

## **ECOPHYSIOLOGICAL STUDY ON THE INVASIVE WEED *Cynanchum acutum* L.**

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### **ABSTRACT**

The invasive weed, *Cynanchum acutum*, was collected from nine different habitat sites from Damietta and Kaliobia governorates. There were 27 associated species with *C. acutum* belonging to 24 genera and related to 15 families. Analysis of variance indicated that soil variables were significantly different at 0.001 level except for pH. The same was also detected for the functional morphological traits of *C. acutum*; all of the traits were significantly different at a level less than 0.001. On the other hand, there were great differences in carbohydrates, phenolics, protein and proline contents among the studied sites. There were differential correlations between functional physiological traits of *C. acutum* and the soil variables in the studied sites. Phenolics content was positively correlated with soil chloride but negatively correlated with soil moisture content. On the other hand, proline content was positively correlated with both soil calcium carbonate and bicarbonate. In addition, there was a close correlation between protein content and soil calcium carbonate and bicarbonate. Meanwhile, soil organic matter and available phosphorus correlated well with various physiological variables. Anyway, there was a positive correlation between available phosphorus and protein content. These present results might provide information about the soil characteristics supporting the plant growth in addition to possible expectations of the plant internal composition. These close correlations tend to indicate that the variation in growth and physiological function of *C. acutum* might be a consequence of the varied edaphic factors.

**Keywords:** invasive weed, *Cynanchum acutum*, soil variables, morphological traits, physiological traits

### **INTRODUCTION**

Weeds are not only a serious problem facing agricultural systems but also ranked as the greatest (Aref and Pike, 1998). They compete with plants for light, water and nutrients. A variety of weed species have potent allelopathic effect on other plants including crops (Weston and Duke, 2003; Xuan *et al.*, 2006; Rudrappa *et al.*, 2007). In natural habitats, invasive plants constitute a serious threat to the natural communities (Timmins and Williams, 1991). *Cynanchum acutum* belongs to the family Asclepiadaceae, commonly known as milkweed family. It is recently noticed to be very widely distributed in the Nile Delta, Egypt (Shaltout and El-Sheikh, 2003).

It contains several alkaloids such as pyrroloisoquinoline, 7-demethoxytylophorine and 7-demethoxytylophorine N-oxide which have antiviral activities against tobacco mosaic virus (An *et al.*, 2001). Lee *et al.* (2003) reported that an extract derived from the root is active in inhibiting the

growth of human cancer cells in culture. Then, they isolated and identified antofine, a phenanthroindolizidine alkaloid, as an active principle for inhibiting human colon cancer cells and human lung cancer cells. Antofine induced arrest in the G2/M phase of the human colon cancer cell cycle after 48 h of incubation. These data suggest the potential of antofine to serve as a cancer chemotherapeutic agent by virtue of arresting the cell cycle. It also contains quercetin 3-O- $\beta$ -galacturonopyranoside and quercetin 7-O- $\beta$ -glucopyranoside which exhibits significant antioxidant and antidiabetic activities (Fawzy *et al.*, 2008).

The increasing invasiveness of *Cynanchum acutum* could be attributed to its ability to adapt with different and diverse environmental variables in several habitats. Therefore, studying the ecophysiological characteristics of this invasive weed in areas of two different governorates (Damietta and Kaliobia) could be a key process in managing efforts in arable, cultivated and natural area habitats. So, the variability in some of the morphological and physiological traits was checked in response to variation in edaphic factors of the habitats supporting the plant growth.

## MATERIALS AND METHODS

Nine sites dominated by the target species *Cynanchum acutum* were selected as follows:

| Site | Locality               | Habitat                    |
|------|------------------------|----------------------------|
| A    | EI-Tawfiqia (Damietta) | Field edge, neglected area |
| B    | EI-Hawashem (Damietta) | Canal bank, neglected area |
| C    | New Damietta           | Sand sheet top             |
| D    | New Damietta           | Neglected sandy area       |
| E    | New Damietta           | Neglected area             |
| F    | New Damietta           | Salt marsh habitat         |
| H    | Kafr Shoker            | Field edge                 |
| I    | Kafr Shoker            | The Nile bank              |
| J    | Kafr Shoker            | Field edge                 |

Samples of *Cynanchum acutum* were collected and leaf area, blade length and width, leaf thickness, fresh weight, dry weight, water content, relative water content, leaf dry matter content, specific leaf area, succulence were determined according to Cornelissen *et al.* (2003) while leaf index was determined according to Tsukaya (2003).

The collected plant samples from each site were separated into leaves and stems and divided into two portions the first was oven-dried at 70-80°C and used for determination of carbohydrates, protein, and proline contents while the second was air-dried for estimation of total phenolic contents.

**Determination of carbohydrate content:** Carbohydrates were extracted with 80% ethanol. After centrifugation, the extract was used for total soluble carbohydrate determination using anthrone reagent (8.6 mM anthrone in 80% v/v H<sub>2</sub>SO<sub>4</sub>). The mixture was heated for 10 min at 80°C and then cooled for 30 min. Absorbance at 623 nm was recorded (Schlüter *et al.*, 2001). Total non-soluble carbohydrates were determined in the residues. An aliquot of the

residues was resuspended in 1.6 M perchloric acid and incubated at 70°C for 2 h. After centrifugation, total non-soluble carbohydrates were determined with anthrone.

**Determination of protein content:** Protein was extracted with 1N NaOH. After centrifugation, aliquots of the extract were treated with Comassie Brilliant Blue G (Bradford, 1976). The absorbance of the resulting blue colour was measured at 595 nm.

**Determination of proline content:** Proline was extracted by homogenization in 3% sulfosalicylic acid. The residue was removed by centrifugation. To aliquots of the supernatant glacial acetic acid and acid ninhydrin were added in the ratio of 1:2:2. Closed test tubes containing the reaction mixture were incubated at 100°C for 1h. The reaction was terminated in ice bath. The reaction mixture was extracted by toluene. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm (Bates *et al.*, 1973).

**Determination of phenolic content:** Total phenolics were extracted in 80% methanol. To aliquots of the extract, diluted Folin-Ciocalteu reagent (1:10, v/v) was added followed by Na<sub>2</sub>CO<sub>3</sub> (7.5% ,w/v) after 3-5 min. The mixture was incubated at 45°C for 15 min and the absorbance was read at 765 nm (Javanmardi *et al.*, 2003). Results were expressed as mg gallic acid equivalent per g of dry weight.

**Physico-chemical analysis of soil:** Composite soil samples (0-25 cm) from the studied sites were collected, dried and sieved (2 mm) after determination of soil moisture content as the difference between soil fresh and oven dry weights (105°C) as a percentage of soil fresh weight.

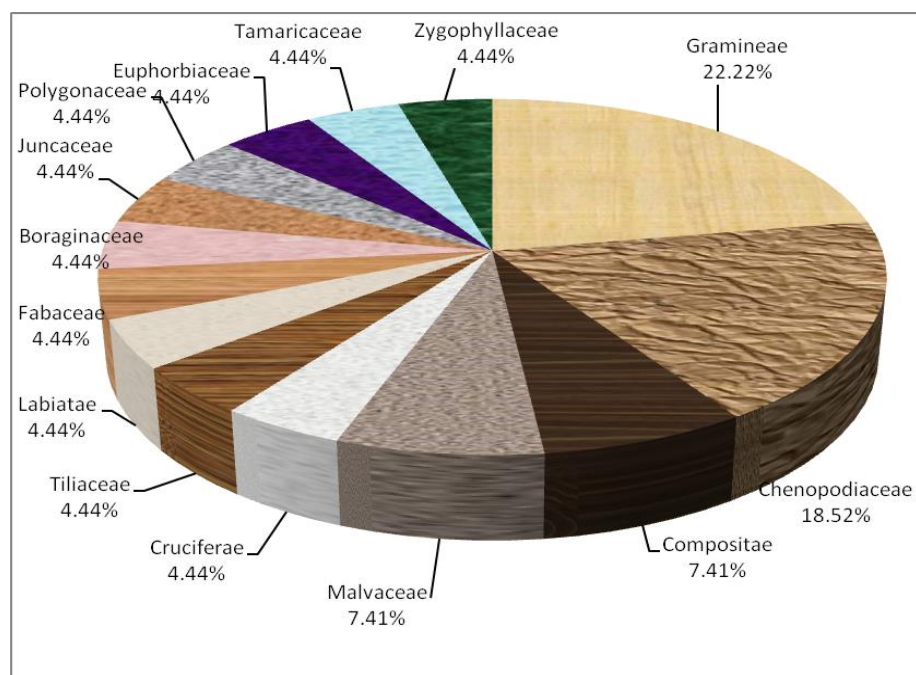
1:5 soil water extract was prepared for measurement of the pH value and determination of soluble carbonates and bicarbonates, by titration against 0.01N HCl using phenolphthalein and methyl orange as indicators respectively (Bashour and Sayegh, 2007). Chloride percentage was also determined in the extract by titration against 1/35.5 AgNO<sub>3</sub> using 5% potassium chromate indicator (Jackson, 1962).

Soil total carbonates, expressed as calcium carbonates, was determined by back-titration of HCl soil extract against sodium hydroxide (Rowell, 1994). Active calcium carbonate content was extracted with 0.2 M ammonium oxalate solution for 2 h (Bashour and Sayegh, 2007). Aliquots of the extract were titrated against 0.02 M KMnO<sub>4</sub> to the endpoint after addition of conc. H<sub>2</sub>SO<sub>4</sub> and heating to 60-70°C. The organic carbon was determined by titration according to Schulte (1995). Chromic acid oxidation was used to measure easily oxidized material in the soil. Available phosphorus was determined in the sodium bicarbonate solution (Watanab and Olsen, 1965). The blue colour formed after addition of ammonium molybdate – ascorbic acid solution was measured at 882 nm.

**Statistical analysis:** All data were calculated as mean ± standard error of mean for at least three replicates. Analysis of variance (ANOVA) was performed using SPSS program (version 11). The Post Hoc Test used to separate the means was the Tukey HSD test and the subset for  $\alpha$  was 0.05. Pearson's correlation was performed using SPSS program (version 11).

## RESULTS

In this study, *Cynanchum acutum* was collected from nine sites including neglected areas, Nile bank, canal bank, field edge and sand formations. The field study indicates that the average total number of the associated wild plant species growing with *Cynanchum acutum* was 27 species belonging to 24 genera and related to 15 families; Gramineae (22.22%), Chenopodiaceae (18.52%), Compositae (7.41%), Malvaceae (7.41%) while Cruciferae, Tiliaceae, Labiatae, Fabaceae, Boraginaceae, Juncaceae, Polygonaceae, Euphorbiaceae, Tamaricaceae and Zygophyllaceae collectively represented 44.44% (4.44% for each one) (Fig. 1).



**Fig. 1: Composition of associated species recorded with *Cynanchum acutum***

Analysis of variance between groups (ANOVA) was performed on soil variables data (Table 1). The results show that all the mean values of the tested soil variables were significantly different at  $< 0.001$  probability level except for the soil pH whose means had significant difference at a probability level of 0.035 but still smaller than  $p = 0.05$ .

**Table 1: Analysis of variance of soil variables.**

| Soil Variables                | Probability Level |
|-------------------------------|-------------------|
| Chloride                      | < 0.001           |
| Calcium Carbonate             | < 0.001           |
| Bicarbonate                   | < 0.001           |
| Soil PH                       | 0.035             |
| Soil Easily-oxidizable Carbon | < 0.001           |
| Organic Matter Content        | < 0.001           |
| Available Phosphorus          | < 0.001           |
| Soil Active Calcium Carbonate | < 0.001           |
| Moisture Content              | < 0.001           |

In table 2, the site J mostly exerted the highest values of calcium carbonate, bicarbonate, soil organic carbon, organic matter content and available phosphorus. However, chloride was highest at site F while pH was highest at site D. On the other hand, soil active CaCO<sub>3</sub> and soil moisture content were highest at site H. Mean soil chloride gradient of soil supporting *Cynanchum acutum* was high 0.06 to 2.17%. Soil calcium carbonate percentage gradient was also high ranging from 5.1 to 12.1% while soil bicarbonate percentage ranged from 0.04 to 0.21%. There was low soil pH gradient in the soil ranging from 7.1 to 7.9 being all in the very weak alkaline range. Soil organic matter content gradient was very high ranging from 0.06 in sand formations to 9.84% in field edge.

**Table 2: Soil variables of the studied areas from which *Cynanchum acutum* was collected. Data are means of at least three replica ± standard error. According to Tukey HSD test values with the same letters are non significantly different.**

|                               | A                | B                  | C               | D                 | E                 | F                 | H                  | I                 | J                 |
|-------------------------------|------------------|--------------------|-----------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|
| Chloride                      | 0.48c<br>±0.03   | 0.06d<br>±0.01     | 0.03h<br>±0.00  | 0.60gh<br>±0.01   | 0.62fg<br>±0.01   | 2.17a<br>±0.01    | 0.33 h<br>±0.01    | 1.04b<br>±0.01    | 0.36ef<br>±0.00   |
| Calcium Carbonate             | 8.13c<br>±0.33   | 8.63c<br>±0.48     | 7.63c<br>±0.17  | 5.05d<br>±0.25    | 9.10c<br>±0.30    | 11.47 b<br>±0.21  | 12.13 b<br>±0.56   | 11.13b<br>±0.21   | 14.60a<br>±0.32   |
| Bicarbonate                   | 0.05de<br>±0.01  | 0.06b<br>±0.01     | 0.04f<br>±0.01  | 0.06cd<br>±0.01   | 0.04f<br>±0.01    | 0.05ef<br>±0.01   | 0.05ef<br>±0.01    | 0.06bc<br>±0.00   | 0.21a<br>±0.01    |
| Soil PH                       | 7.27ab<br>±0.02  | 7.34b<br>±0.13     | 7.63ab<br>±0.23 | 7.86a<br>±0.18    | 7.57ab<br>±0.00   | 7.49ab<br>±0.10   | 7.52ab<br>±0.01    | 7.11b<br>±0.99    | 7.27ab<br>±0.22   |
| Soil oxidizable Carbon        | 1.49b<br>±0.04   | 1.36b<br>±0.06     | 0.02d<br>±0.01  | 0.27cd<br>±0.01   | 0.42c<br>±0.02    | 0.29cd<br>±0.02   | 1.42b<br>±0.05     | 1.62b<br>±0.03    | 3.32a<br>±0.22    |
| Organic Matter Content        | 4.41b<br>±0.10   | 4.03b<br>±0.10     | 0.06d<br>±0.02  | 0.80cd<br>±0.04   | 1.25c<br>±0.05    | 0.85cd<br>±0.07   | 4.22b<br>±0.14     | 4.82b<br>±0.10    | 9.84a<br>±0.65    |
| Available Phosphorus          | 881.00b<br>±0.00 | 647.54c<br>±27.53  | 28.50f<br>±1.50 | 151.00ef<br>±0.00 | 407.00d<br>±27.00 | 240.00e<br>±34.00 | 771.50bc<br>±41.50 | 888.00b<br>±21.00 | 2381.50a<br>±6.50 |
| Soil Active CaCO <sub>3</sub> | 1.22d<br>±0.09   | 0.4892e<br>±0.0002 | 1.58c<br>±0.06  | 1.43cd<br>±0.05   | 2.17b<br>±0.09    | 1.40cd<br>±0.14   | 2.72a<br>±0.06     | 1.56cd<br>±0.05   | 1.48cd<br>±0.00   |
| Moisture Content              | 12.75c<br>±0.18  | 6.97d<br>±0.13     | 0.54h<br>±0.02  | 2.07gh<br>±0.03   | 3.64fg<br>±0.09   | 5.81de<br>±0.07   | 23.72a<br>±0.87    | 16.37b<br>±0.56   | 4.44ef<br>±0.08   |

The available phosphorus also showed the same trend ranging from 28.5 to 2381.5 ( $\mu\text{g P/g soil}$ ). Active calcium carbonate content ranged from 0.49 to 2.72% while the soil moisture content gradient was very high ranging from 0.54 to 23.72%.

It is clear from table 3 that sites E and H were restricted to most of the functional morphological traits of *Cynanchum acutum*. There were the highest leaf length, width, area and specific area. As well the highest values of fresh weight, dry weight, leaf water content, succulence and leaf index were detected for plants collected from these sites. However, some increases for few traits were detected at some other sites. All of these traits were significantly different at a probability level of less than 0.001 (Table 4).

**Table 3: Functional morphological traits of *Cynanchum acutum* at the studied areas. Data are means of 20 samples  $\pm$  standard error. According to Tukey HSD test values with the same letters are non significantly different.**

|  | A                                 | B                                 | C                                | D                                 | E                                | F                                  | H                                  | I                                  | J                                  |
|--|-----------------------------------|-----------------------------------|----------------------------------|-----------------------------------|----------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Leaf Length (cm)                                       | 4.44 <sup>cd</sup><br>$\pm 0.17$  | 4.93 <sup>bc</sup><br>$\pm 0.21$  | 4.96 <sup>bc</sup><br>$\pm 0.13$ | 3.72 <sup>d</sup><br>$\pm 0.11$   | 6.22 <sup>a</sup><br>$\pm 0.17$  | 4.29 <sup>cd</sup><br>$\pm 0.21$   | 5.70 <sup>ab</sup><br>$\pm 0.30$   | 4.48 <sup>cd</sup><br>$\pm 0.15$   | 3.98 <sup>d</sup><br>$\pm 0.12$    |
| Leaf Width (cm)  | 5.02 <sup>bcd</sup><br>$\pm 0.22$ | 5.32 <sup>bc</sup><br>$\pm 0.20$  | 4.94 <sup>cd</sup><br>$\pm 0.13$ | 3.89 <sup>e</sup><br>$\pm 0.14$   | 5.90 <sup>ab</sup><br>$\pm 0.15$ | 4.62 <sup>cdde</sup><br>$\pm 0.26$ | 6.63 <sup>a</sup><br>$\pm 0.35$    | 5.06 <sup>bcd</sup><br>$\pm 0.15$  | 4.32 <sup>de</sup><br>$\pm 0.17$   |
| Leaf Thickness (mm)                                    | 0.69 <sup>a</sup><br>$\pm 0.12$   | 0.37 <sup>c</sup><br>$\pm 0.01$   | 0.58 <sup>ab</sup><br>$\pm 0.02$ | 0.35 <sup>c</sup><br>$\pm 0.02$   | 0.40 <sup>bc</sup><br>$\pm 0.01$ | 0.42 <sup>bc</sup><br>$\pm 0.02$   | 0.26 <sup>bc</sup><br>$\pm 0.02$   | 0.15 <sup>cd</sup><br>$\pm 0.00$   | 0.40 <sup>d</sup><br>$\pm 0.00$    |
| Leaf Area (mm <sup>2</sup> )                           | 2059 <sup>b</sup><br>$\pm 161$    | 1811 <sup>b</sup><br>$\pm 157$    | 1917 <sup>b</sup><br>$\pm 101$   | 1044 <sup>b</sup><br>$\pm 66$     | 3870 <sup>a</sup><br>$\pm 659$   | 1869 <sup>b</sup><br>$\pm 183$     | 4011 <sup>a</sup><br>$\pm 397$     | 1894 <sup>b</sup><br>$\pm 130$     | 1371 <sup>b</sup><br>$\pm 99$      |
| Specific Leaf Area (mm <sup>2</sup> mg <sup>-1</sup> ) | 16.36 <sup>bc</sup><br>$\pm 0.45$ | 20.84 <sup>ab</sup><br>$\pm 1.28$ | 12.62 <sup>c</sup><br>$\pm 0.78$ | 15.85 <sup>bc</sup><br>$\pm 0.62$ | 24.74 <sup>a</sup><br>$\pm 3.88$ | 13.55 <sup>c</sup><br>$\pm 0.54$   | 17.19 <sup>bc</sup><br>$\pm 0.84$  | 26.96 <sup>a</sup><br>$\pm 1.60$   | 25.59 <sup>a</sup><br>$\pm 0.99$   |
| Leaf Fresh Weight (g)                                  | 0.84 <sup>bcd</sup><br>$\pm 0.06$ | 0.49 <sup>de</sup><br>$\pm 0.04$  | 0.86 <sup>bc</sup><br>$\pm 0.07$ | 0.35 <sup>e</sup><br>$\pm 0.03$   | 1.06 <sup>b</sup><br>$\pm 0.06$  | 0.57 <sup>cde</sup><br>$\pm 0.05$  | 1.63 <sup>a</sup><br>$\pm 0.06$    | 0.43 <sup>e</sup><br>$\pm 0.04$    | 0.73 <sup>e</sup><br>$\pm 0.04$    |
| Leaf Dry Weight (g)                                    | 0.13 <sup>bc</sup><br>$\pm 0.01$  | 0.09 <sup>cd</sup><br>$\pm 0.00$  | 0.16 <sup>b</sup><br>$\pm 0.01$  | 0.07 <sup>d</sup><br>$\pm 0.01$   | 0.15 <sup>b</sup><br>$\pm 0.01$  | 0.14 <sup>bc</sup><br>$\pm 0.01$   | 0.24 <sup>a</sup><br>$\pm 0.03$    | 0.07 <sup>d</sup><br>$\pm 0.01$    | 0.05 <sup>d</sup><br>$\pm 0.00$    |
| Leaf Water Con (%)                                     | 84.98 <sup>ab</sup><br>$\pm 0.38$ | 81.26 <sup>cd</sup><br>$\pm 0.56$ | 80.48 <sup>d</sup><br>$\pm 0.70$ | 80.51 <sup>d</sup><br>$\pm 0.45$  | 85.46 <sup>a</sup><br>$\pm 0.42$ | 75.10 <sup>e</sup><br>$\pm 1.23$   | 83.88 <sup>abc</sup><br>$\pm 0.56$ | 82.44 <sup>bcd</sup><br>$\pm 0.74$ | 83.40 <sup>abcd</sup><br>$\pm 0.6$ |
| Leaf Relative Water Con (%)                            | 89.83 <sup>a</sup><br>$\pm 1.25$  | 71.56 <sup>b</sup><br>$\pm 2.02$  | 74.01 <sup>b</sup><br>$\pm 1.65$ | 83.85 <sup>a</sup><br>$\pm 1.23$  | 72.08 <sup>b</sup><br>$\pm 1.67$ | 71.34 <sup>b</sup><br>$\pm 3.39$   | 71.52 <sup>b</sup><br>$\pm 2.00$   | 56.20 <sup>c</sup><br>$\pm 2.36$   | 56.09 <sup>c</sup><br>$\pm 1.68$   |
| Leaf Dry Matter (mg g <sup>-1</sup> )                  | 136.5 <sup>bc</sup><br>$\pm 3.5$  | 140.4 <sup>b</sup><br>$\pm 4.0$   | 151.6 <sup>b</sup><br>$\pm 5.6$  | 169.7 <sup>a</sup><br>$\pm 3.6$   | 107.4 <sup>d</sup><br>$\pm 2.1$  | 185.7 <sup>a</sup><br>$\pm 6.6$    | 119.3 <sup>cd</sup><br>$\pm 2.6$   | 106.4 <sup>de</sup><br>$\pm 2.7$   | 99.6 <sup>e</sup><br>$\pm 3.4$     |
| Succulence   | 6.75 <sup>ab</sup><br>$\pm 0.18$  | 5.44 <sup>cd</sup><br>$\pm 0.18$  | 5.24 <sup>d</sup><br>$\pm 0.18$  | 5.19 <sup>d</sup><br>$\pm 0.13$   | 7.00 <sup>a</sup><br>$\pm 0.22$  | 4.19 <sup>e</sup><br>$\pm 0.19$    | 6.34 <sup>abc</sup><br>$\pm 0.20$  | 5.91 <sup>bcd</sup><br>$\pm 0.27$  | 6.18 <sup>abc</sup><br>$\pm 0.24$  |
| Leaf Index   | 0.88 <sup>cd</sup><br>$\pm 0.02$  | 0.93 <sup>bcd</sup><br>$\pm 0.02$ | 1.01 <sup>ab</sup><br>$\pm 0.02$ | 0.96 <sup>bc</sup><br>$\pm 0.02$  | 1.056 <sup>a</sup><br>$\pm 0.01$ | 0.94 <sup>bcd</sup><br>$\pm 0.02$  | 0.86 <sup>d</sup><br>$\pm 0.01$    | 0.89 <sup>cd</sup><br>$\pm 0.03$   | 0.93 <sup>bcd</sup><br>$\pm 0.01$  |

As shown in table 5, there were great differences in phenolic contents among the nine studied sites. They were highest in leaf and stem of plants collected from the sites C and F. Nevertheless, greater amounts were detected in leaves than in stem. Similarly, protein was higher in leaves relative to stem. The sites I, J and to some extent, site H had plants with the highest protein contents either in leaves and stem. On the other hand, soluble sugars were highest in leaves and stems at sites I and D respectively whereas non-soluble were much greater at sites A and C, respectively than the other sites. Regarding proline, there were varied among the different sites, highest at the site J and least at site C.

**Table 4: Analysis of variance of *Cynanchum acutum* functional traits.**

| Morphological Traits        | Probability Level | Physiological Traits   | Probability Level |
|-----------------------------|-------------------|------------------------|-------------------|
| Leaf Length                 | < 0.001           | Leaf Phenolic          | < 0.001           |
| Leaf Width                  | < 0.001           | Stem Phenolic          | < 0.001           |
| Leaf Thickness              | < 0.001           | Leaf Protein           | < 0.001           |
| Leaf Area                   | < 0.001           | Stem Phenolic          | < 0.001           |
| Specific Leaf Area          | < 0.001           | Leaf Soluble Sugars    | < 0.001           |
| Leaf Fresh Weight           | < 0.001           | Stem Soluble Sugars    | < 0.001           |
| Leaf Dry Weight             | < 0.001           | Leaf Nonsoluble Sugars | < 0.001           |
| Leaf Water Content          | < 0.001           | Leaf Proline Content   | < 0.001           |
| Leaf Relative Water Content | < 0.001           |                        |                   |
| Leaf Dry Matter             | < 0.001           |                        |                   |
| Succulence                  | < 0.001           |                        |                   |
| Leaf Index                  | < 0.001           |                        |                   |

**Table 5: Functional physiological traits of *Cynanchum acutum* at the studied areas. Data are represented as means of at least three samples  $\pm$  standard error of the mean. According to Tukey HSD test values with the same letters are non significantly different.**

|                        | A                                  | B                                 | C                                 | D                                 | E                                  | F                                 | H                                | I                                  | J                                |
|------------------------|------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|-----------------------------------|----------------------------------|------------------------------------|----------------------------------|
| Leaf Phenolic          | 6547 <sup>c</sup><br>$\pm 3$       | 3914 <sup>d</sup><br>$\pm 63$     | 14101 <sup>a</sup><br>$\pm 208$   | 14293 <sup>a</sup><br>$\pm 284$   | 6839 <sup>c</sup><br>$\pm 154$     | 15118 <sup>a</sup><br>$\pm 322$   | 5909 <sup>c</sup><br>$\pm 84$    | 11799 <sup>b</sup><br>$\pm 455$    | 11319 <sup>b</sup><br>$\pm 102$  |
| Stem Phenolic          | 3152 <sup>ef</sup><br>$\pm 60$     | 2599 <sup>g</sup><br>$\pm 39$     | 6435 <sup>a</sup><br>$\pm 135$    | 4106 <sup>c</sup><br>$\pm 61$     | 5024 <sup>b</sup><br>$\pm 101$     | 5073 <sup>b</sup><br>$\pm 72$     | 3517 <sup>d</sup><br>$\pm 50$    | 2998 <sup>f</sup><br>$\pm 39$      | 3447 <sup>de</sup><br>$\pm 5$    |
| Leaf Protein           | 19.28 <sup>f</sup><br>$\pm 0.35$   | 22.96 <sup>d</sup><br>$\pm 0.38$  | 14.83 <sup>g</sup><br>$\pm 0.28$  | 22.46 <sup>d</sup><br>$\pm 0.30$  | 22.35 <sup>de</sup><br>$\pm 0.18$  | 20.05 <sup>ef</sup><br>$\pm 0.16$ | 25.58 <sup>c</sup><br>$\pm 0.23$ | 32.29 <sup>a</sup><br>$\pm 0.76$   | 29.81 <sup>b</sup><br>$\pm 0.94$ |
| Stem Protein           | 15.48 <sup>cde</sup><br>$\pm 0.74$ | 18.11 <sup>ab</sup><br>$\pm 0.38$ | 13.06 <sup>ef</sup><br>$\pm 0.58$ | 15.80 <sup>bcd</sup><br>$\pm 0.6$ | 14.16 <sup>def</sup><br>$\pm 0.49$ | 12.78 <sup>f</sup><br>$\pm 0.40$  | 18.93 <sup>a</sup><br>$\pm 0.50$ | 17.63 <sup>abc</sup><br>$\pm 0.24$ | 20.00 <sup>a</sup><br>$\pm 0.50$ |
| Leaf Soluble Sugars    | 19.00 <sup>c</sup><br>$\pm 0.03$   | 4.12 <sup>f</sup><br>$\pm 0.12$   | 28.99 <sup>b</sup><br>$\pm 0.48$  | 13.10 <sup>d</sup><br>$\pm 0.02$  | 19.11 <sup>c</sup><br>$\pm 0.43$   | 29.34 <sup>b</sup><br>$\pm 0.33$  | 7.90 <sup>e</sup><br>$\pm 0.09$  | 47.78 <sup>a</sup><br>$\pm 0.78$   | 8.51 <sup>e</sup><br>$\pm 0.20$  |
| Stem Soluble Sugars    | 51.06 <sup>c</sup><br>$\pm 0.18$   | 44.92 <sup>d</sup><br>$\pm 1.37$  | 61.00 <sup>b</sup><br>$\pm 0.60$  | 101.91 <sup>a</sup><br>$\pm 0.73$ | 60.12 <sup>b</sup><br>$\pm 0.15$   | 61.83 <sup>b</sup><br>$\pm 2.14$  | 57.44 <sup>b</sup><br>$\pm 0.15$ | 58.55 <sup>b</sup><br>$\pm 1.04$   | 30.98 <sup>e</sup><br>$\pm 1.32$ |
| Leaf Nonsoluble Sugars | 359.0 <sup>a</sup><br>$\pm 3.2$    | 198.5 <sup>ef</sup><br>$\pm 3.41$ | 310.8 <sup>bc</sup><br>$\pm 8.8$  | 290.6 <sup>c</sup><br>$\pm 7.7$   | 238.3 <sup>d</sup><br>$\pm 1.2$    | 317.7 <sup>b</sup><br>$\pm 4.8$   | 194.4 <sup>f</sup><br>$\pm 2.7$  | 221.9 <sup>de</sup><br>$\pm 4.5$   | 232.7 <sup>d</sup><br>$\pm 6.8$  |
| Leaf Proline Content   | 144.1 <sup>h</sup><br>$\pm 4.7$    | 380.9 <sup>d</sup><br>$\pm 2.3$   | 103.9 <sup>i</sup><br>$\pm 1.5$   | 323.1 <sup>e</sup><br>$\pm 6.3$   | 250.6 <sup>f</sup><br>$\pm 5.3$    | 174.8 <sup>g</sup><br>$\pm 4.0$   | 615.9 <sup>b</sup><br>$\pm 5.6$  | 415.4 <sup>c</sup><br>$\pm 9.3$    | 771.3 <sup>a</sup><br>$\pm 4.7$  |

## DISCUSSION

Invasion of weeds to natural ecosystems is considered an activity of damaging consequences to biodiversity and functioning of the native habitat (Downey *et al.*, 2009; Shabbir and Bajwa, 2006; Suding *et al.*, 2004). *Cynanchum acutum* was recorded for the first time in South Sinai in 1995-96 (Abd El-Ghani and Fahmy, 1998). Between October 2004 and November 2005, *Cynanchum acutum* L. was recorded as a ubiquitous species in the four Egyptian new industrial cities: 6th October, El-Sadat, Burg El-Arab and 10th Ramadan (Abd El-Ghani *et al.*, 2011). On the other hand, *Cynanchum acutum* is recorded in one cluster in the salt marsh habitat of the deltaic

Mediterranean coast (Zahran *et al.*, 1990). However, Shaltout and El-Sheikh (2003) reported that Egypt can be categorised into 11 vegetation groups in four of which *Cynanchum acutum* was recorded with a cover equal to or more than 0.1 m/100 m.

The present results showed that phenolics content was positively correlated with soil chloride percentage suggesting a possible role of plant phenolics in the plant tolerance to high salinity levels. Phenolics contribution in response of diverse types of biotic and abiotic stresses is documented (Michalak, 2005). The negatively significant correlation coefficient results between soil moisture content and total phenolics (Table 7) should also support this assumption. Ability of plants to detoxify radicals in response to salinity stress is probably the most critical requirement (Parida and Das, 2005). Phenolics, for their well known antioxidant activities, may participate actively in plant withstanding of the reactive oxygen species damaging effect. Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Ksouri *et al.*, 2007). These findings support the observed wide ecological amplitude of *Cynanchum acutum* and especially the presence in the salt marsh habitats (Zahran *et al.*, 1990).

**Table (6A): Pearson correlation between soil variables in the studied areas.**

|                               | Chloride             | Calcium Carbonate  | Bicarbon-ate         | Soil pH              | Soil oxidizable Carbon | Organic Matter Content | Available Phosphorus | Moisture Content |
|-------------------------------|----------------------|--------------------|----------------------|----------------------|------------------------|------------------------|----------------------|------------------|
| Calcium Carbonate             | 0.22 <sup>NS</sup>   |                    |                      |                      |                        |                        |                      |                  |
| Bicarbonate                   | - 0.16 <sup>NS</sup> | <b>0.61 **</b>     |                      |                      |                        |                        |                      |                  |
| Soil PH                       | - 0.05 <sup>NS</sup> | <b>- 0.46*</b>     | - 0.27 <sup>NS</sup> |                      |                        |                        |                      |                  |
| Soil oxidizable Carbon        | - 0.27 <sup>NS</sup> | <b>0.67**</b>      | <b>0.83**</b>        | <b>- 0.53**</b>      |                        |                        |                      |                  |
| Organic Matter Content        | - 0.27 <sup>NS</sup> | <b>0.67**</b>      | <b>0.83**</b>        | <b>- 0.53**</b>      | <b>1**</b>             |                        |                      |                  |
| Available Phosphorus          | - 0.19 <sup>NS</sup> | <b>0.734 **</b>    | <b>0.91**</b>        | - 0.38 <sup>NS</sup> | <b>0.96**</b>          | 0.96**                 |                      |                  |
| Soil Active CaCO <sub>3</sub> | - 0.02 <sup>NS</sup> | 0.27 <sup>NS</sup> | - 0.15 <sup>NS</sup> | 0.23 <sup>NS</sup>   | - 0.09 <sup>NS</sup>   | - 0.09 <sup>NS</sup>   | - 0.05 <sup>NS</sup> |                  |
| Moisture Content              | - 0.01 <sup>NS</sup> | <b>0.35*</b>       | - 0.19 <sup>NS</sup> | - 0.33 <sup>NS</sup> | 0.30 <sup>NS</sup>     | 0.30 <sup>NS</sup>     | 0.18 <sup>NS</sup>   | 0.43*            |

\*= P < 0.05, \*\*= P < 0.01 and NS= Not significant.

Soil calcium carbonate, bicarbonate and soil pH showed some significant correlation coefficients with physiological variables of *Cynanchum acutum*. Raising the pH of soil is proved to inversely affect the concentrations of major and minor elements in the biomass of *Agrostis capillaris* (Tyler and Olsson, 2001). On the other hand, proline content was positively correlated with both soil calcium carbonate and bicarbonate (Table 7). Proline is known to accumulate in a variety of plant species as a response to various abiotic



stresses including drought, salinity, extreme temperatures, UV radiations and heavy metals (Ashraf and Foolad, 2005). In addition, there was a close correlation between protein content and soil calcium carbonate and bicarbonate. Proteins are known also to be produced in stressed plants in response to various sources of stress, their role includes protein stabilization, water binding/slow desiccation rates; chaperones; protein/membrane stabilization; ion sequestration, reversal/prevention of protein unfolding and translational modulation (Cushman and Bohnert, 2000). Meanwhile, soil organic matter and available phosphorus correlated well with various physiological variables. Both of organic matter and available phosphorus are indicators of soil fertility.

**Table (6B): Pearson correlation between morphological traits of *Cynanchum acutum*.**

|                    | LL           | LW           | LTH          | LA           | SLA          | LFW          | LDW          | LWC          | FTW          | LRWC       | LDMC         | SUC        |
|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|------------|--------------|------------|
| Leaf Length        | 0.88<br>**   |              |              |              |              |              |              |              |              |            |              |            |
| Leaf Width         | 0.18<br>*    | 0.20<br>**   |              |              |              |              |              |              |              |            |              |            |
| Leaf Thickness     | 0.71<br>**   | 0.71<br>**   | 0.15<br>*    |              |              |              |              |              |              |            |              |            |
| Leaf Area          | 0.08<br>NS   | 0.05<br>NS   | - 0.27<br>** | 0.46<br>**   |              |              |              |              |              |            |              |            |
| Specific Leaf Area | 0.78<br>**   | 0.82<br>**   | 0.30<br>**   | 0.71<br>**   | - 0.15<br>*  |              |              |              |              |            |              |            |
| Leaf Fresh Weight  | 0.75<br>**   | 0.80<br>**   | 0.33<br>**   | 0.68<br>**   | - 0.27<br>** | 0.95<br>**   |              |              |              |            |              |            |
| Leaf Dry Weight    | 0.34<br>**   | 0.33<br>**   | 0.05<br>NS   | 0.29<br>**   | 0.33<br>**   | 0.39<br>**   | 0.12<br>NS   |              |              |            |              |            |
| Leaf Water Con     | 0.82<br>**   | 0.85<br>**   | 0.23<br>**   | 0.74<br>**   | - 0.10<br>NS | 0.98<br>**   | 0.95<br>**   | 0.32<br>**   |              |            |              |            |
| Leaf Rel Water Con | - 0.03<br>NS | - 0.02<br>NS | 0.39<br>**   | 0.01<br>NS   | - 0.33<br>** | 0.23<br>**   | 0.15<br>*    | 0.31<br>**   | 0.05<br>NS   |            |              |            |
| Leaf Dry Matter    | - 0.37<br>** | - 0.38<br>** | 0.19<br>*    | - 0.30<br>** | - 0.51<br>** | - 0.25<br>** | - 0.06<br>NS | - 0.71<br>** | - 0.31<br>** | 0.42<br>** |              |            |
| Succulence         | 0.38<br>**   | 0.36<br>**   | 0.06<br>NS   | 0.33<br>**   | 0.35<br>**   | 0.43<br>**   | 0.16<br>*    | 0.94<br>**   | 0.37<br>**   | 0.27<br>** | - 0.70<br>** |            |
| Leaf Index         | 0.23<br>**   | - 0.25<br>** | - 0.03<br>NS | - 0.01<br>NS | 0.03<br>NS   | - 0.06<br>NS | - 0.06<br>NS | 0.01<br>NS   | - 0.06<br>NS | 0.01<br>NS | 0.08<br>NS   | 0.02<br>NS |

\*= P < 0.05, \*\*= P < 0.01 and NS= Not significant.

Soil available phosphorus positively correlated with protein content (Table 7). Phosphorus is an important macronutrient, it is a component of key molecules such as nucleic acids, phospholipids, and ATP, and, consequently, plants cannot grow without a reliable supply of this nutrient (Schachtman, 1998). Smolders *et al.* (1996) reported serious impair of growth in phosphorus deficient seedlings of *Stratiotes aloides* L. accompanied by seriously disturbance of net protein synthesis. Nonetheless, the negative correlation between stem phenolic content and both soil organic matter and available phosphorus could suggest the increase in stem phenolics with decreasing soil fertility. Leaf phenolic content, in contrast, did not show any

significant linear correlation with both soil variables. This finding is important in attempts to use the natural phenolic compounds produced by the plant as the massive biomass found in non fertile neglected desert areas could serve well in this purpose.

**Table 7. Pearson correlation between functional physiological traits of *Cynanchum acutum* and the soil variables in the studied areas.**

|                        | Chloride  | CaCO <sub>3</sub> | HCO <sub>3</sub> <sup>-1</sup> | Soil PH   | Soil oxidizable Carbon | Organic Matter Content | Available P | Soil Active CaCO <sub>3</sub> | Moisture Content |
|------------------------|-----------|-------------------|--------------------------------|-----------|------------------------|------------------------|-------------|-------------------------------|------------------|
| Leaf Phenolic          | 0.49 **   | - 0.08 NS         | 0.08 NS                        | 0.23 NS   | - 0.31 NS              | - 0.31 NS              | - 0.19 NS   | - 0.07 NS                     | - 0.46 *         |
| Stem Phenolic          | 0.15 NS   | - 0.28 NS         | - 0.28 NS                      | 0.44*     | - 0.68**               | - 0.68**               | - 0.54*     | 0.24 NS                       | - 0.56**         |
| Leaf Protein           | 0.08 NS   | 0.61**            | 0.52**                         | - 0.42*   | 0.70**                 | 0.70**                 | 0.66**      | 0.12 NS                       | 0.45*            |
| Stem Protein           | - 0.39*   | 0.53**            | 0.59**                         | - 0.30 NS | 0.82**                 | 0.82**                 | 0.72**      | 0.04 NS                       | 0.47*            |
| Leaf Soluble Sugars    | 0.51**    | - 0.04 NS         | - 0.31 NS                      | - 0.21 NS | - 0.26 NS              | - 0.26 NS              | - 0.27 NS   | - 0.02 NS                     | 0.06 NS          |
| Stem Soluble Sugars    | 0.19 NS   | - 0.72**          | -0.55**                        | 0.57**    | - 0.69**               | - 0.69 **              | - 0.69**    | 0.15 NS                       | - 0.20 NS        |
| Leaf Nonsoluble Sugars | 0.29 NS   | - 0.44*           | - 0.24 NS                      | 0.19 NS   | - 0.39*                | - 0.39*                | - 0.36      | - 0.21NS                      | - 0.37 NS        |
| Leaf Proline Content   | - 0.24 NS | 0.69 **           | 0.73**                         | - 0.21 NS | 0.80**                 | 0.80**                 | 0.78**      | 0.29 NS                       | 0.36 NS          |

\*= P< 0.05, \*\*= P< 0.01 and NS= Not significant.

In conclusion, morphological traits provide relatively easily recognizable indicators for different edaphic variables. This might provide information about the soil characteristics supporting the plant growth in addition to possible expectations of the internal chemical composition of the plant including the phenolic content that might be of considerable medicinal value. The present findings tend to indicate that both the differential growth and the physiological function of *Cynanchum acutum* might be attributed to the varied edaphic factors and would be a consequence of variation in soil characteristics. Moreover, the obtained results also introduce a picture of supporting future control strategies and enables efficient management of this agricultural and natural area invader.

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**دراسة بيئية فسيولوجية على العشب الغازي *Cynanchum acutum***  
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تم تجميع نبات المديد (*Cynanchum acutum*) من تسعة مواقع مختلفة بمحافظتي دمياط والقليوبية. و قد وجد نبات المديد مترافقا مع 27 نوعا ينتمون إلى 24 جنس و 15 فصيلة. و قد أظهر تحليل التباين أن المتغيرات الخاصة بتحليل التربة اختلفت معنويا عند مستوى دلالة أقل من 0.001 فيما عدا درجة الأس الهيدروجيني pH. و قد تم الحصول على نفس النتائج الإحصائية فيما يتعلق بالصفات الوظيفية الظاهرية و الفسيولوجية لنبات المديد حيث كانت كلها مختلفة اختلافا معنويا عند مستوى دلالة أقل من 0.001 و من ناحية أخرى فقد وجدت اختلافات كبيرة في محتوى النبات من الكربوهيدرات و البروتين و المواد الفينولية إضافة إلى البرولين بين المواقع المختلفة. و قد أمكن الحصول على علاقات مختلفة تربط بين الصفات الوظيفية الظاهرية و الفسيولوجية من جهة و متغيرات التربة من جهة أخرى. ارتبط محتوى النبات من المواد الفينولية إيجابيا مع نسبة الكلوريد في التربة بينما ارتبط سلبيا مع المحتوى المائي للتربة. و من ناحية أخرى فقد ارتبط محتوى النبات من البرولين مع محتوى التربة من كربونات الكالسيوم و البيكربونات بينما وجد ارتباط وثيق بين المادة العضوية في التربة و العديد من المتغيرات الفسيولوجية. أثبت النتائج أيضا وجود ارتباط إيجابي بين الفوسفور المتاح في التربة و المحتوى البروتيني للنبات. هذه النتائج توفر معلومات عن خواص التربة التي ينمو بها هذا النبات إضافة إلى توقعات للتركيب الداخلى للنبات. تلك الارتباطات الوثيقة ربما توضح أن التباين في النمو و الوظائف الفسيولوجية لنبات المديد هو نتيجة للتباين في التركيب الكيميائي للتربة.

**قام بتحكيم البحث**

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