Effect of vermicompost and mycorrhiza fungi on yield and growth of milk thistle and antioxidant system activity

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Abstract

In this study, the effect of vermicompost and mycorrhizal fungi was investigated on growth, yield, chlorophyll pigments, leaf antioxidant enzymes, and seed silibinin content of *Silybum marianum*, milk thistle. The seeds were inoculated by two species of mycorrhiza fungi, *Glomus mosseae* and *G. intraradices*, and plants were irrigated and treated with 0, 25, 50, and 75% vermicompost after culturing. The treated plants were then compared to control plants in a greenhouse experiment. The results showed that growth parameters including leaf area, and plant height and yield significantly increased in mycorrhiza fungi treated plants especially along with 75 % vermicompost treatment. The effects of symbiotic relationship between milk thistle and *G. intraradices* were more pronounced than *G. mosseae*. Moreover, combination of mycorrhiza and vermicompost increased the photosynthetic pigments chlorophyll (a), chlorophyll (b), total chlorophyll, and carotenoid. Also, a significant decrease was observed in activities of peroxidase, superoxide dismutase, and catalase after vermicompost and mycorrhiza treatment. The results showed that silibinin decreased significantly in vermicompost application.

Key words: catalase, chlorophyll, organic fertilizers, peroxidase, superoxide dismutase

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Introduction

Milk thistle (*Silybum marianum*) is an annual or a biennial plant of the Asteraceae family which is native to the Mediterranean regions of Europe, North Africa, and the Middle East (Ross, 2008). It is an ancient medicinal herb and some experiment and clinical studies have shown the anticancer, antidiabetic, and cardio protective effects of its extracts (Tamayo and Diamond, 2007). Silymarin, a mixture of flavonoid complexes, is the main essential oil in milk thistle

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which is wildly used to control liver diseases (Hellerbrand et al., 2016). The major active constituent of Silymarin, a standardized extract of the milk thistle seeds is silibinin (Davis-Searles et al., 2005).

Antioxidant system activity plays an important role in Silymarin functions in controlling disease (Post-White et al., 2007). Antioxidant activity consists of enzymatic and non-enzymatic antioxidants. Major antioxidant enzymes include catalase, peroxidase, superoxide dismutase, and glutathione s-transferease (Antolovich et al., 2002; Weydert and Cullen, 2010) which are used to measure endogenous antioxidant activities and fluctuations in plants (Ghanati et al., 2005; Gill and Tuteja, 2010; Sairam et al., 2002). Silymarin and its related compounds are found to be scavengers of active oxygen species such as the superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (Kiruthiga et al., 2007).

The effects of vermicompost amendment on plant growth and physiology varies depending on biological and environmental factors such as species and location. While some studies report the improving effect of vermicompost application on shoot and root dry weights (Paul and Metzger, 2005), others show no effect (Bachman and Metzger, 2007). This is also the case with plant nutrient uptake. Vermicompost contains available forms of nutrients and it is easily absorbed by plants (Edwards and Burrows, 1988). Peyvast et al. (2008) found a significant increase in total nitrogen content of plants following vermicompost treatment. However, Federico et al. (2007) reported no significant effect of vermicompost application on plant total nitrogen contents. Similarly, while some studies on relative water content reported a positive effect of vermicompost application (Verma et al., 2013), others showed no significant changes in this parameter (Jain et al., 2012). However, most studies agree on the positive effect of vermicompost amendment on potassium (K) concentration in plant tissue (e.g., Chamani et al., 2008; Peyvast et al., 2008). Vermicompost application also significantly increased plant leaf area (Peyvast et al., 2008).

Mycorrhiza fungi play an important role in microbial activity, nutrients dynamics, and plant ecology. These fungi are like other beneficial microorganisms such as P solubilizing bacteria (Panhwar et al., 2009) and N fixing bacteria (Naher et al., 2009). Mycorrhizal fungi are natural inhabitants of tropical soils (Smith and Read, 2010). Glomus is a genus of arbuscular mycorrhizal (AM) fungi which are thought to be obligate symbiotes (Wang and Qiu, 2006). The AM association is a relatively non-specific, highly compatible, long lasting mutualism from which both partners derive benefit (Smith and Read, 2010). In addition to its ecological significance, this association may also have applications in agriculture, particularly in sustainable systems (Schreiner and Bethlenfalvay, 1995) where the intimate link between the soil and the plant is created by the mycorrhiza. Since mycorrhiza impact nutrient movement, they may be fully exploited in plant nutrition and soil conservation (Balestrini, 2016). Mycorrhizal symbiosis often leads to changes in the rate of water flow into host plant and influence in tissues hydration and leaves physiological activity (Augé, 2001). It even can increase root adsorption up to 47 times (Smith and Read, 2010). The dry matter of onions in symbiosis with mycorrhiza fungus, *Glomus macrocarpum*, was 5 to 6 times higher than non-In fact, vermicompost and mycorrhiza could have a great potential in sustainable agriculture systems mycorrhizal plants (Thomas et al., 1986).

Vermicompost and mycorrhiza could have a great potential in sustainable agriculture systems. Although there are many studies on antioxidant enzymes activity in plants under stress, little is known on the effect of vermicompost and mycorrhiza on the antioxidant activity in medicinal plants. The main objective of this research was investigation of the impacts of vermicompost and mycorrhiza fungi on growth, antioxidant activity, and chlorophyll pigments in organic cultivation of *Silybum marianum.*

Material and Method

Experimental performance and plant materials

A pot experiment in greenhouse was carried out based on a completely randomized design with three replications in 2015 at the Islamic Azad University, Gorgan Branch. Seeds of *Silybum marianum* were provided from Pakban Bazr Co. Two species of mycorrhiza namely, *Glomus mosseae* and *G. intraradices* procured from Hamoon Morvarid Co., Iran were used for milk thistle seed priming. The seeds were sown in 20-liter pots containing field soil and grown in greenhouse condition (average temperature 25/20°C day/night, RH 75%, photoperiod 12 h, PPF 250 µM m^{-2} s^{-1} (400–700 nm) at the plant level). Vermicompost was used at 25, 50, and 75 % concentrations in irrigation water.

Growth and yield measurement

After 60 days from planting, plant growth traits including plant length and stem diameter were measured by a ruler and caliper. Leaf characters, length, width, and area were measured at 3 to 4 leaf stage by leaf area meter and the average was used for analysis. Number of inflorescence capitule per plant was recorded at the beginning of reproductive season. At the end of vegetative growth and harvesting time, the dry seeds (g) per plant was measured and yield (kg/ha) was calculated with a simple proportion calculation considering pot planting surface. The weight of 1000 seeds was recorded in each pot.

Antioxidant enzymes activity measurement

Fresh leaf samples were harvested two month after planting and immediately were kept in liquid nitrogen at -40° C until antioxidant enzymes and photosynthetic pigments assay. Peroxidase enzyme (PO) was measured in 60 mM Na-phosphate buffer (pH 6.1) by spectrophotometric method and 470 nm absorption according to the method described by Pandolfini et al. (1992). The activity of catalase (CAT) was measured in a reaction mixture consisting 10 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8). The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm within 1 min, using spectrophotometer (T90, Beijing Karaltay Scientific Instruments, China). CAT activity was expressed based on changes in the absorbance against mg of protein in the extract (Ghanati, Morita and Yokota 2005). Protein content was measured by (Bradford, 1976) and BSA standard. The activity of superoxide dismutase (SOD) was measured using the method of (Giannopolitis and Ries, 1977) by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm using the spectrophotometer. SOD activity values were given in units per mg of protein.

Total soluble sugars and photosynthetic pigments assay

Total content of soluble sugars was measured by anthrone reagent (Yemm and Willis, 1954) with a few modifications. Briefly, 0.5 g of samples were homogenized in 95% ethanol and filtered. The residue was twice extracted with 70% ethanol and the filtrates were added and centrifuged at 3500 × g for 15 min. 100 µl of supernatant and 3 mL of anthrone reagent (150 mg anthron+100 mL H_2SO_4 72%) was added and heated in a bath at 100° C for 10 min. The absorbance of the liquid was measured at 625 nm using glucose as a blank.

Photosynthetic pigments were measured in 80% acetone-extracted samples according to (Arnon 1949). The absorbance of chlorophyll and carotenoid content was measured at three-wave lengths 470, 645, and 663 nm using spectrophotometry. The chlorophyll and carotenoid concentrations are calculated as follows:

$$\text{Chlorophyll a} = [(19.3 \times A663) - (0.86 \times A645)]\ V/100W$$

Chlorophyll b = [(19.3 × A645) – (3.6 × A663)] V/100W

Carotenoides = [100(A470) - 3.27(mg chl. a) - 104(mg chl. b)]/227

Total chlorophyll (mgg^{-1}) =

$$[20.2\ (OD645) + 8.02\ (OD663)]V / 1000W$$

where OD is optical density, V is final volume of 80% acetone (ml), and W is dry weight of sample taken (g).

Silibinin content measurement

Exteraction and isolation

Dry powdered seeds (3 g) were placed in a Soxhlet apparatus for 10 hours, petroleum ether (50 ml g) was used as defatting solvent before it was filtered under vacuum. The residue was dissolved in MeOH and extracted for 16 hours. The combined extracts were evaporated to dryness. The yellow remaining powder was dissolved in MeOH up to 50 ml volume and analyzed by HPLC.

HPLC analysis

HPLC (Knauer Co, Germany) was carried out using a K1001 pump, monitored at 280 nm by UV-VIS detector K2501 and quantified. 20 µl of diluted sample, 1:10, was injected. A mixture of acetonitrile-methanol-water was used as mobile phase. Total time of chromatography was 30 min. The silibinin concentration was assayed by comparing obtained peak with the peaks of standard curve for different concentrations of pure silibinin (sigma).

Statistical analysis

Analysis of variance was performed by SAS 6.1 software and differences among the treatments were evaluated by Duncan Test ($P \leq 0.05$).

Results

Growth character and yield

The results of mycorrhiza and vermicompost treatments on milk thistle growth characteristics showed that height of plants, stem diameter, leaf area, and leaf size were affected by mycorrhiza fungi and vermicompost. However, these changes were only statistically significant when mycorrhiza fungi and vermicompost were used together (Table 1). The highest amount of growth characters was observed at 75 % concentration of vermicompost combined by mycorrhiza (Table 1). Although mycorrhizal species did not make statistical differences in growth traits except leaf length, these result showed that *G. intraradices* influenced growth of milk thistle more than *G. mosseae* (Table 1). Results showed that mycorrhiza increased the yield and number of seeds per plant (Table 1); however, this increase only was statistically significant in *Glomus intraradices* (Table 1). *G. intraradices* was more efficient than *G. mosseae* in yield and related traits including the number of capituls, seed weight per plant, and weight of one thousand seeds (Table 1). Vermicompost significantly increased seed weight per hectare, seed weight per plant, the number of capitule, and seed weight and this increase was more

Table 1

The effects of Mycorrhiza fungi and vermicompost on growth and yield of Milk thistle

Mycorrhiza	vermicompost %	Plant Height (cm)	Stem Diameter (mm)	Leaf aria (cm²)	Leaf Width (cm)	Leaf Length (cm)	Yield (kg/h)	Capitule (number)	Weight of 1000 seeds (g)	Seed weight per plant (g)
Glomus mosseae	0	$41.33^{bc} \pm 3.38$	$5.63^{ab} \pm 0.16$	$1.32^{ef} \pm .06$	$3.5^{b} \pm .29$	$8.66^{cd} \pm 0.34$	$550^{e} \pm 39$	$2.1^{cd} \pm 0.35$	$10.46^{cd} \pm 0.51$	$32^{bc} \pm 2.08$
	25	$48^{b} \pm 2.02$	$5.76^{ab} \pm 0.16$	$1.46^{cde} \pm .05$	$3.83^{ab} \pm .14$	$8.66^{cd} \pm 0.33$	$1216^{c} \pm 45$	$2.43^{bc} \pm 0.32$	$11.45^{bc} \pm 0.24$	$43^{a} \pm 1.59$
	50	$50^{b} \pm 2.3$	$5.86^{ab} \pm 0.26$	$1.53^{bcd} \pm .21$	$3.9^{ab} \pm .19$	$9.66^{bc} \pm 0.42$	$1270^{c} \pm 57$	$2.63^{ab} \pm 0$	$11.67^{bc} \pm 0.80$	$44^{a} \pm 2.6$
	75	$66.33^{a} \pm 2.8$	$6.13^{a} \pm 0.2$	$1.72^{ab} \pm .08$	$4.5^{a} \pm .19$	$10.66^{ab} \pm 0.39$	$1765^{a} \pm 81$	$2.93^{a} \pm 0.29$	$13.64^{a} \pm 0.35$	$44.6^{a} \pm 3.33$
G. intraradices	0	$43^{bc} \pm 5.23$	$5.7^{ab} \pm 0.36$	$1.38^{def} \pm .07$	$3.33^{b} \pm .39$	$9^{b} \pm 0.55$	$725^{d} \pm 21$	$2.1^{cd} \pm 0.50$	$10.83^{cd} \pm 0.19$	$36^{b} \pm 2.01$
	25	$52^{b} \pm 3.32$	$5.87^{ab} \pm 0.3$	$1.63^{abc} \pm .08$	$4.33^{a} \pm .28$	$10.33^{ab} \pm 0.58$	$1513^{b} \pm 145$	$2.83^{ab} \pm 056$	$12.82^{ab} \pm 0.38$	$44.3^{a} \pm 2.3$
	50	$64^{a} \pm 2.8$	$5.9^{ab} \pm 0.38$	$1.64^{abc} \pm .08$	$4.46^{a} \pm .17$	$10.66^{ab} \pm 0.57$	$1666^{ab} \pm 155$	$2.86^{ab} \pm 0.47$	$12.98^{ab} \pm 0.31$	$45^{a} \pm 2.8$
	75	$67^{a} \pm 2.3$	$6.13^{a} \pm 0.3$	$1.75^{a} \pm .09$	$4.66^{a} \pm .17$	$11^{a} \pm 0.31$	$1698^{a} \pm 63$	$2.9^{a} \pm 0$	$13.87^{a} \pm 0.41$	$46^{a} \pm 3.54$
Control		$34.33^{c} \pm 3.62$	$5.23^{b} \pm 0.28$	$1.24^{f} \pm .21$	$3.33^{b} \pm .26$	$7.46^{c} \pm 0.25$	$434^{e} \pm 30$	$1.86^{d} \pm 0.23$	$9.59^{d} \pm 0.32$	$28^{c} \pm 1.65$

Values (mean ± SE) followed by different letters are significantly different, according to the Duncan's test

Table 2
The effects of mycorrhiza fungi and vermicompost on chlorophyll pigment, carotenoid and sugars in Milk thistle leaves

Mycorrhiza	vermicompost %	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total Chlorophyll (mg/g Fw)	Carotenoids (mg/g Fw)	Sugars (mg/g Fw)
Glomus mosseae	0	8.381^{a} ± 0.21	4.61^{a} ± 0.13	14.6^{a} ± 0.35	86.8^{a} ± 1.65	1373.2^{bc}± 21.64
	25	6.20^{b} ± 0.49	3.15^{a} ± 0.15	10.7^{c} ± 0.46	65.2^{b} ± 1.27	1378.9^{bc} ± 28.90
	50	6.03^{b} ± 0.18	1.58^{a} ± 0.04	8.69^{bc} ± 0.49	64.7^{b}± 1.43	1283.6^{c}± 29.43
	75	1.26^{d} ± 0.58	0.95^{a} ± 0. 07	4.85^{de} ± 0.25	40.8^{e} ± 1.01	1891 ^{a} ± 18.90
G. intraradices	0	5. 37^{b} ± 0.19	4.3^{a} ± 0.12	11.1^{b} ± 0.38	60.6^{c} ± 1.29	1420.9^{bc} ± 31.36
	25	3.35^{c} ± 0.17	2.40^{a} ± 0.06	7.24^{cd} ± 0.37	36.4^{f}± 1.67	1539^{b} ± 34.66
	50	3.78^{c}± 0.21	2.12^{a} ± 0.14	8.66^{bc} ± 0.19	45^{d} ± 1.43	1885^{a} ± 25.52
	75	6.14^{b} ± 0.53	18.09^{a} ± 0.11	10.38^{bc} ± 0.43	66.4^{b} ± 1.58	1398.2^{bc} ± 19.80
Control		1.12^{d} ± 0.06	0.8^{a}± 0.1	3.23^{e}± 0.03	17.8^{g} ± 1.64	600^{d}± 15.29

Values (mean ± SE) followed by different letters are significantly different according to the Duncan's test.

noticeable in treatments with higher concentrations of vermicompost and in *G. intraradices* (Table 1). Yield, seed weight (kg) per ha, was 1765 kg/ha in combined application of *G. mosseae* with 75 % vermicompost which shows about 4 times yield increase in comparison with control plants (Table 1).

Chlorophyll pigments, sugar and carotenoid

The results showed that both mycorrhiza species, alone or in combination with vermicompost, significantly increased chlorophyll a, b, and total chlorophyll in comparison with control plants (Table 2). The highest level of chlorophyll a, 8.381mg/g FW, was observed in plants treated with *G. mosseae* while the average amount of chlorophyll a in control plants was only 1.12 mg/g FW (Table 2). Comparison of the effects of mycorrhiza on chlorophyll a and total chlorophyll showed that *G. mosseae* species are more effective than *G. intraradices*; however, there were no significant differences in chlorophyll b content between the two species (Table 2). The highest production of chlorophyll b in leaves was observed in *G. mosseae* and 75% vermicompost with G. *intraradices* (Table 2).

Results of leaf carotenoid analysis showed that mycorrhiza fungi significantly increased this pigment more than three times, in comparison with control plants and this increase in *G. mosseae* incubation was significantly more than *G. intraradices* (Table 2). The highest level of carotenoid pigments was 86.8 mg/g FW in *G. mosseae.* Results showed that the amount of soluble sugars significantly increased in mycorrhiza fungi seed inoculation and vermicompost treatments (Table 2). There were significant differences between two types of mycorrhiza in soluble sugar levels. *G. mosseae* was most effective in increasing soluble sugars. The highest amount of soluble sugars in the leaves was related to the treatment with *G. mosseae* mycorrhiza and 75% vermicompost (Table 2).

Leaf protein content

Protein content increased significantly in mycorrhiza fungi treatment. There was a statistically significant difference between two species of mycorrhizal. Vermicompost increased leaf protein content significantly only in 50 g/l fertigation in both mycorrhiza species. The highest leaf protein content, 2.16 mg/g FW, was observed in 25% vermicompost and *G.*

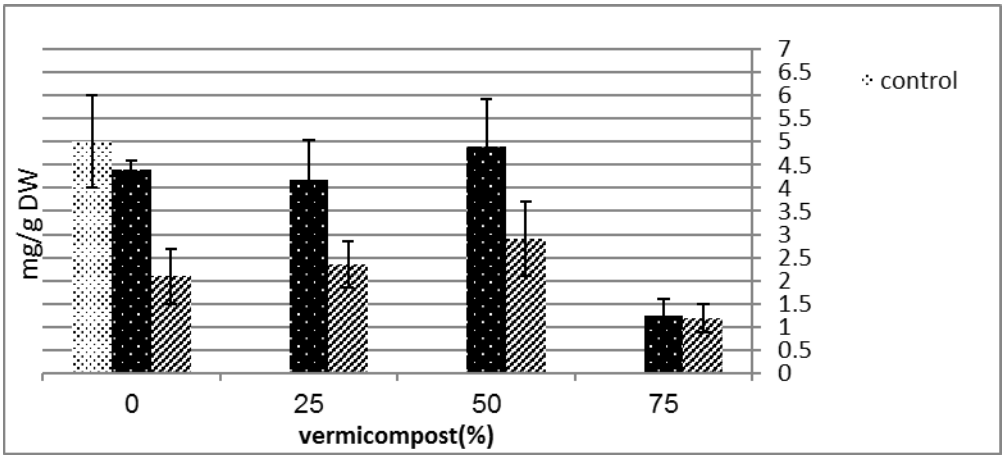

Fig. I. The effects of vermicompost, *Glomus intraradices*, and *Glomus mosseae* on seed Silibinin content of Milk thistle (means ± SE) mg/g DW

intraradices and leaf protein was reduced in higher concentrations of vermicompost treatment (Table 3).

Leaf antioxidant enzymes

Activity of peroxidase enzyme in leaves of milk thistle significantly decreased in mycorrhiza fungi- and vermicompost-treated plants (Table 3). A significant difference in peroxidase enzyme activity was observed in mycorrhiza species (Table 3). Maximum value of peroxidase (50 ΔAbs 470/mg protein) was observed in control plants while minimum value (12.7 ΔAbs 470/mg protein) was observed in G. *mosseae* and 50% vermicompost (Table 3). Catalase activity reduced significantly in mycorrhiza fungi-inoculated plants. Catalase in control treatment reached the maximum value 63. 3 (ΔAbs 240/mg protein). Catalase activity in *G. mosseae* mycorrhiza significantly reduced to the lowest rate of 12.03 (ΔAbs 240/mg protein). Leaf catalase activity raised when vermicompost concentration increased in both mycorrhiza species (Table 3). The SOD activity decreased significantly in vermicompost and mycorrhiza fungi treatments (Table 3). Plants inoculated with *Glomus mosseae* had less SOD activity than *G. intraradices*. The highest and lowest amount of SOD activity were found in control treatment (320 U/protein) and in plants treated with 75% vermicompost and *G. mosseae* (189.2), respectively (Table 3).

Seed silibinin content

The amount of measured silibinin by HPLC method was 4.39±0.19 mg per gram of dry seed of milk thistle (Fig 1). Results showed that silibinin decreased significantly in vermicompost application (Fig I). Mycorrhizal fungi caused a slight decline in silibinin although this reduction was not statistically significant (Fig I). The amount of silibinin was less than 1.19% of seed dry weight in *G. intraradices* and 75% vermicompost treatment.

Discussion

The results showed that the use of vermicompost can have noticeable effects on increasing seed yield and some growth traits of milk thistle which can be a consequence of physiological changes in plant. Vermicompost 75% was more efficient than the other concentrations was it resulted in more increase in leaf size and leaf area expansion.

Many studies have reported positive effects of vermicompost treatments on various plants. Incorporation of pig manure vermicompost enhanced shoot and root weight, leaf area, and shoot/root ratios of tomato and French marigold (Bachman and Metzger, 2008). Joshi and Vig (2010) also reported increase in plant growth parameters (plant height, number of leaves, and plant dry biomass) with application of 45% vermicompost (cattle dung) amended treatment in *L. esculentum*. Also, Gupta et al. (2014) reported that addition of cow dung and household-based vermicompost in appropriate quantities to the potting media resulted in increased growth and flowering of marigold seedlings including plant biomass, plant height, number of buds and flowers. In addition, increase in length, biomass, number of seeds, number of shoots in Vinca rosea and tillers in *Oryza sativa* has been reported by Reddy (1986) in 50:50 soil to vermicompost mixtures. Tomati et al. (1983) showed positive effects of vermicompost on the growth of Begonias sp. and Coleus sp. (Ornamental plants), especially a stimulation of rooting and time of flowering in plots amended with vermicompost. Plant height of maize also increased significantly as compared to the control when grown in soil amended with vermicompost (Gutierrez- Miceli et al. 2008). Azarmi et al. (2008) reported increase in leaf area and shoot dry weight by 43% and 27 %, respectively, in tomato with 15 t/ha sheep manure vermicompost applications, whereas Atiyeh et al. (2001) reported increase in shoot height of tomato plants with the amendment of 5 % pig manure vermicompost.

Mycorrhiza fungi increased yield parameters compared with the control plants and the performance of *G. intraradices* was much more than *G. mosseae*. The increase in growth and yield in milk thistle plants by adding vermicompost treatment to mycorrhiza fungi was very remarkable. Tohidi-Moghaddam et al. (2004) reported that mycorrhiza through expanding roots and increasing accessibility, rose phosphorus absorption and thereby increased the number of seeds per plant as well as other components of yield in soybean. Symbiotic mycorrhiza fungi with wheat roots increased the shoot dry weight, number of tillers per plant, and the root length (Mohammad et al., 1995). Mycorrhiza fungi spread mycelia networks outside roots and expanded the contact surface of the roots to soil and increasing nutrient absorption and transport to roots which are effective in improving yield and their components (Khan, 2006).

Combination of mycorrhiza and vermicompost increases photosynthetic pigments chlorophyll (a), chlorophyll (b), total chlorophyll, and carotenoid. Induction of fungus mycelium of mycorrhiza in plant roots provided access to larger volumes of soil that led to more water and nutrient absorption (Smith et al., 2003). Arbuscular mycorrhiza fungi have hyphae and mycelia with inner and outer root (Zarei et al.,

Table 3
The effects of vermicompost, *Glomus* intraradices, and *Glomus* mosseae on the activities of peroxidase, superoxide dismutase, catalase, and protein in Milk thistle leaves

Mycorrhiza	vermicompost %	Protein (mg/g Fw)	PO (ΔAbs 470/mg protein)	CAT (Δabs240/mg protein)	SOD (U/mg protein)
Glomus mosseae	0	1. 87^{bc} ± 0. 51	27.9^{b} ± 4.65	12.03^{c}± 0. 8	217.7^{d} ± 14.4
	25	1.71^{d} ± 0. 16	13.25^{b} ± 6.41	42^{b} ± 1.10	222.4^{d} ± 24.6
	50	2.07^{a} ± 0. 44	12.7^{b} ± 0.867	15.2^{c} ± 6.31	232.6^{cd} ± 12.6
	75	1. 61^{b} ± 0. 36	17.95^{b} ± 4.91	26^{c} ± 0.4	189.2^{e} ± 15.3
G. intraradices	0	1. 75^{cd} ± 0. 29	13.88^{b} ± 6.34	12.4^{c} ± 3.9	250. 5^{c} ± 5.3
	25	2.16^{a} ± 0. 65	13.87^{b} ± 3.89	12. 8^{c} ± 0.28	280.3^{b} ± 18. 9
	50	2. 11^{a} ± 0. 10	15.3^{b} ± 2.44	12.7^{c} ± 5.1	279. 4^{b} ± 18.7
	75	1.76^{cd} ± 0. 36	12.97^{b} ± 3.56	53^{ab} ± 4.3	224.3^{d} ± 11.6
Control		1. 6^{e} ± 0.018	50^{a}± 4.37	63. 3^{a}± 6.3	320^{a} ± 19.90

Values (mean ± SE) followed by different letters are significantly different according to the Duncan's test.

2006). Using mycorrhiza arbuscular fungi in corn improved the synthesis of chlorophyll and increased photosynthesis in plants (Smith and Read, 2010). The effect of vermicompost and mycorrhiza fungi on sugar and protein contents of leaves was significant (Table 2 and 3). Golchin et al. (2006) demonstrated that chlorophyll content of the leaves of pistachio (*Pistacia vera* L.) and the photosynthesis rate were better in vermicompost treatments relative to the control. Berova and Karanatsidis (2009) found a distinct increase in the content of chlorophyll a and chlorophyll b in comparison with the control. Vermicompost has chelating power through which it affects nutrition. Consequently, the production of plant pigments and transfer of photosynthesis products are made easier for the plant. This is why chlorophyll (a), chlorophyll (b), total chlorophyll, and carotenoid showed increase in the treatment of vermicompost and mycorrhiza. In many plants such as wheat and beans, these compounds increased protein and chlorophyll in plant substances by increasing the rate and extent of food absorption (Abou-Aly and Mady, 2009; El-Bassiony et al., 2010). Vermicompost contains macronutrients, beneficial microorganisms, and hormones which influence the growth and yield of plants (Theunissen et al., 2010). Macronutrients play an important role in crop yield based on their role in activation of enzymes for chlorophyll synthesis, growth, fruit ripening, and maintenance of the plant enzyme system (Grusak and Della Penna, 1999). Vermicompost is known to provide a slow, balanced nutritional release pattern to plants, particularly in terms of release of plant-available N, soluble K, exchangeable Ca, Mg, and P (Edwards and Fletcher, 1988) which is subsequently used by plants efficiently. The beneficial effects of vermicompost treatments in increasing photosynthetic pigments and carbohydrates formation may be explaining by its favourable effect on enhancing growth parameters. Also increased flowering could be attributed to the increased photosynthetic rates as a result of using vermicompost treatments.

Mycorrhiza symbiosis with spearmint was also found to increase water and nutrients absorption through roots which leads to enhanced photosynthesis and this in turn results in more production, improved biological performance, and increased carbohydrates and protein (Canellas et al., 2015). Mycorrhiza fungi also increased the absorption of nitrogen that plays a key role in chlorophyll building and protein synthesis (Balestrini R. 2016). Augé (2001) found that symbiosis with mycorrhiza increased the number of photosynthetic units.

Although the direct effect of vermicompost was not evaluated in this experiment and all effects were on interaction with mycorrhizal symbiosis, differences in the studied traits after vermicompost supplementation were remarkable. Vermicompost as an organic acid derived from humus and other natural resources have an essential role in soil quality. It is the most important part of organic matter that directly plays a major role on the release of nutrients, cation exchange capacity, buffering capacity, and retention of phosphorus metal and toxic organic molecules. Humus substances increase absorption of minerals through stimulating and adding microbiological activities. Organic acids have very small quantities of hormonal substances with beneficial effects on increasing the production and quality of agricultural products (Canellas, 2015).

A significant decrease in activities of peroxidase, superoxide dismutase, and catalase was observed after vermicompost and mycorrhiza treatment. Antioxidant enzyme activity in cells over expressing plants growing in the face of increased environmental stress and thereby can reduce the damage caused by oxygen free radicals. Research has shown that inoculation of mycorrhiza fungi plays an important role in increasing plant resistance against environmental stress (Augé, 2001). Silibinin content also was significantly reduced by vermicompost treatment. There are reports showing that drought stress enhanced accumulation of silibinin in milk thistle seeds (Afshar et al., 2015). On the other hand, vermicompost and mycorrhiza reduce water stress effects and loss. Therefore, antioxidant enzyme activity and silibinin reduced in proper growth condition provided by biostimolators.

Conclusion

Vermicompost and mycorrhizae with increasing photosynthetic pigments enhanced growth and yield of milk thistle plant; however, antioxidant activity per unit significantly decreased. Combination of mycorrhiza and vermicompost can increase the photosynthetic pigments chlorophyll (a), chlorophyll (b), total chlorophyll, and carotenoid. Simultaneous use of vermicompost and mycorrhiza puts the plant in an ideal condition where it spends less energy for production of peroxidase, superoxide dismutase, and catalase enzymes. Silibinin content also was significantly reduced by vermicompost treatment. Despite bio-stimulators such as vermicompost and mycorrhiza potential for increasing growth and yield parameters of milk thistle, they decrease antioxidant activity and secondary metabolism production.

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