

Response of *Silybum marianum* plant to irrigation intervals combined with fertilization

**SABER F. HENDAWY^{1,✉}, MOHAMED S. HUSSEIN¹, ABD-ELGHANI A. YOUSSEF²,
REYAD A. EL-MERGAWI³**

¹Medicinal and Aromatic Plants Research Department, National Research Centre, Dokki 12311, Giza, Egypt. Tel. +202-3366-9948, +202-33669955, Fax: +202-3337-0931, ✉email: hendawysaber@yahoo.com

²Departement of Chemistry, Faculty of Science, Jazan University, Saudi Arabia

³Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, P.O.Box 6622, Buhrida 51452, Al-Qassim; Saudi Arabia

Manuscript received: 11 April 2013. Revision accepted: 5 May 2013.

Abstract. *Hendawy SF, Hussein MS, Youssef AA, El-Mergawi RA. 2013. Response of Silybum marianum plant to irrigation intervals combined with fertilization. Nusantara Bioscience 5: 22-29.* This study was investigated to evaluate the influence of different kinds of organic and bio fertilization under different irrigation intervals on the growth, production and chemical constituents of *Sylibium marianum* plant. Data indicated that all studied growth and yield characters were significantly affected by the duration of irrigation intervals. Fertilizer treatments had a primitive effect on growth and yield characters. The interaction between irrigation intervals and fertilizer treatments has a clear considerable effect on growth and yield characters. The obtained results indicated the favorable effect of organic and bio fertilizers which reduce the harmful effect of water stress. Different treatments had a pronounced effect on silymarin content.

Key words: *Sylibium marianum*, silymarin, bio fertilization and irrigation intervals.

Abstrak. *Hendawy SF, Hussein MS, Youssef AA, El-Mergawi RA. 2013. Respons tanaman Silybum marianum terhadap interval irigasi yang dikombinasi dengan pemupukan. Nusantara Bioscience 5: 22-29.* Penelitian ini bertujuan untuk mengevaluasi pengaruh berbagai jenis pupuk organik dan hayati dengan interval irigasi yang berbeda terhadap pertumbuhan, produksi dan kandungan kimia tanaman *Sylibium marianum*. Data menunjukkan bahwa semua sifat pertumbuhan dan produksi yang diteliti secara signifikan dipengaruhi oleh durasi interval irigasi. Perlakuan pemupukan berpengaruh nyata terhadap karakter pertumbuhan dan hasil panen. Interaksi antara interval irigasi dan perlakuan pemupukan berpengaruh besar pada karakter pertumbuhan dan hasil panen. Hasil yang diperoleh menunjukkan efek menguntungkan dari pupuk organik dan hayati yang dapat mengurangi efek berbahaya cekaman air. Perlakuan yang berbeda berpengaruh kuat terhadap kandungan silymarin.

Kata kunci: *Fagus orientalis*, serat, sifat biometri, pohon unggul.

INTRODUCTION

Milk thistle (*Silybum marianum* L. Gaertn.), a member of the Mediterranean Basin, as a crop and weed on agricultural plantations, it occurs in many European countries, North Africa, South and North America, Central and Western Asia and southern Australia (Carrier et al. 2002). The pharmaceutical compound of milk thistle is derived from its fruits, which are achenes (Fructus silybi mariani). In their dry pericarp and seed coat the plant accumulates a group of flavonolignans commonly referred to as silymarin (Cappelletti and Caniato 1984). Taxifolin is their precursor. The main flavonolignans of milk thistle are silybinin, isosilybinin, silydianin and silychristin. Several other compounds of that type have also been identified, but their importance in the silymarin complex is insignificant (Kurkin et al. 2001). Silymarin, derived from the seeds of milk thistle plant has been used widely for the treatment of toxic liver damage (Dewick 1998). Silymarin primarily consists of an isomeric mixture of six phenolic compounds:

silydianin, silychristin, diastereoisomers of silybin (silybin A and B), and diastereoisomers of isosilybin (isosilybin A and B) (Lee et al. 2007).

The compost must be added to conventional NPK fertilizer to improve soil structure, making the soil easier to cultivate, encouraging root development, providing plant nutrients and enabling their increased uptake by plants. Moreover, compost aids water absorption and retention by the soil, reducing erosion and run-off and thereby protecting surface waters from sedimentation, help binding agricultural chemicals, keeping them out of water ways and protecting ground water from contamination (leaMaster et al. 1998). Compost has already been established as a recommended fertilizer for improving the productivity of several medicinal and aromatic plants, as peppermint (O'Brien and Barker 1996), *Tagetes erecta* (Khalil et al. 2002), *Sideritis montana* (El-Sherbeny et al. 2005), *Ruta graveolens* (Naguib et al. 2007) and *Dracocephalum moldavica* L. (Amer 2008). Compost tea is a highly concentrated microbial solution produced by extracting



Figure 1. Inflorescence of milk thistle (*Silybum marianum* L. Gaertn.)

beneficial microbes from vermicompost and or compost. Compost tea provides direct nutrition as a source of foliar and soil organic nutrient and as chelated micronutrients for easy plant absorption. Also, compost tea provide microbial functions, that compete with disease causing microbes, degrade toxic pesticides, produce plant growth hormones, mineralize plant available nutrients and fix nitrogen (Hendawy 2008).

Arbuscular mycorrhiza (AM) fungi (Endogonaceae) form a mutualistic relationship with the roots of most plant species. This plant-fungus association involves the translocation of carbon from the plant to the fungus and enhanced uptake and transport of soil nutrients, primarily phosphorus, to the plant via the fungus (Newman and Reddel 1987). Other potential benefits of AM fungal colonization to host plants include improved uptake of poorly mobile nutrients such as zinc (Gildon and Tinker 1983), improved plant water relations (Allen and Allen 1986) and reduced pathogenic infections (Newsham, et al. 1995). AMF can also benefit plants by stimulating the production of growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance to pests and soil borne diseases (Al-Karaki 2006).

However, water deficit is a limiting factor in production of many field crops (Kafi and Mahdavi Damghani 2001; Munns 2002) and water stress causes different morphological, physiological and biochemical changes including: leaf area reduction, leaf senescence and reduction in cell development (Kafi and Mahdavi Damghani 2001), stomatal closure (Safar-Nezhad 2003) and photosynthetic limitation (Kafi and Mahdavi

Damghani 2001). It appears that the effect of water stress on economic yields of medicinal plants which are mainly secondary metabolites, are somehow positive (Baher et al. 2002). In many cases, a moderate stress could enhance the content of secondary metabolites.

This current experiment targeted the evaluation of the influence of different kinds of organic and bio fertilization under different irrigation intervals on the growth, production and chemical constituents of *Silybum marianum* plant.

MATERIALS AND METHODS

Field experiment

Location

The field experiment was carried out at El-Nubareia Research Station (El-Behira Governorate, Egypt), National Research Centre, to investigate the influence of Chemical, organic and bio fertilizers under different irrigation intervals on growth, yield and chemical constituents of milk thistle.

Soil

The experiment was set up on sand loam soil as shown in Table 1.

Table 1. Main characteristics of soil

| Characteristics | Value |
|----------------------------------|------------|
| Mechanical analysis | |
| Sand% | 68.08 |
| Silt% | 16.00 |
| Clay% | 15.92 |
| Texture | Sandy loam |
| Chemical analysis | |
| PH 1:2.5ext. | 8.50 |
| Ca Co3 | 21.70 |
| Electrical conductivity 1:2.5ext | 0.61 |
| Soluble cations meq/l | |
| Ca ⁺⁺ | 3.38 |
| Mg ⁺⁺ | 3.62 |
| Na ⁺ | 3.23 |
| K ⁺ | 0.49 |
| Soluble anions meq/l | |
| HCO ₃ ⁻ | 1.12 |
| Cl ⁻ | 1.5 |
| SO ₄ ⁻ | 9.1 |
| Macro-elements (ppm) | |
| N | 30.00 |
| P | 20.00 |
| K | 368.00 |
| Micro-elements (ppm) | |
| Zn | 0.28 |
| Mn | 2.50 |
| Fe | 3.70 |
| Cu | 0.96 |

Experiment design and agronomic practices

The fertilization factor experiment was set up in a randomized design in three replicates.

Experimental treatments**A. Irrigation every 3 days**

1. NPK (100 kg super phosphate+150 kg nitrate ammonium+50 kg potassium sulphate).
2. Compost 20m³/feddan
3. Compost 20m³/feddan+mycorrhiza
4. Compost 20m³/feddan+compost tea 20 L/feddan
5. Compost 20m³/feddan+compost tea 20 L/feddan+mycorrhiza

B. Irrigation every 6 days

6. NPK (100 kg super phosphate+150 kg nitrate ammonium+50 kg potassium sulphate) as control.
7. Compost 20m³/feddan
8. Compost 20m³/feddan+mycorrhiza
9. Compost 20m³/feddan+compost tea 20 L/feddan
10. Compost 20m³/feddan+compost tea 20 L/feddan+mycorrhiza

C. Irrigation every 9 days

11. NPK (100 kg super phosphate+150 kg nitrate ammonium+50 kg potassium sulphate) as control.
12. Compost 20m³/feddan
13. Compost 20m³/feddan+mycorrhiza
14. Compost 20m³/feddan+compost tea 20 L/feddan
15. Compost 20m³/feddan+compost tea 20 L/feddan+mycorrhiza

The seeds were directly sown in 20th of October 2010. Each plot was 13.5 m² consisting of 9 rows with a distance of 50 cm between the rows and 30 cm between each successive plant. Weeding and thinning was done after 30 days of plantation. Recommended agronomic practices were adopted.

Super phosphate or compost was added during preparing soil. The other chemical fertilizers (Ammonium nitrate and Potassium sulphate were divided into two equal portions during the growing season, the 1st portion was added after one month of sowing, while the second one was applied after one month from the 1st. Tea compost (Table 2, 3) was sprayed after 60 days from sowing and repeated after 15 days. Vesicular arbuscular mycorrhiza (VAM) fungi which contained 3 effective strains representing *Glomus etunicatum*, *Glomus fasciculatum* and *Glomus intraradices*. VAM fungi was used for soil inoculation. The VAM inoculation was applied into sowing hills at a rate of 5 mL/hill. The amount contained about 200 VAM spores/hill.

The effect of the above treatments was measured by plant height, branches number, capitula number/plant, seed yield and silymarin content.

Table 2. Microbial population of organic compost tea

| Constituent | Value |
|----------------------------------|-----------------------|
| Bacterial Plate Count (CFU/ml) | 7.1 X 10 ⁷ |
| Bacterial Direct Count (Cell/ml) | 6.4 X 10 ⁸ |
| Spore Forming Bacteria (CFU/ml) | 7 X 10 ⁴ |
| Total Fungi (CFU/ml) | 2.8 X 10 ⁵ |

Table 3. Chemical analysis of organic compost tea

| Constituent | Value |
|--------------------------------|-------|
| Bulk Density kg/m ³ | 510 |
| Moisture Content% | 18.2 |
| Electrical conductivity dS/m | 9.65 |
| PH | 7.6 |
| Total Organic Carbon% | 24.6 |
| Total Organic Matter% | 42.41 |
| Total Nitrogen% | 1.35 |
| C/N Ratio | 18.22 |
| NH ₄ -N, mg/kg | 880 |
| NO ₃ -N, mg/kg | 450 |
| Total Phosphorus% | 1.6 |
| av. Phosphorus mg/kg | 410 |
| Total Potassium% | 2.3 |
| av. Potassium mg/kg | 620 |
| Trace Element (ppm) | |
| Fe | 960 |
| Zn | 280 |
| Mn | 320 |
| Cu | 140 |

Note: Nematodes (nil), Weeds germination (nil), Parasites (nil), Pathogenic (nil), Humus value (5).

Extraction procedure

Silymarin content was extracted according to (Cacho et al. 1999). Gram of seeds were defated in a Soxhlet apparatus with 50 mL of petroleum-ether at 40-60 °C for 12 h. The residue was extracted with 50 mL of methanol at 65-70 °C over 8 h. The methanolic solution was concentrated to a dry residue. The extract was dissolved in 10 mL of methanol.

HPLC analysis

HPLC was carried out using an HPLC pump monitored at 280 nm by a UV detector and quantified by an integrator. A Shim-pack C18 (1250 x 4.6 mm ID) column was used, eluting with MeOH-H₂O-AcOH 40:60:5, at a flow rate of 2 mL/min. Mixture of flavonolignans obtained from Alex Pharm, Egypt (specifications: Silychristin 25% R_t 2.94 min, silydianin 9.7% R_t 3.64 min, silybin A 21.3% R_t 7.84 min, silybin B 32% R_t 9.18 min, isosilbin A 8.7% R_t 13.61 min and isosilybin B 3% R_t 15.18 min).

RESULTS AND DISCUSSION**Vegetative growth and yield***Irrigation intervals*

Data tabulated in Table 4 indicated that all studied growth and yield characters were significantly affected by the duration of irrigation intervals.

By increasing the severity and duration of drought from 3 days to 9 days, plant height (cm) showed significant reduction. Such reduction in plant height in response to drought may be due to blocking up of xylem and phloem vessels thus hindering any translocation through (Lovisolo and Schuber 1998). Similar results were obtained by Singh et al. (2006) and Khalil et al. (2010).

Table 4. Effect of irrigation intervals on vegetative growth and yield of *Silybum marianum*

| Seed yield (g/Plant) | Flowers heads no/plant | Branches no/plant | Plant height (cm) | Irrigation intervals |
|----------------------|------------------------|-------------------|-------------------|----------------------|
| 20.91 | 20.60 | 6.60 | 179.00 | 3 days |
| 17.39 | 18.20 | 7.20 | 169.40 | 6 days |
| 14.89 | 15.80 | 8.60 | 166.2 | 9 days |
| 0.477 | 0.582 | 0.576 | 0.504 | LSD at 5% |

Data on hand, illustrated also that, number of branches/plant increased significantly with decreasing of irrigation, this may be due to that drought reduced cycling-dependent kinase activity results in slower cell division as well as inhibition of growth (Schuppler et al. 1998). This supported by the results of (Rahmani et al. 2008) on *Calendula officinalis* L. and (Taheri et al. 2008) on *Cichorium intybw* L.

Significant higher numbers of flowers head/plant and seed yield (g/plant) were recorded with the shortest irrigation interval (3 days) followed by (6 days). The decrease in yield attributes under the longest irrigation interval (9 days) may be due that water stress changing the hormonal balance of mature leaves, thus enhancing leaf senescence and hence the number of active leaves decreased, as well as leaf area was reduced by water shortage, which was attributed to its effect on cell division and lamina expansion. When the number of active leaves decreased the light attraction and CO₂ diffusion inside the leaf decreased and the total capacity of photosynthesis decreased, therefore, the photosynthetic materials that transferred to seeds will decreased (Ahmed and Mahmoud 2010; Moussavi et al. 2011).

Fertilizer treatments

Data tabulated in Table 5 show that fertilizer treatments had a significant effect on growth and yield characters of *Silybum marianum* plants. The mean values of plant height were 174.33, 164.33, 168.33, 171.0 and 179.67 cm as a result of NPK, compost, compost+mycorrhiza, compost+compost tea and compost+compost tea+mycorrhiza treatments, respectively. So, the highest value of plant height was obtained as a result of compost+compost tea+mycorrhiza treatment.

Table 5. Effect of fertilizer treatment on vegetative growth and yield of *Silybum marianum*

| Seed yield (g/plant) | Flowers heads no/plant | Branches No/plant | Plant height (cm) | Fertilizer treatments |
|----------------------|------------------------|-------------------|-------------------|--------------------------------|
| 18.15 | 21.33 | 8.33 | 174.33 | NPK |
| 15.74 | 17.00 | 6.33 | 164.33 | Compost |
| 18.90 | 16.67 | 7.33 | 168.33 | Compost+mycorrhiza |
| 16.49 | 17.00 | 7.33 | 171 | Compost+compost tea |
| 19.37 | 19 | 8.00 | 179.67 | Compost+compost tea+mycorrhiza |
| 1.054 | 0.318 | 0.449 | 0.825 | LSD at 5% |

The results in Table 5 reveal that, fertilizer treatments had a pronounced effect on branches number. It can be noticed that, mean values of branches number recorded 8.33, 6.33, 7.33, 7.33 and 8.00/plant were obtained from NPK, Compost, Compost+mycorrhiza, compost+compost tea and compost+compost tea+mycorrhiza treatments, respectively. Thus, the maximum mean value of branches number/plant (8.33) was obtained as a result of NPK treatment followed by compost+compost tea+mycorrhiza treatment, which recorded 8.00/plant. There is no significant difference between NPK treatment and compost+compost tea+mycorrhiza treatment.

The averages of heads flowers number were 21.33, 17.00, 16.67, 17.00 and 19.00/plant as a result of NPK, Compost, Compost+mycorrhiza, compost+compost tea and compost+compost tea+mycorrhiza treatments, respectively. Thus, the maximum mean value of flowers heads number/plant (21.33) was obtained from NPK treatment followed by compost+compost tea treatment, which recorded 19.00/plant.

It is evident from data in Table 5 that fertilizer treatments had a significant effect on seed yield (g/plant). In this respect, mean values of seed yield (g/plant) were 18.15, 15.74, 18.90, 16.49 and 19.37 g/plant as a result of NPK, compost, compost+mycorrhiza, compost+compost tea and compost+compost tea+mycorrhiza treatments, respectively. Therefore, compost+compost tea+mycorrhiza treatment gave the highest mean value of seed yield (19.37g/plant) followed by compost+mycorrhiza treatment which recorded (18.90 g/plant).

The promotion effect of compost on the growth and yield of plant could be explained through the role of organic materials including composts in improving soil P availability (Gichangi et al. 2009). Since during composting, labile nutrients are converted into stabilized organic material (Zucconi and De Bertoldi 1987), therefore a large proportion of nutrients are labile. Composts provide microbes not only with P but also C and N and are therefore likely to induce changes in P pools that differ from those of inorganic P addition (Hassan et al. 2012).

The favorable effects of the combination between compost +compost tea+mycorrhiza may be explained based on the beneficial effects of them on the improvement soil physical and biological properties and also, the chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant root and its effect on the physiological processes such as photosynthesis activity as well as the utilization of carbohydrates. A similar suggestion was made by Hanafy et al. (2002) on rocket plants. Furthermore, this stimulative effect may be related to the good equilibrium of nutrients and water in the root medium (Abdelaziz and Balbaa 2007) or to the beneficial effects of mycorrhiza on vital enzymes and hormonal, stimulating effects on plant growth and yield.

Interaction treatments

The interaction between irrigation intervals and fertilizer treatments has a clear considerable effect on growth and yield characters (Table 6). It can be observed

that the maximum mean value of plant height (190.00 cm) was obtained from the combination treatment between irrigation intervals every 3 days and fertilized with compost+compost tea+mycorrhiza. On the other hand, the lowest average of plant height (158.00 cm) was obtained from the combination between irrigation intervals every 9 days and compost treatment. The variation in plant height between maximum and the minimum values reached to 20.25%.

For branches number/plant, it can be observed that, the highest mean value of branches number/plant (10.00/plant) against the lowest value (5.00/plant) were obtained as a result of the combination between irrigation intervals every 9 days and NPK treatment and the combination irrigation intervals every 3 days with compost treatment, respectively. The variation in branches number/plant between maximum and the minimum values reached to 100%.

Data shown in Table 6 indicated that, the combination between irrigation intervals every 3 days and NPK treatment gave the highest mean value of flowers heads number (25.00/plant), while the combination between irrigation intervals every 9 days and compost+compost tea

treatment gave the lowest mean value (13.00/plant). The variation in flowers heads number/plant between maximum and the minimum values reached to 92.31%.

Concerning the interaction treatments, it can be noticed that the combination between irrigation intervals every 3 days and compost+compost tea+mycorrhiza treatment resulted in the maximum mean value of seed yield (23.40 g/plant) while the interaction between irrigation intervals every 9 days and compost+compost tea treatment gave the lowest one (13.00 g/plant). The variation in seed yield (g/plant) between maximum and the minimum values reached to 78.49%.

The obtained results indicated the favorable effect of organic and bio fertilizers which reduce the harmful effect of water stress through their effect on improving the soil texture. The structural improvement can encourage the plant to have a good root development by improving the aeration in the soil. The favorable effects of these fertilizers may be due to the role of organic material for continues supply of nutrients, which improve some physical properties of soil and increase water retention (Abd-Elmoez et al. 1995; Fliessbach et al. 2000).

Table 6. Effect the interaction treatments between irrigation intervals and fertilization on growth and yield of *Silybum marianum*

| Seed yield (g/plant) | Flowers heads no/plant | Branches no/plant | Plant height (cm) | Fertilizer treatments | Irrigation intervals |
|----------------------|------------------------|-------------------|-------------------|--------------------------------|----------------------|
| 19.50 | 25.00 | 7.00 | 183.00 | NPK | 3 days |
| 18.70 | 19.00 | 5.00 | 170.00 | Compost | |
| 22.60 | 18.00 | 6.00 | 175.00 | Compost+mycorrhiza | |
| 20.33 | 21.00 | 8.00 | 177.00 | Compost+compost tea | 6 days |
| 23.40 | 20.00 | 7.00 | 190.00 | Compost+compost tea+mycorrhiza | |
| 18.55 | 20.00 | 8.00 | 174.00 | NPK | |
| 15.40 | 17.00 | 7.00 | 165.00 | Compost | |
| 19.60 | 18.00 | 7.00 | 167.00 | Compost+mycorrhiza | |
| 15.50 | 17.00 | 6.00 | 166.00 | Compost+compost tea | |
| 17.90 | 19.00 | 8.00 | 175.00 | Compost+compost tea+mycorrhiza | 9 days |
| 16.40 | 19.00 | 10.00 | 166.00 | NPK | |
| 13.11 | 15.00 | 7.00 | 158.00 | Compost | |
| 14.50 | 14.00 | 9.00 | 163.00 | Compost+mycorrhiza | |
| 13.65 | 13.00 | 8.00 | 170.00 | Compost+compost tea | |
| 16.80 | 18.00 | 9.00 | 174.00 | Compost+compost tea+mycorrhiza | |
| 1.067 | 1.301 | 1.288 | 1.126 | LSD at 5% | |

Table 7. Effect irrigation intervals on silymarin content (mg/g seed) of *Silybum marianum*

| Irrigation Intervals | Silychristin | Silydianin | Silybin A | Silybin B | Isosilybin A | Isosilybin B | Total |
|----------------------|--------------|------------|-----------|-----------|--------------|--------------|--------|
| 3 days | 17.952 | 11.182 | 11.092 | 18.576 | 7.216 | 2.814 | 68.832 |
| 6 days | 18.584 | 12.032 | 12.086 | 19.332 | 7.538 | 3.078 | 72.65 |
| 9 days | 22.028 | 13.352 | 14.776 | 23.34 | 8.734 | 3.184 | 85.414 |

Table 8. Effect of fertilizer treatment on silymarin content (mg/g seed) of *Silybum marianum*

| Fertilizer treatments | Silychristin | Silydianin | Silybin A | Silybin B | Isosilybin A | Isosilybin B | Total |
|---------------------------------|--------------|------------|-----------|-----------|--------------|--------------|-------|
| NPK | 19.62 | 11.76 | 12.61 | 20.82 | 7.64 | 3.07 | 75.52 |
| Compost | 19.49 | 13.10 | 12.35 | 20.00 | 7.92 | 3.09 | 75.95 |
| Compost+mycorrhiza | 20.49 | 12.53 | 13.35 | 21.48 | 8.21 | 2.79 | 78.85 |
| Compost+compost tea | 19.37 | 12.34 | 12.80 | 20.27 | 8.18 | 3.36 | 76.32 |
| Compost+compost tea+ mycorrhiza | 18.34 | 11.22 | 11.96 | 19.51 | 7.20 | 8.47 | 76.7 |

Table 9. Effect the interaction treatments between irrigation intervals and fertilization on silymarin content (mg/g seed) of *Silybum marianum*

| Total | Isosilybin B | Isosilybin A | Silybin B | Silybin A | Silydianin | Silychristin | Fertilizer treatments | Irrigation intervals |
|-------|--------------|--------------|-----------|-----------|------------|--------------|--------------------------------|----------------------|
| 71.74 | 3.02 | 7.49 | 19.45 | 11.66 | 11.48 | 18.64 | NPK | 3 days |
| 69.64 | 2.95 | 7.26 | 18.46 | 11.02 | 11.83 | 18.12 | Compost | |
| 64.86 | 2.03 | 6.98 | 17.70 | 10.79 | 10.34 | 17.02 | Compost+mycorrhiza | |
| 72.00 | 3.38 | 7.57 | 19.14 | 11.38 | 12.03 | 18.50 | Compost+compost tea | 6 days |
| 65.92 | 2.69 | 6.78 | 18.13 | 10.61 | 10.23 | 17.48 | Compost+compost tea+mycorrhiza | |
| 72.24 | 3.29 | 7.33 | 18.74 | 11.71 | 12.80 | 18.37 | NPK | |
| 68.96 | 2.69 | 7.22 | 18.51 | 11.30 | 11.30 | 17.94 | Compost | 9 days |
| 75.95 | 3.13 | 8.15 | 20.21 | 12.55 | 12.39 | 19.52 | Compost+mycorrhiza | |
| 71.3 | 2.92 | 7.63 | 19.35 | 12.48 | 10.81 | 18.11 | Compost+compost tea | |
| 74.8 | 3.36 | 7.36 | 19.85 | 12.39 | 12.86 | 18.98 | Compost+compost tea+mycorrhiza | 9 days |
| 82.58 | 2.89 | 8.11 | 24.27 | 14.47 | 10.99 | 21.85 | NPK | |
| 89.24 | 3.64 | 9.28 | 23.02 | 14.74 | 16.16 | 22.40 | Compost | |
| 96.29 | 3.20 | 9.50 | 26.54 | 17.25 | 14.87 | 24.93 | Compost+mycorrhiza | 9 days |
| 85.64 | 3.77 | 9.33 | 22.32 | 14.54 | 14.17 | 21.51 | Compost+compost tea | |
| 73.32 | 2.42 | 7.45 | 20.55 | 12.88 | 10.57 | 19.45 | Compost+compost tea+mycorrhiza | |

Silymarin content

Data tabulated in Tables 7, 8 and 9 indicated that total silymarin content (mg/g seed) ranged from 64.86 to 96.29 mg/g. The main constituent of silymarin were Silybin B (17.70-26.54 mg/g) followed by Silychristin (17.48-24.93 mg/g). In this connection, dried extracts of milk thistle seeds contain approximately 60% silymarin, where silymarin consists of four flavonolignans of silybinin (~ 50 to 60%), isosilybinin (~ 5%), silychristin (~ 20%) and silydianin (~ 10%) (Burgess, 2003). (Ibrahim et al. 2007) found that the concentration and total yield of six silymarin compounds showed wide variations between lines, varieties and generations ranged from 11.92 to 62.85 mg/g seed and between 329.8 to 2121.3 mg/plant, respectively. Six silymarin compounds: silychristin, silydinin, silybin A, silybin B, isosilybin A and isosilybin B were detected in the extract of all tested treatments. These results were in agreement with (Ibrahim et al. 2007).

Irrigation intervals

Data tabulated in Table 7 show that, the mean values of total Silymarin content (mg/g seed) were 68.83, 72.65 and 85.41 mg/g were obtained as a result of irrigation intervals at 3, 6 and 9 days, respectively.

Silybin B followed by silychristin were the main components of silymarin. The maximum mean values of Silybin B (23.34 mg/g) and Silychristin (22.03 mg/g) were observed as a result of irrigation intervals every 9 days.

Drought stress increases the secondary products percentage of more medicinal and aromatic plants, because in case of stress, more metabolites are produce in the plants and substances prevent from oxidization in the cells, but secondary products content reduce under drought stress, because the interaction between the amount of the secondary products percentage and mass production is consider important as two components of the secondary products content and by exerting stress, increases the secondary products percentage but mass production decreases by the drought stress, therefore secondary products content reduces. The data from (de Abreu and

Mazzafera 2005) showed that also the total amount of some secondary plant products per plant indeed is significantly higher in plants grown under drought stress than in those cultivated under normal conditions. Although stressed plants had been quite smaller, the product of biomass and substance concentration yields in a 10% higher amount of phenolic compounds; however, the total content of betulinic acid was nearly the same in plants when grown under drought stress or under standard conditions. Also the studies published by Nogues et al. (1998), who found a massive increase of phenolic compounds in stressed peas, allow calculating the overall yield of the related substances. Despite the fact that the total biomass of pea plants grown under drought stress is just about one third of those cultivated under standard condition, the overall amount of anthocyanins (product of biomass and anthocyanin concentration) is about 25% higher in the stressed plants. Apart from that, the overall yield of total flavanoids was nearly the same in *Pisum sativum* plants grown under drought stress or under non-stress conditions.

Fertilizer treatments

Data tabulated in Table 8 indicated the effect of different fertilizer treatments on silymarin content (mg/g). Total silymarin content ranged from 75.52 to 78.85 mg/g. Compost+mycorrhiza treatment gave the maximum mean values of total silymarin content (78.85 mg/g) followed by Compost+compost tea+mycorrhiza treatment which gave 76.70 mg/g. The highest mean values of Silybin B (21.48 mg/g) and Silychristin (20.49 mg/g) were obtained as a result of compost+mycorrhiza treatment compared with other treatments.

As for the favorable effect of applying organic and/or bio fertilizers on silymarin content may be due to effect of these fertilizers on accelerating metabolism reactions as well as stimulating enzymes. Application of bio fertilizers and compost significantly improved secondary products such as essential oil, rutin and coumarin (El-Sherbeny et al. 2007 a, b). Variations in plant growth and active principles in mycorrhizae inoculated plants have been reported for

many other medicinal plants (Sailo and Bagyara 2005; Copetta et. al. 2006).

Interaction treatments

It can be noticed that compost+ mycorrhiza treatment under 9 days irrigation intervals gave the maximum value of total silymarin content (96.29 mg/g) followed by compost treatment under the same irrigation intervals which gave 89.24 mg/g (Table 9). The lowest value of Silymarin content (64.86 mg/g) was obtained as a result of compost+mycorrhiza treatment under 3 days irrigation intervals.

Moreover, the highest values of Silybin B (26.54 mg/g) and Silychristin (24.93 mg/g) were observed as a result of compost+ mycorrhiza treatment under 9 days irrigation intervals. In this respect, mycorrhiza fungi play a critical role in interest cycling and ecosystem function. They improve plant growth and survival through a mutuality relationship in which photosynthates are exchanged for increased access to water and nutrients (Kernaghan 2004). These effects may be played an important role to increase the secondary metabolites accumulation.

CONCLUSION

All presented data indicated that all studied growth and yield characters were significantly affected by the duration of irrigation intervals also organic and bio fertilizer showed a primitive effect on growth and yield characters. The interaction between irrigation intervals and fertilizer treatments has a clear considerable effect on growth and yield characters. Organic and bio fertilizers can reduce the harmful effect of water stress.

REFERENCES

- Abdel Aziz NG, Balbaa LK. 2007. Influence of tyrosine and zinc on growth, flowering and chemical constituents of *Salvia farinacea* plants. J Appl Sci Res. 3:1479-1489.
- Abd-Elmoez MR, Ghali MH, Abd-El-Fatth A. 1995. Conditioning of a sand soil by organic wastes and its impact on N-concentration and yield of broad bean. Zagazig J Agric Res 22 (11): 223-233.
- Ahmed ME, Mahmoud FA. 2010. Effect of irrigation on vegetative growth, oil yield and protein content of two sesame (*Sesamum indicum* L.) cultivars. Res J Agric Biol Sci 6 (5): 630-636.
- Al-Karaki GN. 2006. Nursery inoculation of tomato with arbuscular mycorrhiza fungi and subsequent performance under irrigation with saline water. Scientia Horticulture 109: 1-7.
- Allen EB, Allen MF. 1986. Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. New Phytol 104: 559-571.
- Amer HM. 2008. Effect of sowing date and organic manure on the growth, production and active ingredients of dragonhead plant (*Dracocephalum moldavica* L.). [Thesis]. Fac Agric Cairo Univ, Egypt.
- Baher ZF, Mirza M, Ghorbani M, et al. 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L. Flav Frag J 17: 275-277.
- Burgess CA. 2003. *Silybum marianum* (milk thistle). J Pharm Soc Wincons, Mar/Apr: 38-40.
- Cacho M, Moran M, Corchete P et al. 1999. Influence of medium composition on the accumulation of flavonolignans in cultured cells of *Silybum marianum* (L.) Gaertn. Plant Sci 144 : 63-68.
- Cappelletti EM, Caniato R. 1984. Silymarin localization in the fruit and seed of *Silybum marianum* (L.) Gaertn. Herba Hungar 23, 53-62.
- Carrier DJ, Crowe T, Sokhansanj S, et al. 2002. Milk thistle, *Silybum marianum* L. Gaertn., flower head development and associated marker compound profile. J Herbs Spices Med Plants 10: 65-74.
- Copetta A, Lingua G, Berta G. 2006. Effects of three AM fungi on growth, distribution of glandular hairs and essential oil production of *Ocimum basilicum* L. var. *Genovese*. Mycorrhiza 16 (7): 485-494.
- de Abreu IN, Mazzafera P. 2005. Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. Plant Physiol Biochem 43: 241-248.
- Dewick DM. 1998. Medicinal natural products; A biosynthetic approach. John Wiley & Sons, Canada.
- El-Sherbeeney SE, Hussein MS, Khalil, MY. 2007a. Improving the production of *Ruta graveolens* L. plants cultivated under different compost levels and various sowing distance. Amer-Eur J Agric Environ Sci 2 (3): 271-281.
- El-Sherbeeney SE, Khalil EMY, Naguib NY. 2005. Influence of compost levels and suitable spacing on the productivity of *Sideritis montana* plants recently cultivated under Egyptian conditions. Bull Fac Agric Cairo Univ 56: 373-392.
- El-Sherbeeney SE, Khalil M.Y, Hussein MS.. 2007b. Growth and productivity of rue (*Ruta graveolens* L.) under different foliar fertilizers application. J Appl Sci Res 3 (5): 399-407.
- Fliessbach A, Mader P, Dubois D, et al. 2000. Results from 21 year old field trial; Organic farming enhances soil fertility and biodiversity. Bull Res Org Agric 1: 15-19.
- Gichangi EM, Mnkeni PN, Brookes PC. 2009. Effects of goat manure and inorganic phosphate addition on soil inorganic and microbial biomass phosphorus fractions under laboratory incubation conditions. Soil Sci Pl Nutr 55: 764-771.
- Gildon A, Tinker PB. 1983. Interactions of vesicular-arbuscular mycorrhiza infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol 95: 247-261.
- Hanafy AH, Mishrik JF, Khalil MK. 2002. Reducing nitrate accumulation in lettuce (*Lactuca sativa* L) plants by using different biofertilizers. Ann Agric Sci 47 (1): 27-41.
- Hassan FAS, Ali EF, Mahfouz SA. 2012. Comparison between different fertilization sources, irrigation frequency and their combinations on the growth and yield of coriander plant. Aust J Basic Appl Sci 6 (3): 600-615.
- Hendawy, SF. 2008. Comparative study of organic and mineral fertilization on *Plantago arenaria* plant. J Appl Sci Res 4 (5): 500-506.
- Ibrahim AK, Khalifa S, Khafagi I. et al. 2007. Stimulation of oleandrin production by combined *Agrobacterium tumefaciens* mediated transformation and fungal elicitation in *Nerium oleander* cell cultures. Enzym Microb Technol 41: 331-336
- Kafi M, Damghani MM. 2001. Mechanisms of environmental stress resistance in plants. Ferdowsi University, Mashhad.
- Kernaghan G. 2004. Mycorrhiza diversity: Cause and effect International Symposium on Impacts of Soil Biodiversity on Biogeochemical Processes in Ecosystems, Taipei, Taiwan, 2004
- Khalil MY, Naguib YN, El-Sherbeney SE. 2002. Effect of *Tagetes erecta* L. to some foliar application under compost levels. J Agric Sci 10 (3): 939-964.
- Khalil SE, Nahed G, Aziz A, et al. 2010. Effect of water stress and ascorbic acid on some morphological and biochemical composition of *Ocimum basilicum* plant. J Amer Sci 6: 33-46.
- Kurkin VA, Zapesochnaya GG, Volotsueva AV et al. 2001. Flavonolignans of *Silybum marianum* fruit. Chem Natural Comp 37: 315-317.
- Lea IL, Narayan M, Barrett JS. 2007. Analysis and comparison of active constituents in commercial standardized silymarin extract by liquid chromatography-electrospray ionization mass spectrometry. J Chrom B 845: 95-103.
- leaMaster B, Hollyer JR, Sullivan JL. 1998. Composted animal manures: precautions and processing. Anim Waste Manage 6: 100-105.
- Lovisollo C, Schubert A. 1998. Effects of water stress on vessel size and xylem specific hydraulic conductivity in *Vitis vinifera* L. J Exp Bot 49: 693-700.
- Moussavi SM, Salari M, Mobasser HR, et al. 2011. The effect of different irrigation intervals and mineral nutrition on seed yield of Ajowan (*Trachyspermum ammi*). Ann Biol Res 2 (6): 692-698.
- Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ 25: 239-250

- Newman EI, Reddell P. 1987. The distribution of mycorrhizas among families of vascular plants. *New Phytol* 106: 745-751.
- Newsham KK, Fitter AH, Watkinson AR. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *J Ecol* 83: 991-1000.
- Nogués S, Allen DJ, Morison JIL, et al. 1998. Ultraviolet-B radiation effects on water relations, leaf development, and photosynthesis in droughted pea plants. *Plant Physiol* 117: 173-181.
- O'Brien TA, Barker AV. 1996. Growth of peppermint in compost. *J Herbs Spices Med Pl* 4 (1): 19-27.
- Rahmani N, Aliabadi Farahani H, Valadabadi SAR. 2008. Effects of nitrogen on oil yield and its component of *Calendula officinalis* L.) in drought stress conditions. Abstracts Book of The World Congress on Medicinal and Aromatic Plants, South Africa.
- Safar-Nezhad A. 2003. A review on methods of plant selection for drought resistance. *Agric Arid Drought* 45: 7-13.
- Sailo GL, Bagyaraj DJ. 2005. Influence of different AM fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. *Mycol Res* 109 (7): 795-798.
- Schuppler U, He PH, John PCL, et al. 1998. Effect of water stress on cell division and cell-division-cycle 2-like cell-cycle kinase activity in wheat leaves. *Plant Physiol* 117: 667-678.
- Singh-Sangwan N, Farooqi AHA, Singh-Sangwan R. 2006. Effect of drought stress on growth and essential oil metabolism in lemon grasses. *New Phytol* 128 (1): 173-179.
- Taheri AM, Daneshian J, Valadabadi SAR, et al. 2008. Effects of water deficit and plant density on morphological characteristics of chicory (*Cichorium intybus* L.). Abstracts Book of 5th International Crop Science Congress & Exhibition.
- Zucconi F, De Bertoldi M. 1987. Compost specifications for the production and characterization of compost from municipal solid wastes. In: De Bertoldi M (ed) *Compost: Production, quality and use*. Elsevier, London.