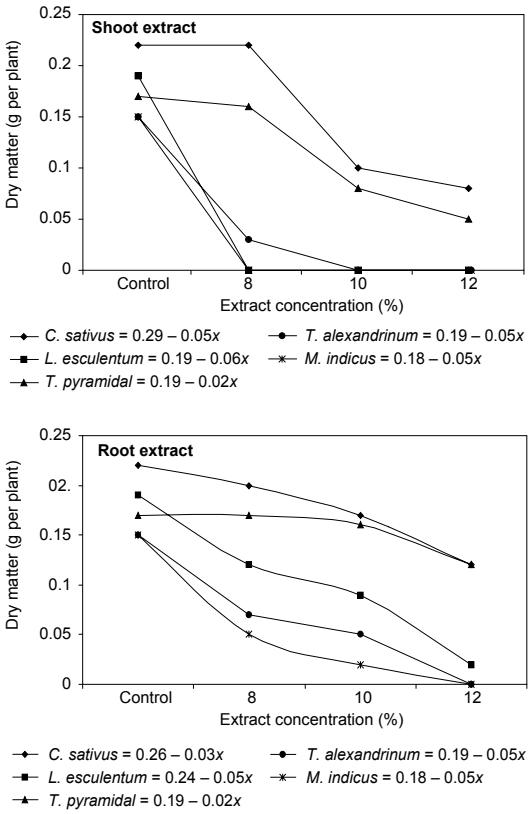

Fig. 4. Effect of aqueous tissue extract of *C. murale* on the dry matter production by the different test species.

($P < 0.01$) between the control and test treatments at the different concentrations of *Chenopodium murale* extract. Some authors (Leather & Einhellig 1985, Hegazy & Fadl-Allah 1995, El-Khatib 1999, 2000) have reported that, in the seedling stage, the susceptibility to allelochemicals increased and some morphological abnormalities occurred. They suggested that seedling growth is more affected by the allelopathic interaction than is seed germination.

There was a wide variation in the average leaf area as expressed in cm^2 $plant^{-1}$ along the concentration gradient, and type of tissue extract (Fig. 3). The results of regression analysis showed that there is an inverse relationship between the leaf area of the test species as a response variable and extract concentration as an independent variable. In general, the leaf area of the test species has the same trend of decreasing with the increases of extract concentration, regardless of the extract type. Shoot extract appeared to be lethal to *Lycopersicon esculentum, Trifolium alexandrinum* and *Melilotus indicus*, even at the low concentrations, where the determinant coefficient (R^2) in the regression model indicates the highest amount of variation in their leaf area with the increase of extract concentration, being 93%, 95% and 98%, respectively. For the root extract, there was only significant difference from the control ($P < 0.05$) at the high extract concentrations.

Concerning dry matter production, no differences were detected ($P > 0.05$) with most of the test species at the low extract concentrations (Fig. 4). However, a highly significant difference was found with higher concentrations. In the case of *Triticum pyramidal*, the decrease in dry

matter production was not affected by the extract type, since the regression coefficient for shoot and root treatment is the same. This reduction suggests interference by the toxic substances from the extract with the cell division, causing a reduction in the root cell growth. This may lead to a decrease in mineral uptake, nutrient absorption, and the transport of nutrients from the root to other plant parts. The reduction in growth and development also resulted in the reduction of dry matter production.

When the pot plants were treated with different concentrations of *Chenopodium murale* tissue extract, pigment content of all test species exhibited significant differences from control (Fig. 5). In comparison with other test species, chlorophyll *b* and carotenoide contents of *Triticum pyramidal* and *Cucumis sativus* were more negatively affected than chlorophyll *a*, their slope value in the regression model being –0.18 and –0.29 with shoot and root extract treatment, respectively. Based on regression slope analysis, pigment contents of most test species demonstrated more susceptibility to the shoot extract of *C. murale* than root extract. The degree of inhibition in the pigment content varied according to the variation in the concentrations, where no significant differences ($P > 0.05$) were recorded with root extract concentration of 8% and 10%, with some of the test species. In general, shoot extract was more severe in its inhibition effect on the pigment content of all test species than root extract. Colton and Einhellig (1980), and Einhellig and Rasmussen (1993) reported that allelochemicals caused marked reduction in the chlorophyll content of the test plants through their effect on the biosynthesis and denaturation of chlorophyll molecules.

Concerning total carbohydrate content, the linear regression analysis for the data of all test species has reflected the descending function in the content of the different plant parts with the increase of the tissue extract concentration (Fig. 6). The slope of the regression line varied for different test species, plant parts, and extract types and concentrations. The carbohydrate content of *Cucumis sativus* parts exhibited a significant reduction compared to those of other test species. *Triticum pyramidal* was the most tolerant species tested, and its slope values were smaller than those of the other species. The reduction in the carbohydrate content may operate through inhibition of stomatal opening and CO_2 uptake, and/or through inhibition of coupled electron transport and both cyclic and non-cyclic photophosphorylation. Gonzalez *et al.* (1997) reported the inhibition effect of allelochemicals on photosystem II electron transfer reaction. The results of this investigation are in agreement with those of many others (Zelitch 1967, Einhellig 1971, Arntzen *et al.* 1974, Inderjit & Dakshini 1992, Hegazy & Fahmy 1999), who reported inhibitory effects of allelochemicals on the net photosynthetic rate causing reduction in the carbohydrate content.

Soluble protein content varied widely among the different parts of the test species with variation of the extract concentration. The soluble protein content of *Lycopersicon esculentum* and *Melilotus indicus* treated with shoot extract of *Chenopodium murale* was more negatively affected than that of the other test species, even at low extract concentrations (Fig. 7). These species were comparatively the most inhibited in their contents of soluble protein. Concerning *Triticum pyramidal*, shoot extract has a similar inhibition effect as root extract on the production of soluble protein within different plant parts. The reduction in the protein content may be due to inhibiting the incorporation of many amino acids into protein (van Sumere *et al.* 1971).

Phytotoxicity appears to be associated with allelochemicals contained within aqueous extracts of *Chenopodium murale*. This is indicated by its inhibitory action on the test species. Inhibition covers all stages of plant growth from seeds to adult plants. The inhibitory effects are reflected in decreased seed germination, seedling growth, leaf area, dry matter production, pigment content, and carbohydrate and protein contents of the adult plants. These effects result in an inhibition of plant development and growth, which is not significant only for the individual plant, but can influence an ecosystem by changing the pattern of vegetation. The negative association of *C. murale* with some of the test species under field conditions can be explained by its allelopathic effect on these species.

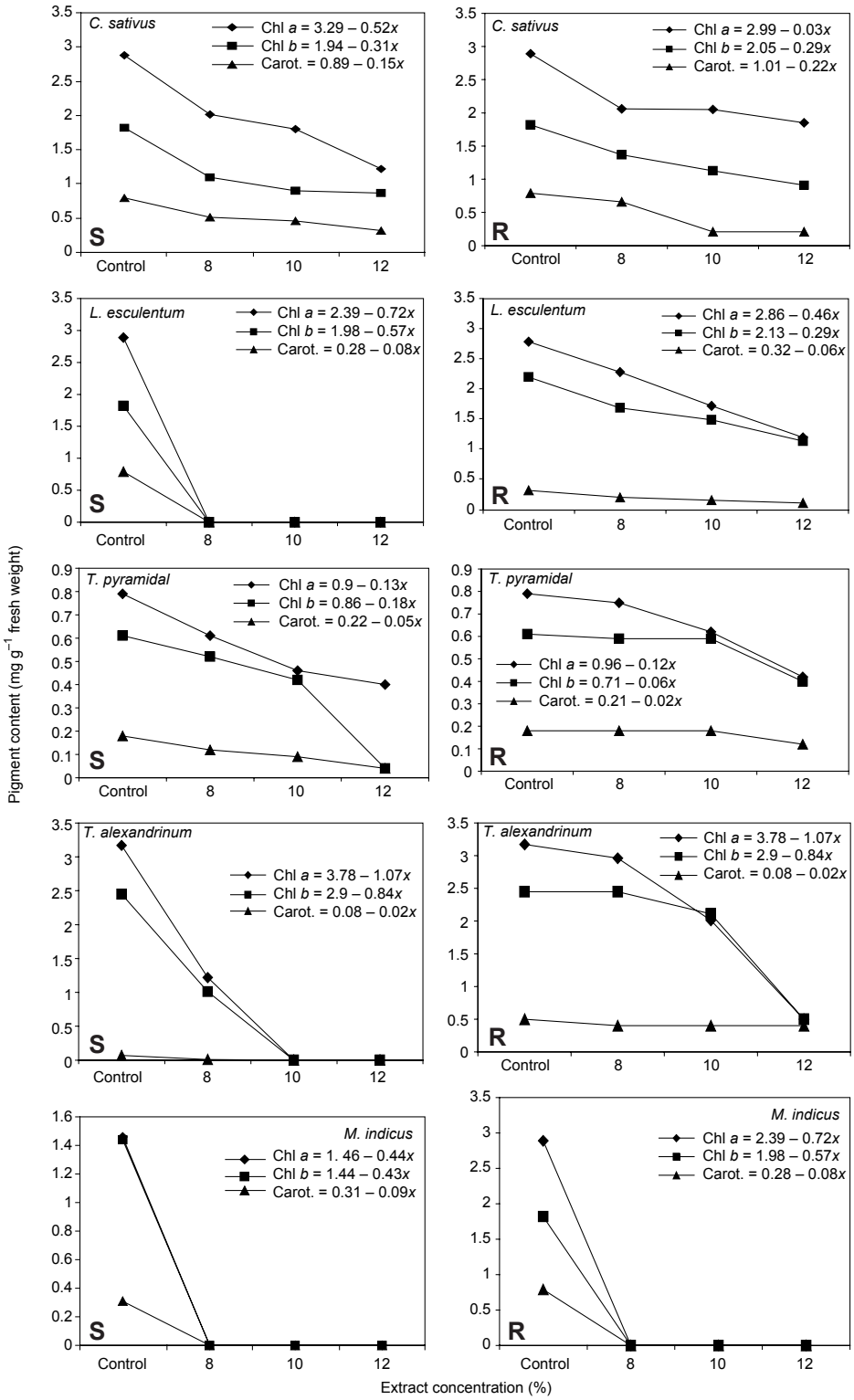

Fig. 5. Effect of different tissue extracts of *C. murale* on the pigment content of the test species. S = shoot extract, R = root extract.

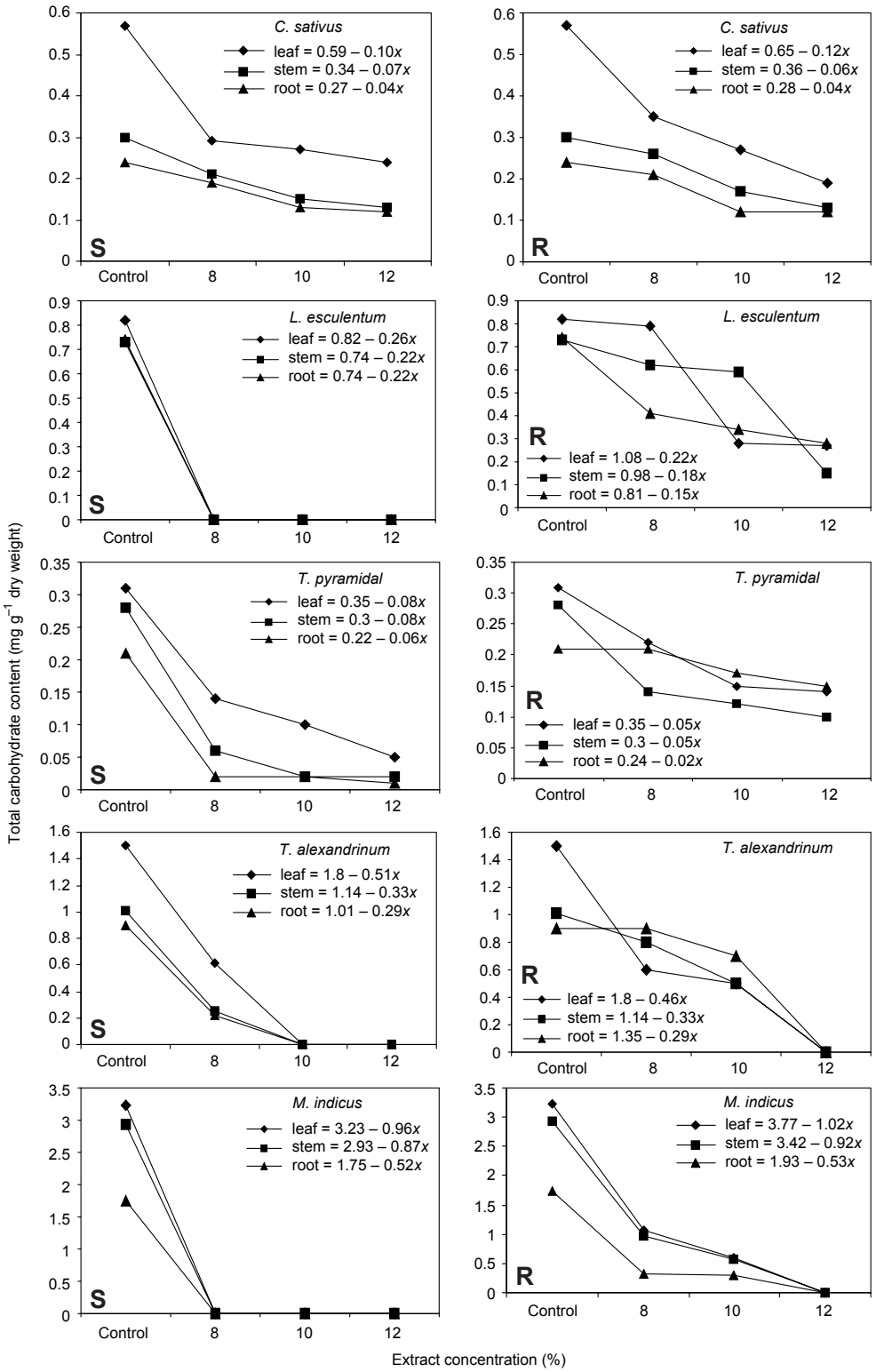

Fig. 6. Effect of different tissue extracts of *C. murale* on total carbohydrate contents of the test species. S = shoot extract, R = root extract.

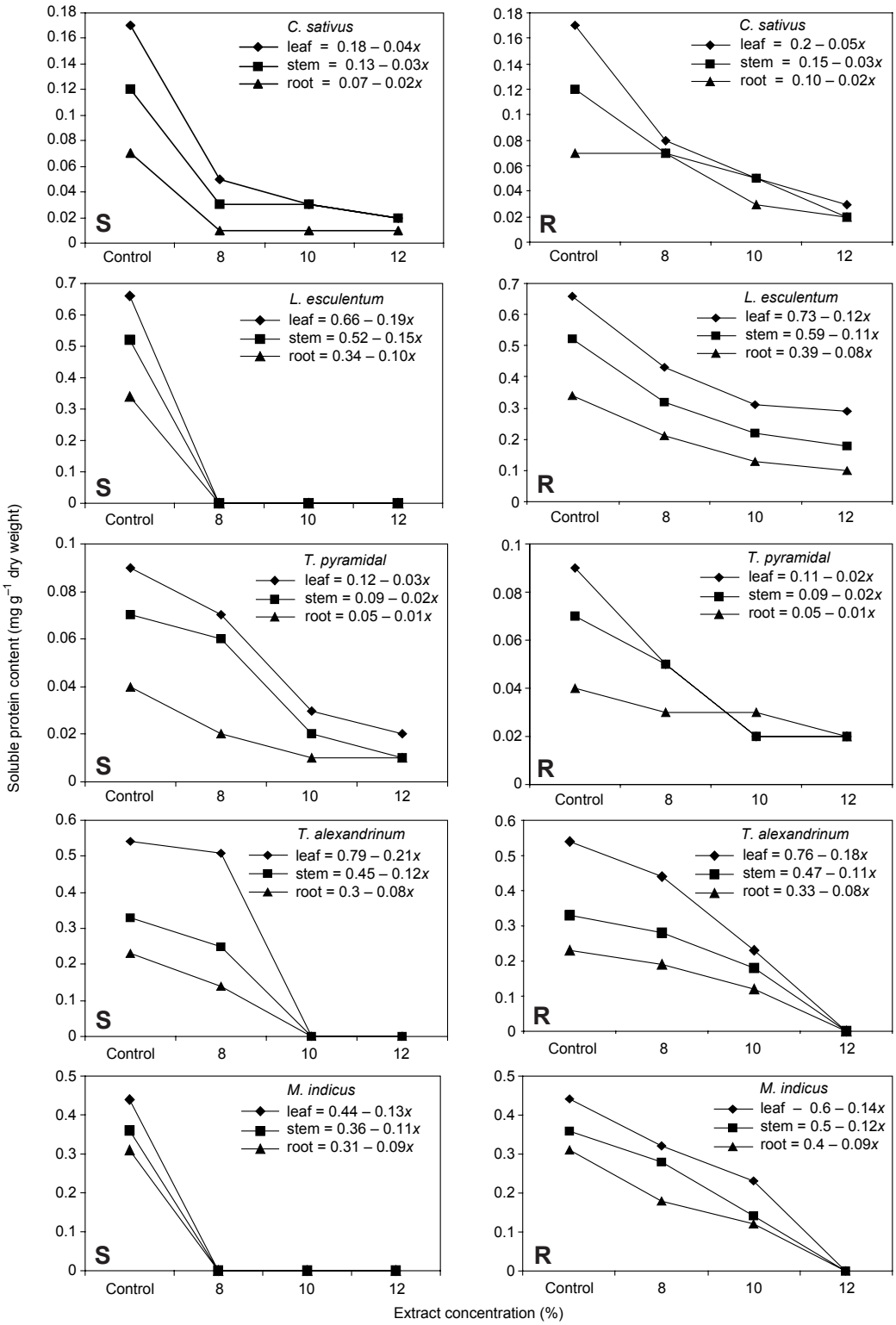

Fig. 7. Effect of different tissue extracts of *C. murale* on soluble protein contents of the test species. S = shoot extract, R = root extract.

References

Arntzen, C. J., Falkenthal, S. V. & Bobick, S. V. 1974: Inhibition of photophosphorylation by kaempferol. — *Plant Physiol*. 53: 304–306.

Badour, S. S. 1959: *Analytisch-Chemisch Untersuchung des Kaliummangels bei Chlorella in Vergleich mit anderen Mangel zustanden*. — Diss. Göttingen Universität, Göttingen.

Colton, C. E & Einhellig, F. A. 1980: Allelopathic mechanisms of velvetleaf (*Abutilon theophrasti* Medic. Malvaceae) on soybean. — *Am. J. Bot*. 67: 1407–1413.

Einhellig, F. A. 1971: Effects of tannic acid on growth and stomatal aperture in tobacco. — *Proc. South Dakota Acad. Sci*. 50: 205–209.

Einhellig, F. A. 1995: Mechanism of action of allelochemicals in allelopathy. — In: Inderjit, Dakshini, K. M. & Einhellig, F. A. (eds.), *Allelopathy: organisms, processes and applications*: 96–116. Am. Chem. Soc., Washington, DC.

Einhellig, F. A. & Rasmussen, J. A. 1993: Effect of root exudate sorgoleone on photosynthesis. — *J. Chem. Ecol*. 19: 369–375.

El-Khatib, A. A. 1997: Former and present vegetation of Kraman Island. River Nile, Sohag, Egypt. — *J. Arab. Gulf Sci. Res*. 15: 261–274.

El-Khatib, A. A. 1998: Does allelopathy involve in the association pattern of *Trifolium resupinatum*? — *Biol. Plantarum* 40: 425–431.

El-Khatib, A. A. 1999: An ecological overview on the allelopathy of water hyacinth (*Eichhornia crassipes*), a strategy of weed control. — In: Macias, F. A., Galindo, J. C. G., Molinillo, J. M. G. & Cutler, H. G. (eds.), *Recent advances in allelopathy, vol. I. A science for the future*: 471–478. Service de publications, Universidad de Cadiz.

El-Khatib, A. A. 2000: The ecological significance of allelopathy in the community organization of *Allhagi graecorum*. — *Biol. Plantarum* 43: 427–431.

El-Khatib, A. A. & Abd-Elaah, G. A. 1998: Allelopathic potential of *Zilla spinosa* on growth of associate flowering plants and some rhizosphere fungi. — *Biol. Plantarum* 41: 461–467.

El-Khatib, A. A. & Hegazy, A. K. 1999: Growth and physiological response of wild oats to the allelopathic potential of wheat. — *Acta Agron. Hung*. 47: 11–18.

Galal, H. K. 2000: *Ecological and allelopathy of Chenopodium murale L. in different habitat*. — M.Sc. thesis, Bot. Dept., Fac. Sci., Sohag.

Gonzalez, V. M., Kazimir, J., Nimbal, C., Weston, L. A. & Cheniae, G. M. 1997: Inhibition of photosystem II electron transfer reaction by the natural product sorgoleone. — *J. Agric Food Chem*. 45: 1415–1421.

Hegazy, A. K. 1999: Allelopathy in desert ecosystems: an overview, problems and prospects. — In: Narwal, S. S. (ed.), *Allelopathy update: basic and applied aspects*: 163–175. Sci. Publ. Inc., Enfield.

Hegazy, A. K. & Fadl-Allah, E. M. 1995: Inhibition of seed germination and seedling growth by *Cleome droserifolia* and allelopathic effect on rhizosphere fungi in Egypt. — *J. Arid Environ*. 29: 3–13.

Hegazy, A. K. & Fahmy, G. M. 1999: Host-parasite allelopathic potential in desert plants. — In: Macias, F. A., Galindo, J. C. G., Molinillo, J. M. G. & Cutler, H. G. (eds.), *Recent advances in allelopathy, vol. I. A Science for the future*: 301–312. Service de publications, Universidad de Cadiz.

Inderjit & Dakshini, K. M. M. 1992: Hesperetin 7-rutinoside (heperidin) and taxifolin 3-arabinoside as germination and growth inhibitors in soils associated with the weed *Pluchea lanceolata* (DC.) C. B. Clarke (Asteraceae). — *J. Chem. Ecol*. 17: 1585–1591.

Khandakar, A. L. & Bradbeer, J. W. 1983: *Jute seed quality*. — Bangladesh Agric. Res. Council, Dhaka.

Kosinova, A. 1975: Weed communities of winter crops in Egypt. — *Preslia* 47: 58–74.

Leather, G. R. & Einhellig, F. A. 1985: Mechanisms of allelopathic action in bioassay. — In: Thompson, A. C. (ed.), *The chemistry of allelopathy, biochemical interaction among plants*: 197–205. ACS Symp. Ser. 268., Amer. Chem. Soc. Washington, DC.

Lowery, O. H., Rosebrough, N. J., Farr, A. & Randall, R. J. 1951: Protein measurement with the folin phenol reagent. — *J. Biol. Chem*. 193: 265–275.

Metzner, H., Rau, H. & Senger, H. 1965: Untersuchunger zur synchronisierbarkeit einzelner pigment-mangel mutanten von *Chlorella*. — *Planta* 65: 186–194.

Rice, E. L. 1972: Allelopathic effects of *Andropogon virginicus* and its persistence in old fields. — *J. Amer. Bot*. 59: 752–755.

SAS Institute 1985: *SAS User's Guide: Basics*, 5th ed. — SAS Inst. Inc., Cary.

Shaltout, K. H, Sharaf El-Din, A. & El-Fahar, R. A. 1992: Weed communities of the common crops in the Nile Delta region. — *Flora* 187: 329–339.

van Sumere, C. F., Cottenie, J., De Greef, J. & Kint, J. 1971: Biochemical studies in relation to the possible germination regulatory role of naturally occurring coumarine and phenolics. — *Rec. Adv. Phytoch*. 4: 165–221.

Vokou, D. 1992: The allelopathic potential of aromatic shrub in Phryganic (east Mediterranean ecosystem). — In: Rizivi, S. J. H. & Rizivi, V. (eds.), *Allelopathy: basic and applied aspects*: 303–320. Chapman & Hall, London.

Wardle, D. A., Nicholson, K. S. & Ahmed, M. 1992: Comparison of osmotic and allelopathic effects of grasses leaf extracts on grass seed germination and radicle elongation. — *Plant Soil* 140: 315–319.

Watson, D. J. & Watson, M. A. 1953: Studies in potatoes agronomy. I. Effect of variety seed size and spacing on growth, development and yield. — *J. Agric. Sci*. 66: 241–246.

Zelitch, I. 1967: Control of leaf stomata: their role in transpiration and photosynthesis. — *Am. Sci*. 55: 72–86.