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## Ecology and Phytochemistry of Stinking Chamomile (*Anthemis cotula* L.) in Egypt

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### ABSTRACT

The present study comprises a detailed ecological study on *Anthemis cotula* community type. Vegetation analysis showed that most of the associated species are annual weeds, mainly therophytes and Mediterranean taxa. The soil supporting *A. cotula* was wet, essentially fine sand, non-saline and neutral-slightly alkaline. Biochemical analysis of the stinking chamomile revealed that, it contained many metabolic products have a commercial application in pharmaceutical, cosmetics and food craft. The main components being flavonoids, polyphenols and volatile oils. According to the present study; *A. cotula* is over wintering weed aggressively spread through fields of winter crops in El Dakahlia Governorate. It has nutritive value of 244.39 cal/100 g in addition to many bioactive metabolic products. Thus, it may be concluded how far the economic future of *A. cotula*.

**Key words:** Anatomy, flavonoids, polyphenols, volatile oils, *Anthemis*

### INTRODUCTION

*Anthemis* L., is the second largest genus of tribe Anthemideae (family Asteraceae). It comprises a heterogenous assemblage of annual and perennial herbs with beautiful and attractive capitula. Some species of *Anthemis* are economically important being used as herbal tea to cure anxiety, flatulence, stomach disorders and toothache (Vakili *et al.*, 2013; Ghafoor, 2010). Eight investigated *Anthemis* taxa contained essential oils ranged from 0.01-0.13% (Saroglou *et al.*, 2006).

*Anthemis cotula* is one of the best phytochemically active plants of family Asteraceae; polyacetylenes flavonoid and sesquiterpene lactones are the main classes of secondary metabolites of the genus (Vuckovic *et al.*, 2006; Williams *et al.*, 2001). Two endemic *Anthemis* species in Turkey have shown antioxidant and antimicrobial activities (Albayrak and Akosy, 2013).

Rashid and Reshi (2010) found that, natural populations of *A. cotula* growing in habitats differ in disturbance. Number of disc florets per plant and per capitulum and number of capitula per plant (used as the measure of fitness) was highest in riparian populations and lowest in populations growing in habitats with relatively low disturbance. The phenotypic plasticity has been reported as the primary mechanism

enabling aliens to colonize environmentally diverse habitats (Valladares *et al.*, 2006; Sultan, 2004; Parker *et al.*, 2003).

The objectives of this study were to give a detail account on *A. cotula* with respect to morphological, anatomical, taxonomical and biogeographical features and the favoring environmental conditions for growth and phytosociological behavior. Phytochemical analyses was conducted to look at the different metabolic products of the plant.

### MATERIALS AND METHODS

**Macromorphology:** The morphological features of *A. cotula* were described according to Foster and Gifford (1974), Hickey (1979) and LAWG (1999).

**Micromorphology:** For anatomical investigation, thin sections of stem, leaf and root were prepared according to Peacock and Bradbury (1973), then examined by light microscope and photographed.

### Ecological characteristics (vegetation analyses)

**Analytical characters:** *Anthemis cotula* community was surveyed in El Dakahlia Governorate. One representative stand was studied to detect the analytical characters using 5 random quadrats (1 m<sup>2</sup> each).

**Synthetic characters:** Twenty stands were chosen to look at the floristic components. Each species was assigned by two figures (cover-abundance estimate and phenological aspect) as described by Kent and Coker (1992). Identification, classification, life-span, life-forms and chorotypes for the species were followed (Boulos, 1995, 1999, 2000, 2002, 2005).

**Soil analyses:** Twenty soil samples representing the different stands of *A. cotula* community were collected, air dried and analyzed according the methods described by Klute (1986), Margesin and Schinner (2005), Pansu and Gautheyrou (2006) and Carter and Gregorich (2007). The mean values of soil variables and standard error were calculated.

**Bioactive constituents:** For the phytochemical analysis, the plant samples were collected, handley cleaned, air dried and ground to fine powder. The mean values of primary metabolites were determined as described by Harborne (1973), Trease and Evans (1989), Sofowora (1993), Sadasivam and Manickam (2008) and AOAC (2000). The nutritive value was calculated as cited by Indrayan *et al.* (2005). Quantitative estimation of the mean values polyphenols and flavonoids were follow (Boham and Kocipai-Abyazan, 1994; Sadasivam and Manickam, 2008). The determination of volatile oils and identification of its components were performed using GC/MS (Adams, 2007).

## RESULTS AND DISCUSSION

*Anthemis cotula* L. is a winter-spring active annual, herbaceous weed belonging to family Asteraceae (subfamily: Asteroideae, tribe: Anthemideae and subtribe: Anthemidineae). It is a floral element originated and adapted to the Mediterranean climate and is native to Southern Europe-West Siberia (Erneberg, 1999; Josifovic, 1975). It has been widely introduced as a weed of cultivation in temperate areas (Christensen, 1992).

The genus *Anthemis* is represented by 12 species in Egypt. *Anthemis cotula* is found in the Nile region and Eastern Desert (Boulos, 2002).

**Macromorphology:** *Anthemis cotula* is commonly referred to as stinking chamomile (ribyaan in arabic). It is an over wintering weed growing to 18 inches tall. It is an invasive species and aggressively spread through fields of winter crops. It is erect, herbaceous weed, with pent angular, grabrescent and corymbosely stem. Leaves are finely pinnatisect. Inflorescences are solitary, terminal capitula with convex receptacle. Capitulum is heterogeneous with yellow disc florets and white ray ligulate florets (Fig. 1).

**Micromorphology:** Microscopic examination of the cross-sections in stem, leaf and root of *A. cotula* revealed that, the stem was circular and wavy in outline (Fig. 2). It showed secondary thickening. The epidermis is a single layer of



Fig. 1: Dense growth of *Anthemis cotula* plant at the edge of Phaseolus field near El Mansoura

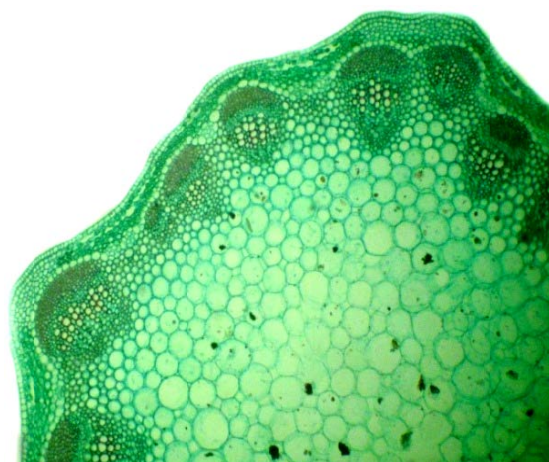


Fig. 2: Cross section in stem of *Anthemis cotula*

oval-rectangular cells with the outer wall lightly cutinized. Collenchyma zone present just beneath the epidermis, followed by few parenchymatous cortical cells. The innermost layer is the endodermis. The collateral vascular bundles are arranged in a ring.

The sclerenchymatous pericyclic cap is found above each vascular bundle. The inter vascular cambium gives few secondary tissues, as character of herbaceous dicot stems. A large parenchymatous pith is seen in the center.

The transverse section of *A. cotula* leaf (Fig. 3) showed that, both epidermis consist of oval cells. The leaf of bifacial type with the mesophyll of one-layered palisade parenchyma and isodiametric spongy parenchyma. The midrib forms a projection part at the abaxial surface while the adaxial surface is flat. The bundle sheath is made of hexagonal cells.

The root is circular in transverse section (Fig. 4). It grows in thickness by development of secondary tissues.

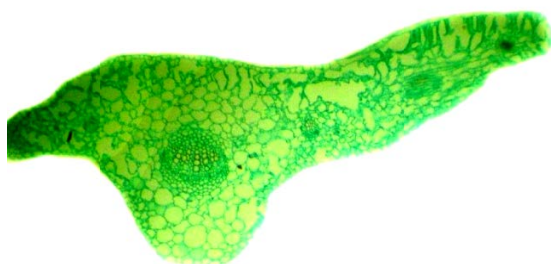


Fig. 3: Cross sections in leaf of *Anthemis cotula*

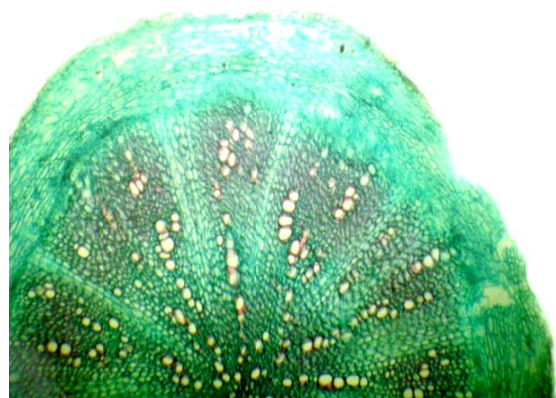


Fig. 4: Cross section in *Anthemis cotula* root

Multilayered periderm is formed in the cortical zone. The secondary vascular tissues form a continuous cylinder. The central zone occupied by narrow pith. The results of the present study are agree with that of Metcalfe and Chalk (1979) and Dickison (2000).

#### Ecological characteristics (vegetation analysis)

**Analytical characters:** Table 1 gives the data of vegetation that was analyzed in detail on 10 March 2014, using five random quadrats (1 m<sup>2</sup> each). *Anthemis cotula* is dominant and has importance value of 122.7. It has the highest values of relative frequency, relative density, relative cover and relative biomass. *Rumex dentatus* is abundant member being present in all quadrats and has importance value of 44.6. *Capsella bursa-pastoris*, *Anagallis arvensis*, *Convolvulus arvensis* and *Chenopodium murale* are common associates with importance values ranged between 26 and 36. Other nine associates were recorded in the fine quadrats having IV = 4.5-22.3. Comparable plant communities have been described by Nile Delta by El-Shazly (2013) and El-Ashri (1996).

**Synthetic characters:** Table 2 gives the floristic composition of fifteen stands representing this community in El Dakahlia Governorate. It is obvious that *A. cotula* is the dominant, has presence value of 100% and the highest cover-abundance

estimate. This community type comprised 27 plant species belonging 16 families. Poaceae, Brassicaceae, Apiaceae and Asteraceae are major families as they contribute more than 60% of the species. This agrees with the studies of Shaltout *et al.* (1992) and Sadek (2008).

Of these species 77.8% are dicot and 22.2% are monocots. On the basis of life-span, the floristic components are 22 annuals and 5 perennials. Also classified according to their life-forms into 23 therophytes, 3 geophytes and one hemicryptophyte. It is worth to mention that, the floristic composition of *A. cotula* community is mainly represented by therophytes (85%). The dominance of therophytes may be due to their life cycles that enable them to resist the instability of the cultivation system and have the ability to produce heteromorphic seeds. Therophytes are less adapted to drought and salinity and their presence is a seasonal phenomenon. They are abundant during the rainy season only. Comparing the obtained results with findings of many authors such as El-Sheikh (1996) reported 59%, El-Kady *et al.* (2000) reported 68% and Sadek (2008) reported 54%. Mediterranean taxa are the main chorotype (13 species) followed by cosmopolitan (10 species) Irano-Toronian (10 species) and Euro-Siberian (9 species) in addition to other three categories are poorly represented. This is confirmed by Zaki and Mashaly (1992).

#### Environmental conditions

**Edaphic factors:** Table 3 gives the results of physical and chemical analysis of fifteen soil samples supporting the growth of *Anthemis* community. The soil deposits are essentially fine sand (43.42-52.41%). The percentage of silt is relatively high with mean value of 26.9% while the clay fraction is low with mean value of 2.1%. The soil moisture content varied from 0.74-3.79%. Porosity varied within a narrow range from 39.80-41.34%. The water holding capacity was high and varied from 52.02-59.25% and increases with increase of fine ingredients.

The organic carbon content was low and varied between 0.4-1.4%. CaCO<sub>3</sub> content was generally low and ranged from 1.0-7.0%. Electric conductivity of the soil extract (1:5) fluctuated between 146 and 252 μmohs cm<sup>-1</sup>. Bicarbonates contributes the main soluble salts; the soil reaction (pH) was slightly alkaline. Consequently, *A. cotula* is salt non-tolerant plant. Similar results had been obtained by El-Halawany *et al.* (2010) and Abu Ziada *et al.* (2008).

**Climatic factors:** Regarding the climatic conditions of EL-Dakahlia Governorate where the stinking chamomile was successfully naturalized and thriving. As Mediterranean type of climate, it is characterized by a long hot dry season (April-October) and a short cold mild one (November-March). This weedy plant recorded during the cold mild period of the year, at maximum temperature (19.1-23.2°C), minimum temperature (6.8-9.3°C), relative humidity (64.0-70.0%), monthly rainfall (6.8-10.4 mm) and evaporative power of air (2.0-3.4 mm day<sup>-1</sup>).



Table 3: Physical and chemical analyses of fifteen soil samples supporting *Anihemis cotula* L. inhabiting different stands of the study area (February-March, 2014)

| Sample No. | Physical characteristics                       |              |              |              |              |              |              |              |        |         | Chemical characteristics        |           |            |                       |                |                     |                                  |                                  |                                   |                |
|------------|--|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------|---------|---------------------------------|-----------|------------|-----------------------|----------------|---------------------|----------------------------------|----------------------------------|-----------------------------------|----------------|
|            | Mechanical analysis of particles size (mm) (%) |              |              |              |              |              |              |              |        |         | Analysis of 1 : 5 water extract |           |            |                       |                |                     |                                  |                                  |                                   |                |
|            | >2.057   | 2.057- 1.003 | 1.003- 0.500 | 0.500- 0.211 | 0.211- 0.104 | 0.104- 0.053 | 0.053- 0.104 | 0.104- 0.053 | <0.053 | M.C (%) | Por (%)                         | W.H.C (%) | Org. C (%) | CaCO <sub>3</sub> (%) | E.C (µmohs/Cm) | Cl <sup>-</sup> (%) | SO <sub>4</sub> <sup>-</sup> (%) | CO <sub>3</sub> <sup>-</sup> (%) | HCO <sub>3</sub> <sup>-</sup> (%) | TDS (mg/100 g) |
| 1          | 0.00   | 1.48         | 5.70         | 16.49        | 43.42        | 28.89        | 3.01         | 3.79         | 41.13  | 55.92   | 0.90                            | 2.00      | 252.00     | 0.007                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 132.00                            | 6.95           |
| 2          | 0.19   | 1.92         | 4.37         | 18.76        | 45.67        | 25.61        | 2.92         | 2.56         | 41.34  | 57.52   | 0.80                            | 1.50      | 201.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 104.00                            | 6.91           |
| 3          | 0.52   | 1.58         | 4.03         | 15.05        | 46.21        | 30.21        | 2.54         | 3.39         | 41.26  | 52.02   | 0.40                            | 4.50      | 198.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 104.00                            | 6.89           |
| 4          | 0.00   | 1.84         | 4.78         | 18.64        | 45.05        | 27.07        | 2.64         | 1.37         | 40.53  | 54.85   | 1.30                            | 1.50      | 168.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 87.00                             | 7.19           |
| 5          | 0.21   | 1.35         | 5.60         | 18.16        | 46.10        | 26.08        | 2.57         | 0.74         | 40.44  | 59.25   | 1.20                            | 7.00      | 170.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.061                            | 90.00                             | 6.94           |
| 6          | 0.11   | 0.21         | 5.30         | 15.86        | 48.54        | 27.62        | 2.40         | 2.49         | 40.22  | 55.96   | 1.40                            | 4.00      | 182.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 97.00                             | 6.95           |
| 7          | 0.00   | 0.35         | 5.37         | 19.53        | 46.32        | 26.91        | 1.52         | 3.77         | 39.80  | 58.84   | 0.90                            | 4.50      | 149.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.061                            | 78.00                             | 6.90           |
| 8          | 0.00   | 0.31         | 5.41         | 17.76        | 50.34        | 24.74        | 1.57         | 1.77         | 40.86  | 54.09   | 0.80                            | 4.00      | 146.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 76.00                             | 6.70           |
| 9          | 0.00   | 0.30         | 4.60         | 19.77        | 47.89        | 26.41        | 1.33         | 3.09         | 39.91  | 58.35   | 0.80                            | 4.50      | 169.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 89.00                             | 6.79           |
| 10         | 0.00   | 0.36         | 4.19         | 16.47        | 53.41        | 25.50        | 0.16         | 2.26         | 40.66  | 56.18   | 1.00                            | 6.00      | 167.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.061                            | 88.00                             | 6.90           |
| 11         | 0.12   | 1.58         | 4.81         | 17.51        | 46.50        | 27.73        | 2.53         | 1.99         | 40.62  | 55.57   | 1.30                            | 1.00      | 189.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 98.00                             | 6.98           |
| 12         | 0.10   | 0.60         | 5.43         | 17.82        | 47.81        | 25.76        | 2.15         | 2.73         | 40.36  | 56.73   | 0.70                            | 7.00      | 159.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.050                            | 84.60                             | 6.84           |
| 13         | 0.00   | 0.51         | 4.76         | 17.90        | 48.88        | 26.40        | 1.54         | 3.46         | 40.41  | 56.66   | 0.60                            | 4.00      | 171.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.050                            | 89.80                             | 6.88           |
| 14         | 0.20   | 1.42         | 5.20         | 17.70        | 46.33        | 27.11        | 2.52         | 2.51         | 41.01  | 55.90   | 1.00                            | 1.00      | 194.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.050                            | 102.00                            | 6.91           |
| 15         | 0.00   | 0.90         | 4.96         | 16.26        | 47.31        | 26.93        | 1.84         | 3.00         | 40.60  | 56.50   | 0.80                            | 4.50      | 182.00     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.052                            | 95.00                             | 6.96           |
| Mean       | 0.01   | 0.96         | 4.96         | 17.58        | 47.31        | 26.90        | 2.10         | 2.60         | 40.54  | 56.30   | 0.93                            | 3.80      | 180.50     | 0.005                 | 0.005          | 0.000               | 0.000                            | 0.045                            | 94.20                             | 6.91           |
| S.E        | 0.04   | 0.16         | 0.13         | 0.25         | 0.16         | 0.36         | 0.20         | 0.23         | 0.19   | 0.48    | 0.07                            | 0.57      | 6.69       | 0.000                 | 0.000          | 0.010               | 0.000                            | 0.030                            | 3.58                              | 0.03           |

M.C: Moisture content, Por: Porosity, W.H.C: Water-holding capacity, Org. C: Organic carbon, E.C: Electric conductivity, TDS: Total dissolved salts and pH: Soil reaction

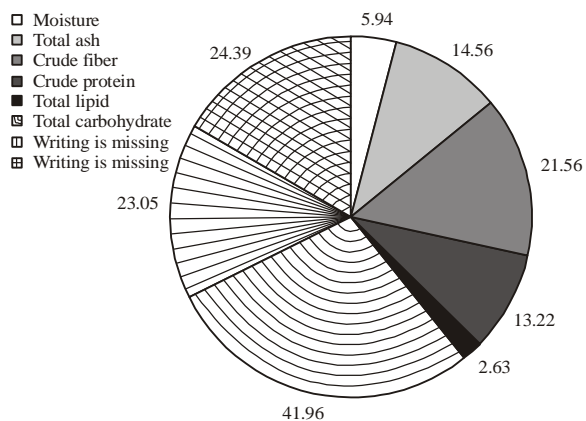


Fig. 5: Mean values of the primary constituents of *Anthemis cotula* aerial parts

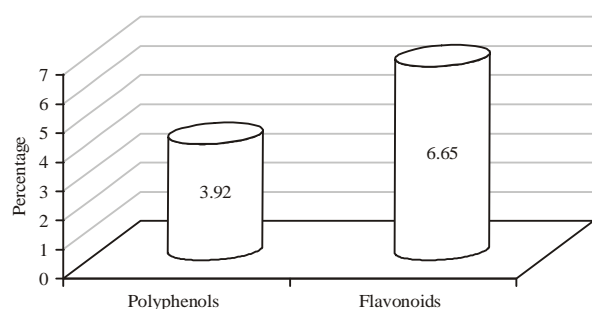


Fig. 6: Quantitative estimation of phenolic and flavonoid compounds

Table 4: Qualitative estimation of the secondary metabolites

| Secondary metabolites | Effect |
|-----------------------|--------|
| Phenols               | ve+    |
| Flavonoids            | ve+    |
| Saponins              | ve-    |
| Sterols               | ve+    |
| Alkaloids             | ve-    |

Table 5: Phytochemical composition of the volatile oil of *Anthemis cotula* fruits determined by GC/MS analysis

| No. | Compound                      | Concentration (mg/100 g) |
|-----|-------------------------------|--------------------------|
| 1   | $\alpha$ -Bisabolol oxide A   | 204.7                    |
| 2   | $\alpha$ -Bisabolol           | 160.2                    |
| 3   | Bisabolol oxide               | 62.8                     |
| 4   | $\beta$ -farnesene            | 57.3                     |
| 5   | (+) spathulenol               | 13.2                     |
| 6   | Chamazulene                   | 9.2                      |
| 7   | Germacrene D                  | 7.6                      |
| 8   | $\alpha$ -epiCadinol          | 3.4                      |
| 9   | $\gamma$ -elemene             | 2.9                      |
| 10  | Heptacosane                   | 2.2                      |
| 11  | $\alpha$ -Bisabolene          | 2.0                      |
| 12  | Caryophyllene Oxide           | 1.9                      |
| 13  | 9, 12-octadecadienoic acid    | 1.5                      |
| 14  | Hexadecanoic acid ethyl ester | 1.3                      |
| 15  | Unknown                       | 29.7                     |

### Bioactive constituents

**Primary metabolites and nutritive value:** The primary metabolites are of prime importance and essentially required

for the growth, development and reproduction of plants (Sagwan *et al.*, 2010). As shown in Fig. 5, *A. cotula* aerial parts contained relatively high values of carbohydrates, soluble sugars, proteins and lipids. Consequently, the nutritive value was 244.39 cal/100 g. On the basis of these results, *A. cotula* is promising weed as renewable natural resource for forage and medicinal purposes.

Sterols, flavonoids and phenols were recorded while alkaloids and saponins were absent in *A. cotula* (Table 4). Polyphenols and flavonoids were determined with mean values of 3.92 and 1.51%, respectively (Fig. 6). Vuckovic *et al.* (2006) reported that, polyacetylenes, flavonoids and sesquiterpene lactones are the three main classes of secondary metabolites of *A. cotula*. Secondary metabolism in a plant not only plays a role for its survival by producing chemical defense against predators and diseases. Many secondary metabolites are also an important trait for food (taste, colour, scent) while others have a commercial application in the pharmaceutical and bio-medical fields and are part of drugs, dyes, flavors and fragrances.

Qualitative and quantitative assessments of volatile oils revealed that, the yield of volatile oils isolated from *A. cotula* capitula has blue colour with mean value of 0.56 g/100 g dry powder. Analysis of oils by GC/MS allowed to identification of 14 constituent compounds (Table 5). The prevailing components were  $\alpha$ -Bisabolol oxide A,  $\beta$ -Farnesene, Chamazulene, Germacrene, etc.

Chamomile oil has been reported to be beneficial for relief of sleeping disorders, colic, mucositis and eczema (McKay and Blumberg, 2006). The quality and yield of oils extracted from Anthemideae plants are influenced by the harvesting season (Williams *et al.*, 2001); fertilizer and pH of soil (Alvarez-Castellanos and Pascual-Villalobos, 2003); drying conditions (Tateo and Riva, 1991); geographic location (Maffei *et al.*, 1994) and extraction method (Scalia *et al.*, 1999).

### CONCLUSION

*Anthemis cotula* is a herbaceous corymbosely branched weedy species aggressively spread through fields of winter crops in El-Dakahlia Governorate. It has a noticeable and strong unpleasant odor and prefers medium (loamy) and heavy (clayey) agricultural soil in sun. It grows best in fertile, moist, well drained and slightly alkaline soils. It is native to the Mediterranean region and reproduces by seed. *Anthemis cotula* belongs to family Asteraceae, subfamily Asteroideae, tribe Anthemideae and subtribe Anthemidineae. The polyphenols and flavonoids contents in *A. cotula* were 3.92 and 6.65%, respectively. The volatile oil obtained from *A. cotula* flowers heads or capitula (0.56 g/100 g) has blue colour and comprises 14 identified components and a single unknown compound. The prevailing components were  $\alpha$ -Bisabolol oxide A,  $\alpha$ -Bisabolol, Bisabolol oxide,  $\beta$ -farnesene, (+) spathulenol, chamazulene and Germacrene D. According to the results of the present study, it may be concluded how far the economic future of *A. cotula* and the ability of its use in traditional and modern medicines.

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