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Research Paper

Does salinity enhance allelopathic effects of *Tribulus terrestris* L. in *Citrullus vulgaris* schrad. Agroecosystems at Nobaria, Egypt?

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Field observations in summer season at year 2013 showed that growth and yield of *Citrullus vulgaris* at Nobaria district, Egypt was highly affected by some unidentified stressful conditions. Presumably, the problem was confined considerably to the two combined factors; soil salinity and suppressive effects of *Tribulus terrestris* (a common weed dominant in *C. vulgaris* fields in the study area). Field and laboratory experiments were carried out to confirm the preceding hypothesis.The interaction between the two factors was found to be harmful to *C. vulgaris* plant. Field experiment revealed that phytomass and leaf area index (LAI) of the study plant were significantly reduced in the vicinity of *T. terrestris* (poor weed management) than in good weed management. Likewise, in laboratory test the interaction between both salinity and allelochemicals watery extracted from *T. terrestris* plant severely affected germination efficiency, hypocotyl-radicle length and germination index of *C. vulgaris* compared to the effect of just one factor. In conclusion, *T. terrestris* had a considerably suppressive effect on the growth and yield of *C. vulgaris*, which was increased under slight or moderate salinity. Importantly, weed management in desert agro-ecosystems is an essential strategy to avoid a wide array of interactions between crop-weed from one side and weed-external climatic and edaphic factors from the other side.

Key words: Allelopathy, Germination efficiency, LAI, Phytomass, Plant density, Salinity, *Tribulus terrestris*, *Citrullus vulgaris*.

INTRODUCTION

The biological active concentration of allelopathic compounds in soil is often influenced by its biological and chemical characteristics such as nutrients, microorganisms, organic matter pH, moisture content and salinity (Kruse *et al*., 2000). The relative density between donor and recipient species has been suggested to be an important factor in the degree of expression of allelopathy (Thijs *et al*., 1994).

Weeds are considered as an integral part of the natural and agroecosystems and its allelopathic activity has been suggested to be part of their success. It is well known that some annual and perennial weed species (e.g. *Tribulus terrestris*, *Artemisia* spp., *Pluchea lanceolata, Lantana camara*, *Buchloe dactyloides, Achillea santolina, Eucalyptus* spp. and many others) exert growth inhibiting substances from the tissues of their living or dead shoots and roots (Friedman *et al*., 1977; El-Ghareeb, 1991; Inderjit and Dakshini, 1994; Wu *et al.*, 1998; El-Darier and Youssef, 2000; El-Darier, 2002; Hatata and El-Darier, 2009; Tammam *et al.* 2011). These inhibitors greatly retard and reduce the growth and physiology of associated crop plants in contact with them (El-Khatib *et al*., 2004; El-Darier and Tammam, 2012; El-Kenany and El-Darier, 2013).

Citrullus vulgaris Schrad. is one of the most important summer vegetable crops cultivated in the new reclaimed areas at Nobaria district. High soil salinity causes plants to absorb water less easily from the soil, aggravating water stress conditions. It can also achieve nutrient imbalances, resulting in the accumulation of elements toxic to plants, and reducing water infiltration if the level of one salt element (e.g. sodium) is high (Kotuby-Amacher *et al*.,2000). Problems arising from high salt level are more severe when they are accompanied with other stresses such as inadequate drainage, hot and dry conditions, occurrence of allelochemicals released from adjacent plants and also when plants are subjected to diseases, insects and nutrient deficiency. Each of these factors is exaggerated by the existence of the other (Migahid and Elkhazan, 2002). A current hypothesis suggests that salinity may alter the nature of allelopathic interaction and also modify the expression of the allelochemicals (Singh *et al*., 2001). Surveying the literature, less conclusive evidences have been obtained to support this hypothesis. Therefore, the present study is an endeavor aiming to confirm the hypothesis that salinity may perhaps magnitude the allelopathic effects of *T. terrestris* in *C. vulgaris* agroecosystems at Nobaria, Egypt.

MATERIALS AND METHODS

Field observations were set up during summer 2013 (poor weed management) and extended to the next season during 2014 (good weed management) in an area of about 24 feddans (10.082 hectare) cultivated with *C. vulgaris* (crop plant) at Nobaria district (about 65 km southwest of Alexandria city) (Figure 1). During each season, twenty-seven quadrates (2 m^2) were randomly distributed throughout the entire farm whereas T. terrestris (allelopathic plant) individuals were counted in each quadrate and their density was expressed as number per m^2. As well, the neighboring *C. vulgaris* individuals in the same quadrat were harvested for the determination of phytomass and leaf area index (LAI). Soil collected at depths of 0-30 and 30-60 cm from the soil surface and the water applied for irrigation were analyzed for some of their physical and chemical properties (Allen *et al*., 1984). Sodium adsorption ratio (SAR) was calculated according to the following equation:

$$SAR = Na^{+} \Big/ \sqrt{\frac{(Ca^{+2} + Mg^{+2})}{2}}$$

Laboratory experiment

Laboratory experiment aimed to investigate: (1) the allelopathic effects of *T. terrestris* aqueous extract, (2) the effect of different levels of salinity and (3) the effect of a combination of both *T. terrestris* extract and salinity on the germination characteristics of *C. vulgaris*.

To achieve the first part, fresh complete samples (Aerial shoots + roots) of *T. terrestris* were collected from natural agro-fields in June 2014. The samples were divided into two lots where the first was handled fresh and the second was air- dried then cut into 0.5 – 1 cm pieces. Stock aqueous extract was obtained by soaking the plant materials (dry or fresh) in 10% (w/v) distilled water at room temperature (28 ±2°C) for 24 hours with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper and the purified extract was adjusted to pH 6.8 with 1M HCl. Under such optimal pH, no significant growth inhibition occurs (Macias *et al*., 2000; Singh *et al*., 2003). Different concentrations (1, 2 and 4%) were prepared from the stock solution in addition to the control (distilled water). Different levels (0, 50, 100, 200 mM) of NaCl solution were prepared to perform the second part. A combination from the *T. terrestris* extract and NaCl solution was obtained by diluting the extract in 50,100 and 200 mM NaCl solution in addition to the control to verify the third part.

To accomplish the three mentioned parts, twenty seeds of *C. vulgaris* were arranged in 18-cm diameter covered glass Petri dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 27-30°C and night temperature from 18-20°C. 15 cm^3 of each level of the *T. terrestris* extract, salt concentration and the mixture of both were added daily to four replicates related to three separate sets of Petri dishes. Seed germination rate (%) and hypocotyl-radicle length were recorded daily for seven successive days after the beginning of the experiment and seed germination index (SGI) was calculated according to the following equation (Scott *et al*., 1984):

$$SGI = \sum Ti\ Ni / S$$

Where,
Ti = is the number of days after sowing
Ni = is the number of seeds germinated on day i
S = is the total number of seeds planted

Relative reduction (percentage inhibition) or stimulation of seed germination and hypocotyl–radicle length as affected by the allelopathic substance and salinity were calculated according to the general equations:Relative reduction = [1- (allelopathic/control) 100] = [1 (salinized/control) 100]

Statistical analysis: data of the present study were subjected to standard one-way analysis of variance

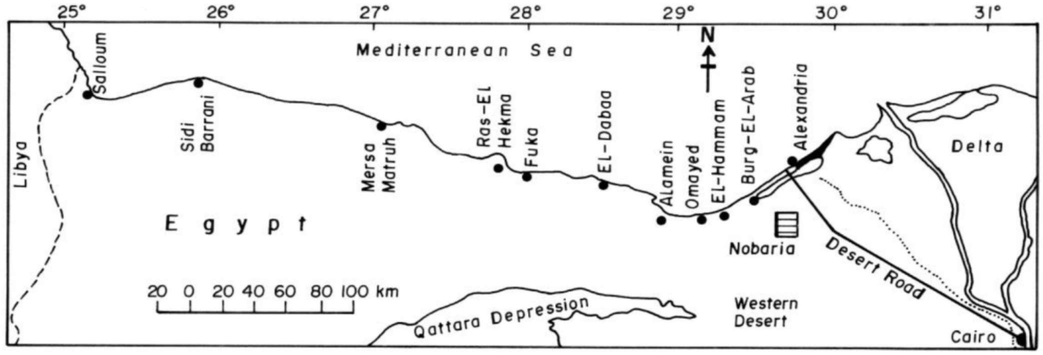

Figure 1. Map of the western Mediterranean desert of Egypt indicating the location of the study area (hatched area).

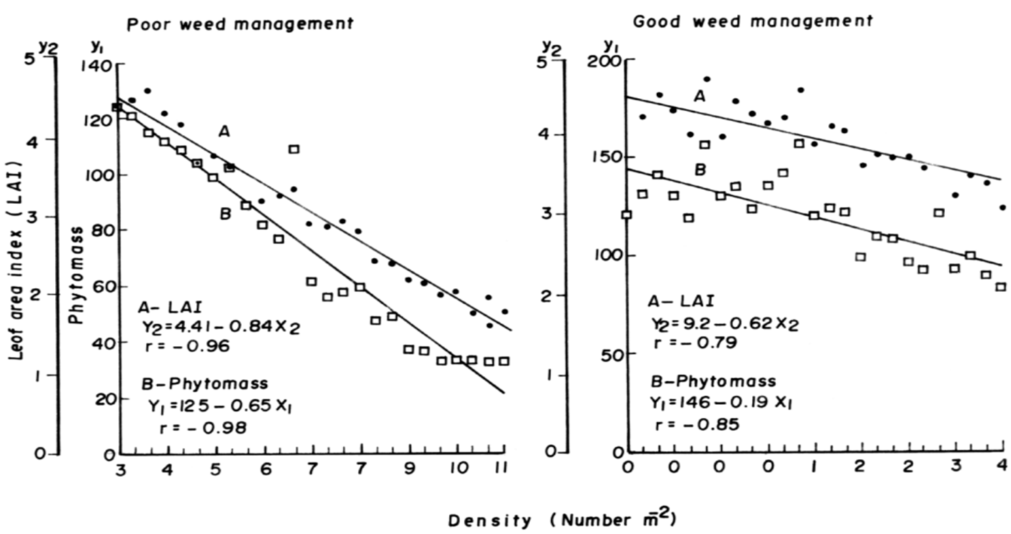

Figure 2. Density (number m^{-2}) of *Tribulus terrestris* plants versus phytomass (g $plant^{-1}$) and leaf area index (LAI) (cm^2 cm^{-2}) of *Citrullus vulgaris* at 70 days after sowing recorded in 27 randomly distributed quadrates ($2m^2$) in two different weed management practices.

(ANOVA) and student's t-test (Zar, 1984) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company (1986).

RESULTS

Data regarding physical and chemical characteristics of soil collected from 27 stands in the *C. vulgaris* agroecosystem at Nobaria district as well as water currently applied for crop irrigation (Nile water) are illustrated in (Table 1). Generally, the soil was sandy loam with an average pH of 7.85 and EC=2.22 mmhos cm^{-1} and optimum levels of different cations and anions.

In the field experiment, greater abundance of *T. terrestris*, yet reduced phytomass and leaf area index (LAI) of the neighboring *C. vulgaris* plants at year of poor weed management treatment (Figure 2). Explicitly, the slopes (Tan b) of the two regression lines calculated by simple regression equation "y= a + bx" concerning the two mentioned growth parameters (0.30 and 0.28 respectively) were significantly lower at the year of good weed management treatment compared to that of poor weed management treatment (0.90 and 0.72 respectively) indicating more inhibitory effects in the latter.

The effect of different levels of *T. terrestris* extract, NaCl solution and the interaction of both on germination efficiency (Figure 3) and hypocotyl-radicle length (Figure 4) of *C. vulgaris* was highly significant ($p< 0.05$) as evaluated by one-way ANOVA. The maximum of the two measured parameters (105 and 107% respectively relative to the control) was attained at 1% *Tribulus* extract level and the values progressively decreased to about 61 and 55% respectively as the extract level increased (4%) (Figures 3 and 4A).

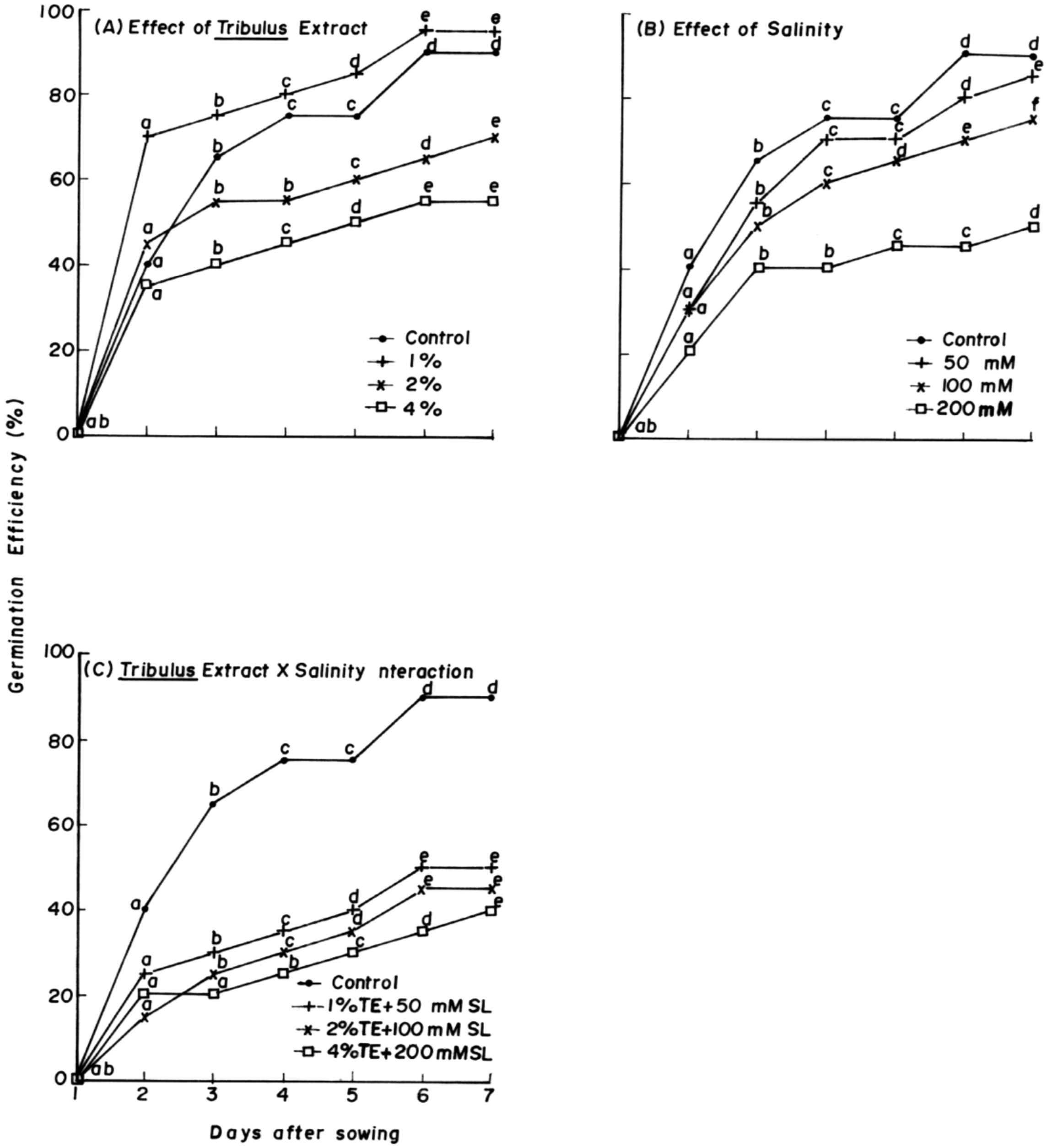

Figure 3. Effect of different concentrations of (A) *Tribulus terrestris* extract, (B) salinity and (C) extracts X salinity interaction on germination efficiency (%) of *Citrullus vulgaris*. Different letters for each treatment indicate a significant difference at $p<0.05$ according to one way ANOVA.

Remarkably, there was an inverse relationship between the values of the two mentioned parameters and the applied concentration levels of NaCl (Figures 3 and 4B). A reduction of about 55.5% in germination efficiency and 52.4% in hypocotyl-radicle length (relative to the control) was achieved at 200 mM NaCl concentration level. Interestingly, conclusive evidence was obtained from the present study relating to the mutual relationship between allelopathy and salinity. The interaction of the two factors virtually caused severe reduction in the two concerned parameters (44 and 41% respectively) compared with the effect of each individually factor (Figures 3 C and 4 C).

Seed germination index (SGI) and percentage inhibition of germination and hypocotyl-radicle length as influenced by the two main factors beside their combination are presented in (Table 2). Data indicate a longer germination time (small values of SGI) with the increase of the two main factors and their interactions except at

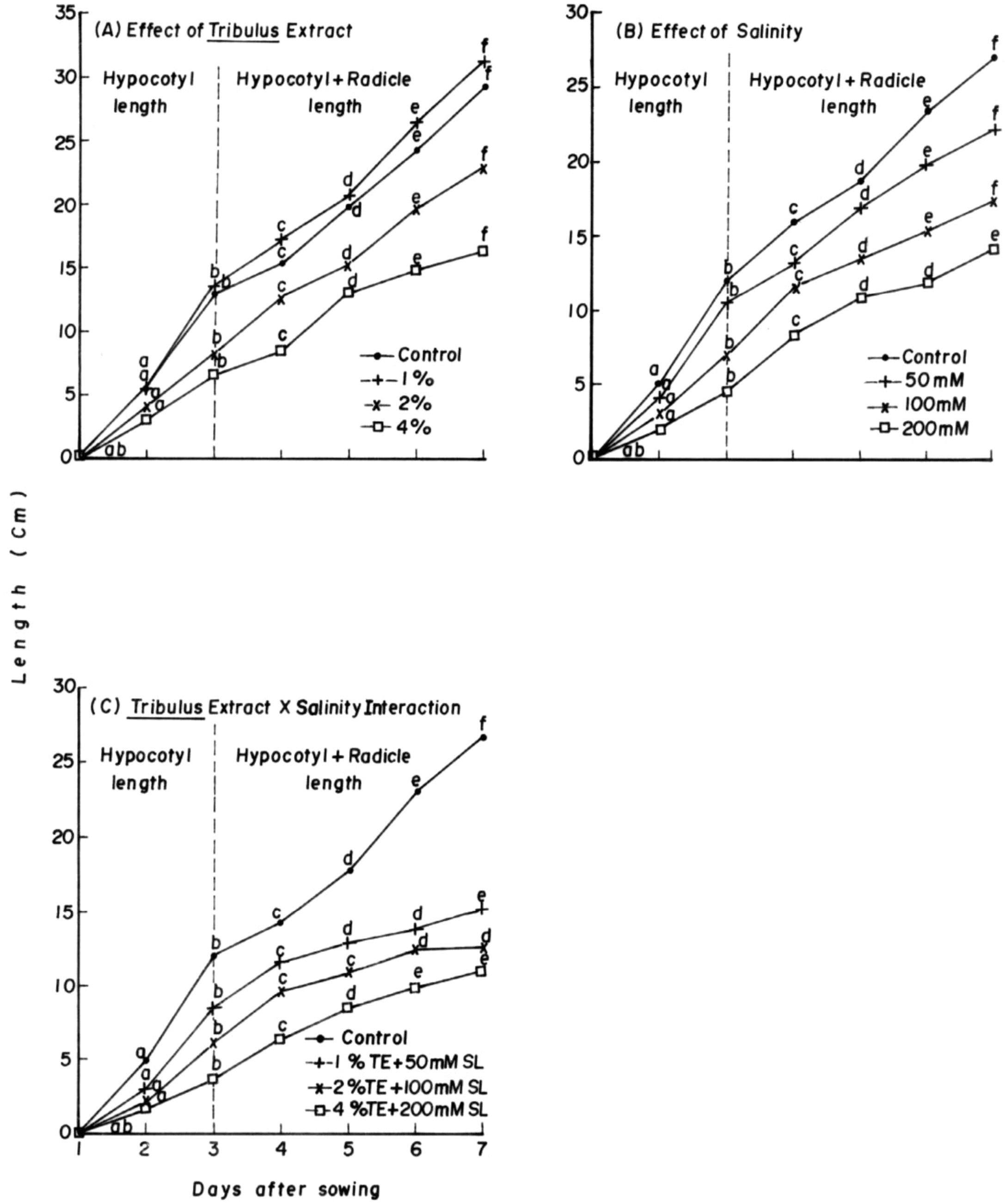

Figure 4. Effect of different concentrations of (A) *Tribulus terrestris* extract, (B) salinity and (C) extract X salinity interaction on hypocotyl-radicle length of *Citrullus vulgaris*. Different letters for each treatment indicate a significant difference at $p < 0.05$ according to one-way ANOVA.

1% *T. terrestris* extract. Finally, the variation between the totally effect of *T. terrestris* extract from one hand and the combined effect of *T. terrestris* extract and NaCl from the other hand on the germination percentage and hypocotyl-radicle length was significant as evaluated by t-test. Similarly, the test was significant for the phytomass and LAI between the two weed management treatments.

DISCUSSION

In Egypt, weeds in agricultural lands increased to represent approximately 22.5% of the total flora (El-Hadidi, 1993). Weeds allelopathy plays an important role in agroecosystems leading to a wide array of interactions between crop-crop, crop-weed and tree-crops (Singh *et*

Table 1. Soil (A) and water (B) analysis of *Citrullus vulgaris* agroecosystems at Nobaria region (average of the years 2013 and 2014). Numbers between brackets indicate the values of SD.

Depth (cm)	Soil Texture (%)	PH	EC (mmhos cm^{-1})	OM (%)	$CaCO_3$ (%)	Soluble cations and anions (meq/l) K^+ Na^+ Ca^{2+} Mg+2 Cl^- SO^{-4}
0-30	Sand=52	7.9	1.95	0.32	17.14	0.65 7.6 5.2 6.4 7.2 8.1
	Silt=16	-0.1	-0.3	-0.05	-2.1	(0.1) (1.2) (0.9) (1.4) (2.0) (2.1)
30-60	Clay=32	7.8	2.5	0.38	17.16	0.49 12.4 8.7 5.2 10.2 10.9 (0.05)
	Sandy loam Soil	-0.1	-0.2	-0.03	-3	(2.7) (1.9) (1.0) (3.0) (2.8)

A: Soil analysis

B: Water analysis

TSS (mg/l)	PH	EC (mmhos cm-1)	SAR	Soluble cations and anions (meq/l) K^+ Na^+ Ca^{+2} Mg^{+2} Cl^- SO^{-4}
806	8	1.26	2.27	0.48 4.5 4.4 3.4 3.9 5.9 (0.06) (0.7) (0.5) (0.4) (0.6) (0.8)
75	1.2	(0.02)	(0.3)	

Table 2. Seed germination index and percentage inhibition of seed germination and hypocotyl-radicle length of *Citrullus vulgaris* as influenced by different concentration levels of *Tribulus terrestris* extract, salinity and extract x salinity interaction. Different letters for each row indicate a significant difference at $p < 0.05$ according to one-way ANOVA.

Parameter	Concentration Level (%)			
	1	2	4	Control
Seed germination index				
Extract	23.45^a	16.55^b	13.35^c	21.20^b
Salinity	19.30^a	17.20^b	11.65^c	21.20^a
Extract x Salinity	1.30^a	9.85^b	8.40^c	21.20^c
Germination Efficiency				
Extract	5.55^a	22.22^b	38.88^c	-
Salinity	5.55^a	16.66^b	44.44^c	-
Extract x Salinity	44.44^a	50.00^b	55.55^c	-
Hypocotyl-radicle length				-
Extract	5.84^a	21.91^b	44.52^c	-
Salinity	18.08^a	35.79^b	47.60^c	-
Extract x Salinity	43.07^a	52.80^b	59.17^c	-

Table 3. Results of paired t-test between the effect of both *Tribulus terrestris* extract and extract x salinity interaction on germination (%) and hypocotyl- radicle length (df = 12) and between poor and good weed management for phytomass and leaf area index (df = 52).

		Concentration level (%)		
Variable	Control	1	2	4
Germination (%)	0.00NS	3.747**	3.051**	2.794*
Hypocotyl-radicle length	0.22NS	2.501*	2.915*	2.180*
Phytomass		4.004**		
Leaf area index		3.721**		

NS : not significant,* : Significant at 0.05,** : Significant at 0.01

al., 2001). Occurrence of allelopathy under field conditions may depend on size and density of plants (Weidenhamer *et al*., 1989; Thijs *et al*., 1994); soil and climatic conditions (del Moral and Muller, 1970; Rice,

1984) time of the year (Hegazy *et al*., 1990) and the dominant of other environmental stresses (El-Darier and Youssef, 2000; Migahid and Elkhazan, 2002) that control accumulation or dispersion of allelopathic compounds.

Soils salinity levels at which plants begin to experience yield-reducing effects are indicated by the threshold values. According to the international guidelines for plant response to soil salinity, C. *vulgaris* is considered as a sensitive crop and its growth begin to be affected at EC value = 2 mmhos cm^{-1} and loss about 50% of its yield at EC = 4.5 mmhos/cm (A.S.A.M., 1990). The salinity level in *C. vulgaris* agroecosystems in the present study was slightly above the normal range (1.9 - 2.5 mmhos/cm) but the growth was greatly reduced which may be often attributed to the allelopathic effect of the neighbor dominant species *T. terrestris* that magnitudes under moderate salt stress.

In the present study, a significant reduction ($p < 0.05$) in plant phytomass and leaf area index (LAI) of *C. vulgaris* plants was exhibited at poor management treatment (removal of only about 30% of *T. terrestris* stands) as compared to the removal of about 90% of *T. terrestris* individuals (good management treatment). Such reduction cannot be attributed to less favorable physical environmental conditions as in fact the results indicate optimum organic matter, pH and adequate amount of all most cations and anions. In addition, the osmotic potential of *T. terrestris* as about -2.5 bars was found to be not an osmotically inhibitory concentration (del Moral and Cates, 1971). The mechanism of allelopathy in such field condition is incompletely known, but *T. terrestris* as a prostrate creeping plant the standing dead materials together with the live organs of *T. terrestris* are subjected directly to repeated washing by irrigation water. Consequently, the readily water-soluble phytotoxins are liberated and released into the soil, may remain concentrated around the plant for a time enough to affect the neighboring *C. vulgaris* plants. Such effects may be directly on receiver plant itself or indirectly through alteration of soil properties, nutrient status and population and/or activity of harmful or beneficial organisms like microorganisms, insects and nematodes (Kruse *et al*., 2000). In similar field studies, seedling growth of two susceptible species was negatively correlated to increasing density of *Lantana camara* (Gentle and Duggin 1997). It is noteworthy that, in India, the species under discussion caused a 15 to 20% loss in grain yield of pearl millet (*Pennisetum typhoides*) (Misra, 1962). To go through with this, aqueous extract of *T. terrestris* inhibited seedling growth of the latter plant species but germination percentage was not affected (Sen *et al*., 1969). Otherwise, *T. terrestris* is able to exert effective allelopathic interference on the phytomass and stature of certain annuals in the surrounding flora in Kuwait (El-Ghareeb, 1991).

Allelopathy is tightly coupled with competition for resources and stresses from diseases, temperature and salinity extremes, moisture deficit and herbicides (Migahid and Elkhazan, 2002). Phytochemical screening of *T. terrestris* (Rizk, 1986) revealed that the plant is rich in saponins, alkaloids, flavonoids and other secondary metabolites, which may have an allelopathic effect and play an important role in crop agroecosystems (Kefeli and Kadyrov, 1971; Rice, 1984). The present study indicated that in the laboratory experiment, the germination efficiency and hypocotyl-radicle length of *C. vulgaris* was strongly inhibited (38.88 and 44.52% respectively) by applying 4% aqueous extract of *T. terrestris,* indicating a possibility that chemical inhibition might be responsible for the reduction of the germination capacity. This was also established in other studies with other allelopathic species (Al-Saadawi and Al-Rubeaa, 1985; El-Darier, 2002); Hegazy and Fadl-Allah, 1995). As is true for any chemical based response, allelopathic interactions are also concentration specific. Data also clarified that the germination efficiency was stimulated at 1% concentration level of the aqueous extract of *Tribulus* compared to the control. The stimulation occurred was supported by data provided by other studies (El-Darier, 2002; El-Darier and Youssef, 2000). Interestingly, germination and seedling growth of some parasitic species; in response to their host extracts, were promoted at low concentrations (up to 1%), and reduced at higher concentrations (Hegazy and Fahmy, 1999). Synergistic effects were confirmed when control values were less than any of the test treatment (Leatham *et al*., 1980; Rice, 1984), which would be expected when multiple allelopathic agents are present (Patrick, 1986).

Generally, vegetables are more salt sensitive than forage or field crops (especially under hot conditions) and such sensitivity are more pronounced during seedling stage, immediately when subject to other stresses (allelochemicals, insects, nutrient, and disease) (Kotuby-Amacher *et al*., 2000). The percentage of germination inhibition increased to about 44.44% under the effect of 200 mM concentration level of NaCl and to 55.55% as influenced by the interaction of both *T. terrestris* extract and NaCl. Such inhibition may be due to the combined effects of both allelochemicals and NaCl on the rate of water uptake, which are confirmed by the welting of *C. vulgaris* individuals in the field or by altering its metabolism and mobilization of storage compounds during germination (El-Darier and Youssef, 2000, El-Khatib, 1997, Malibari, 1993). At Nobaria region (hot dry ecosystems), salts originate mainly from inorganic fertilizers, manure and irrigation water. Salt accumulation also has resulted from frequent irrigation processes especially in regions where rainfall is so light (El-Darier and Youssef, 2000).

In conclusion, the allelopathic effects of *T. terrestris* on plant density and leaf area index in the field and on germination efficiency, seed germination index and hypocotyl- radicle length, of *C. vulgaris* plants in the laboratory were exaggerated by the incidence of low level of salinity. Such conclusion sustains the hypothesis that salinity may synergize the allelopathic effects of *T. terrestris* in *C. vulgaris* agroecosystems at Nobaria, Egypt. Importantly, weed control must be properly experienced to minimize its drastic effects on the economy of the cultivated lands. Efforts should, therefore, be made to devise a strategy for controlling *T. terrestris* in *C. vulgaris* agroecosystems.

Authors' declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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