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

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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 biofertilization under different irrigation intervals on the growth, production and chemical constituents of *Silybum 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 considerable clear effect on growth and yield characters. The obtained results indicated the favorable effect of organic and biofertilizers which reduce the harmful effect of water stress. Different treatments had a pronounced impact on silymarin content.

Keywords: *Silybum marianum,* silymarin, biofertilization, 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 *Silybum 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) (Lea 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 waterways and protecting groundwater 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-Sherbeeny 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 provides 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, moderate stress could enhance the content of secondary metabolites.

This current experiment targeted the evaluation of the influence of different kinds of organic and biofertilization 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, Egypt to investigate the influence of Chemical, organic and biofertilizers 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 20m^3/feddan
3. Compost 20m^3/feddan+mycorrhiza
4. Compost 20m^3/feddan+compost tea 20 L/feddan
5. Compost 20m^3/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 20m^3/feddan
8. Compost 20m^3/feddan+mycorrhiza
9. Compost 20m^3/feddan+compost tea 20 L/feddan
10. Compost 20m^3/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 20m^3/feddan
13. Compost 20m^3/feddan+mycorrhiza
14. Compost 20m^3/feddan+compost tea 20 L/feddan
15. Compost 20m^3/feddan+compost tea 20 L/feddan+mycorrhiza

The seeds were directly sown in 20th of October 2010. Each plot was 13.5 m^2 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.

Superphosphate or compost was added during preparing soil. The other chemical fertilizers (Ammonium nitrate and Potassium sulfate 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 were 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 was defatted in a Soxhlet apparatus with 50 mL of petroleum-ether at 40-60ºC for 12 h. The residue was extracted with 50 mL of methanol at 65-70ºC 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-H_2O-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, isosilybin 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-Sangwan 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 cyclin-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 intybus* 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 CO_2 diffusion inside the leaf decreased and the total capacity of photosynthesis decreased, therefore, the photosynthetic materials that transferred to seeds will decrease (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 improved 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 (Abdel Aziz and Balbaa 2007) or 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 considerable clear 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 of 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 biofertilizers 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	
23.40	20.00	7.00	190.00	Compost+compost tea+mycorrhiza	
18.55	20.00	8.00	174.00	NPK	6 days
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	
16.40	19.00	10.00	166.00	NPK	9 days
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	
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	6 days
68.96	2.69	7.22	18.51	11.30	11.30	17.94	Compost	
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	
82.58	2.89	8.11	24.27	14.47	10.99	21.85	NPK	9 days
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	
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 was 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 percentage of the secondary products of medicinal and aromatic plants, because, in case of stress, more metabolites are produced in the plants and substances prevent oxidization in the cells, but secondary products content reduce under drought stress, because interaction between the amount of secondary products percentage and mass production is considered important; as two components of the content of secondary product 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 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 flavonoids was nearly the same in Pisum sativum plants grown under drought stress or 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 biofertilizers on silymarin content may be due to effect of these fertilizers on accelerating metabolism reactions as well as stimulating enzymes. Application of biofertilizers and compost significantly improved secondary products such as essential oil, rutin, and coumarin (El-Sherbeeny 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 of 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 biofertilizer 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 biofertilizers can reduce the harmful effect of water stress.

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