Journal of Applied Research on Medicinal and Aromatic Plants 1 (2014) 148–151

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Short communication

Phenological response of milk thistle (*Silybum marianum* [L.] Gaertn.) to different nutrition systems

Samieh Eskandari Nasrabadi*, Reza Ghorbani, Parviz Rezvani Moghaddam, Mehdi Nassiri Mahallati

Department of Agronomy and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 14 October 2014
Accepted 14 October 2014
Available online 20 November 2014

Keywords:
Biosulfur
Developmental stage
Fertilizer
Growth
Mycorrhiza

ABSTRACT

Nowadays, growing medicinal plants in sustainable agricultural systems in which sustainable plant nutrition strategies play an important role are more focused. Seeds of milk thistle plants (*Silybum marianum* [L.] Gaertn.) are the main source of silymarin which is known as the biological active component helpful in treating liver and biliary diseases and also preventing liver cancer. Phenological stages, especially the reproduction time and maturity of milk thistle, might be affected by management strategies. In order to investigate the potential effects of different nourishment systems on phenological stages of milk thistle, a field experiment was conducted during 2010–2011, at Research Station, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. Twelve nutrition systems comprising single and integrated nutrition systems of vermicompost, poultry manure, chemical fertilizer, mycorrhiza (*Glomus mosseae*), and bio-sulfur (*Thiobacillus* sp.) were compared in a completely randomized block design with three replications. According to the results, growth development stages of milk thistle in Mashhad climate were found to have four discrete stages; vegetative, elongating, flowering, and seed maturation stage. Analysis of variance of heat units, expressed in growing degree-days (GDDs), showed that elongating and flowering stages were significantly affected by different nutrition systems ($P < 0.05$). Milk thistle plants treated with mycorrhiza started the elongating stage and flowering stage with less GDDs (845 and 1001 °C, respectively) compared with other treatments. The highest amount of GDDs for starting the elongating stage was recorded for chemical fertilizer treatment and plants under integrated use of mycorrhiza and vermicompost required more GDDs to start flowering stage.

1. Introduction

Milk thistle (*Silybum marianum* [L.] Gaertn.) is a member of Asteraceae family which has large prickly edged leaves covered with undulating white patches and stems containing a milky juice. Milk thistle is an annual plant that grows naturally in warm climates and different provinces of Iran such as Kurdistan, Kermanshah, Lorestan and Ahvaz (Shamsi, 2009). Seeds of milk thistle plants are the main source of silymarin, which is used in pharmaceutical industries. Silymarin is a flavonoid complex which consists

* Corresponding author. Current address: Department of Agronomy and Soil Science, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia. Tel.: +61 267732715; fax: +61 267733238.

E-mail addresses: samie.eskandari@gmail.com, seskanda@une.edu.au (S. Eskandari Nasrabadi), reza-ghorbani@um.ac.ir (R. Ghorbani), prm93@yahoo.com (P. Rezvani Moghaddam), mnassiri@ferdowsi.um.ac.ir (M. Nassiri Mahallati).

http://dx.doi.org/10.1016/j.jarmap.2014.10.002

Table 1
Physicochemical characteristics of the experimental soil collected from depth of 0–30 cm.

Soil texture	Organic carbon (%)	Total N (%)	P (ppm)	K (ppm)	pH	EC (dS m^{-1})
Loam	0.5	0.063	13.2	135	7.24	3.21

Table 2
Nutrient contents of vermicompost and poultry manure.

Organic fertilizer	Total N (%)	P (%)	K (%)
Vermicompost	1.3	1.3	1.2
Poultry manure	2.49	2.33	1.67

of different compounds including silybinin, isosylibinin, silydianin, and silychristin, with the common name flavanolignans (Guz and Stermitz, 2000). It is believed that silymarin extracted from the ripe seeds of milk thistle is the biologically active component. The whole plant is used for medicinal purposes, but the highest content of silymarin is accumulated in the seeds. The main reason for the wide cultivation of this plant is due to its importance in treating liver and biliary diseases and also preventing liver cancer (Tamayo and Diamond, 2007). Increased in crop production largely depends on the type of fertilizers used to supplement essential nutrients for plants. The use of chemical fertilizer, organic fertilizer or bio-fertilizer has their own advantages and disadvantages but the advantages need to be incorporated in order to make best use of each type of fertilizer and attain fair nutrient management for crop growth (Chen, 2006). Crop management will definitely be improved by knowledge of crop phenology. Phenology regulation is important for the synchronization on the plant reproductive process resulting in simultaneously seed maturation and harvest. This knowledge is based on observation of the growth and the development stages of the plant. Phenology can also be considered as one of the major component in the adaptation of plants to any given environment (Desclaux and Roumet, 1996). In order to have a constant and stable index for estimating the crop growth and determining the accurate time of phenological stages, GDD or growing degree days seems to be more reliable and has been confirmed by Clarke and Simpson (1978). While the effect of nitrogen fertilizer, seed rate, plant spacing and salinity on grain yield and active substances in milk thistle have been well documented (Ghavami and Ramin, 2008; Omer et al., 1990; Omidbaigi and Nobakht, 2001). No information about phenological response of this medicinal species to a range of nutrition systems is available; therefore this experiment was conducted to understand the single and integrated effects of chemical, biological and organic fertilizers on phenology of milk thistle in Mashhad climate, Iran.

2. Materials and methods

2.1. Experimental site preparation

The field experiment was conducted during 2010–2011, at Research Station, Faculty of Agriculture, Ferdowsi university of Mashhad, Iran (36°16′ N, 59°36′ E, elevation 985 m). Before conducting the experiment, soil samples were taken from the depth of 0–30 cm to determine the physicochemical characteristics of the experimental site (Table 1). The initial plowing and tillage procedure were performed in Autumn 2010, after 12 month fallow, and second tillage procedure was done in early 2011, after that plots (3 m × 4 m) were designed for furrow irrigation with 7 rows per plot. Forty days before sowing, the rows in organic treatment plots were thoroughly mixed with organic manures, vermicompost and poultry manure. Plots were separated from each other by two rows spacing. The monthly total amount of natural precipitation for the first 3 months of the growing period were 17.72, 14.35, and 10.52 mm, respectively. No precipitation was recorded in the last 5 weeks of the growing period in Mashhad, Iran.

2.2. Experimental design and treatments

A complete randomized block design with twelve treatments and three replications was used. Treatments were: Control, Vermicompost (V), poultry manure (H), Chemical fertilizer (CH), Mycorrhiza (M), Bio-sulfur (B), M + V, M + CH, M + H, B + V, B + CH, B + H. Detailed information about treatments is presented in Table 3. Vermicompost and poultry manure values were calculated based on their nutrient contents (Table 2) and milk thistle nitrogen requirement in Mashhad region, Iran (Malakooti and Tehrani, 1993). Bio-fertilizers including mycorrhiza and bio-sulfur were added based on factory instruction and recommended dozes at the time of sowing. Integrated treatments contained the same amounts of fertilizers as single treatments. The amounts of chemical fertilizer were determined based on recommended doses in Mashhad soil and weather condition for milk thistle as a medicinal plant (Malakooti and Tehrani, 1993). Urea was distributed three times during the growth period to avoid leaching.

2.3. Phenological stage recordings in field

Five plants were randomly selected in each plot and growth development stages were recorded every few days from emergence. In order to have a better understanding of milk thistle phenological stages, Growing Degree-Days (GDDs) were used and heat units earned at the end of each phenological stage were calculated using the following formula (McMaster and Wilhelm, 1997).

$$GDD = \left[\frac{T_{\min} + T_{\max}}{2}\right] - T_b$$

In which $T_{\max}$ and $T_{\min}$ are daily maximum and minimum air temperature, respectively, and T_b is the base temperature of milk thistle (9 °C) (Omidbaigi, 1995).

2.4. Statistical analysis

Data analysis was done by SAS statistical package. Analysis of variance was computed using the general linear model and mean comparison was based on Duncan's multiple test with significance defined at 5% probability level.

Table 3
Detailed information on treatments used in the experiment.

Treatments	Fertilizers	Amount of fertilizer (kg ha^{-1})	Sum of the supply of nutritional elements[a] (kg ha^{-1})		
			N	P	K
C	Control	–	–	–	–
V	Vermicompost	13,000	169	169	156
H	Poultry manure	8000	199.2	186.4	133.6
CH	Chemical fertilizer (NPK)	400 kg urea + 100 kg superphosphate + 150 kg potassium sulfate	184	18	64.5
M	Mycorrhiza	1750 kg inoculated soil by *Glomus mosseae*	NA	NA	NA
B	Bio-sulfur	5 kg *Thiobacillus* sp. + 250 kg bentonite organic sulfur	NA	NA	NA
M + V	Mycorrhiza + Vermicompost	1750 + 13,000	NA	NA	NA
M + CH	Mycorrhiza + Chemical fertilizer	1750 + 400 + 100 + 150	NA	NA	NA
M + H	Mycorrhiza + Poultry manure	1750 + 8000	NA	NA	NA
B + V	Bio-sulfur + Vermicompost	5 + 250 + 13,000	NA	NA	NA
B + CH	Bio-sulfur + Chemical fertilizer	5 + 250 + 400 + 100 + 150	NA	NA	NA
B + H	Bio-sulfur + Poultry manure	5 + 250 + 8000	NA	NA	NA

Sections marked as NA were not available due to the unknown percentage of bio-fertilizer contributions to the supply of pure nutritional elements.
[a] Amount of nutritional elements is given as pure elements (NPK).

3. Results

Milk thistle plants completed their growth from emergence to maturation (producing seeds) in 4 phases including vegetative, elongating, flowering, and seed maturation stage. Analysis of the variance of accumulative GDDs received by milk thistle plants at different growth stages showed that single and integrated nutrition systems affected GDDs received at elongating and flowering stages significantly ($P < 0.05$), while vegetative and seed maturation stage were not influenced by treatments statistically (Table 4). Mean comparison of accumulative GDDs of plants under different nutrition systems presented in Table 5 showed that those milk thistle plants treated with mycorrhiza started the elongating stage by less GDDs (845 °C) than those plants under a group of treatments including vermicompost, chemical fertilizer, mycorrhiza + vermicompost, mycorrhiza + poultry manure, biosulfur + chemical fertilizer and control. The flowering stage in milk thistle plants treated with pure mycorrhiza was started sooner than milk thistle plants under all single treatments with the exception of plants received poultry manure (GDDs 1040 °C), and among integrated systems only sooner than those plants under treatments of mycorrhiza + vermicompost and mycorrhiza + chemical fertilizer. The highest amount of GDDs (971 °C) for starting the elongating stage was recorded in chemical fertilizer treatment. Milk thistle plants under integrated use of mycorrhiza + vermicompost gained more GDDs to start the flowering stage than those plants under pure use of mycorrhiza and integrated use of mycorrhiza and poultry manure, bio-sulfur + vermicompost as well as bio-sulfur + chemical fertilizer. Mycorrhiza + poultry manure treatment had the least amount of GDDs (1021 °C) at flowering stage compared with other integrated nutrition systems, however there was no significant difference, statistically, between this treatment and other integrated systems with the exception of mycorrhiza + vermicompost (Table 5). No significant differences between accumulative GDDs received by milk thistle plants under integrated nutrition systems were observed at elongating stage. The results indicate that, milk thistle needed approximately 1200–1500 GDD to complete its growth phases.

4. Discussion

According to Table 5 milk thistle plants treated with mycorrhiza started elongating and flowering stage with less GDDs compared with some other treatments; this might be due to the potential effects of biological fertilizers on decreasing the vegetative growth period of plants which itself is associated with the plant growth mechanism improvement by stimulating the production of plant growth-regulating substances by *G. mosseae* (Barea and Azcón-Aguilar, 1982). On the other hand, mycorrhiza is well-known because of its remarkable effects on increasing P uptake, which is an important nutrient to start the reproductive stage. Akbari et al. (2009) found the positive effects of biological fertilizer on decreasing the growth period of sunflower (*Helianthus annuus* L.).

Table 4
Analysis of variance (mean squares) of accumulative GDDs earned by milk thistle at different phenological stages.

Source of variation	Degree of freedom	Vegetative stage	Elongating stage	Flowering stage	Seed maturation stage
Block	2	50.6	10,695	2082	14,858
Treatment	11	70.3[ns]	4244.68*	2346*	11,102[ns]
Error	22	60.5	1592.00	887	7347
CV %		12.2	4.38	2.84	6.15

ns, not significant.
* Significant at $\alpha = 0.05$.

Table 5
Mean comparison of accumulative GDDs (°C) gained by milk thistle plants under different nutrition systems.

Nutrition systems	Vegetative stage	Elongating stage	Flowering stage	Seed maturation stage
Mycorrhiza	57.0	845c	1001d	1413
vermicompost	69.3	944ab	1075abc	1392
Chemical fertilizer	58.8	971a	1076abc	1463
Bio-sulfur	70.8	894abc	1083ab	1365
Poultry manure	65.1	913abc	1040abcd	1400
Mycorrhiza + Vermicompost	60.6	944ab	1089a	1462
Mycorrhiza + Chemical fertilizer	57.0	912abc	1062abc	1435
Mycorrhiza + Poultry manure	64.8	944ab	1021 cd	1294
Bio-sulfur + Vermicompost	69.0	874bc	1029bcd	1413
Bio-sulfur + Chemical fertilizer	64.8	933ab	1027bcd	1383
Bio-sulfur + Poultry manure	66.6	874bc	1034abcd	1266
Control	57.0	938ab	1047abcd	1435

Mean values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple test.

High N availability increases the vegetative growth period; therefore, plants treated with chemical fertilizer received the maximum amount of GDDs at the elongating stage and seed maturation stage, which is a sign of vegetative stage lengthening; Furthermore, gradual release of nitrogen and plant nutrition improvement in mycorrhiza + vermicompost treatment during growth period of milk thistle might be responsible for delaying the reproductive stages. Bakht et al. (2010) reported that days to inflorescences begin to open and physiological maturity of sunflower increased with an increase in fertilizer (NPK) level. The least amount of GDDs gained by milk thistle plants under integrated application of mycorrhiza and poultry manure compared with other integrated systems might be explained by increasing P availability by poultry manure (Table 2) accompanied by extensive P absorption through hyphal network. Multi-annual experiments are desirable to confirm current findings.

5. Conclusion

The results achieved in this study showed that milk thistle plants treated with those fertilizers which potentially provide the plants with better P uptake, mycorrhiza and mycorrhiza + poultry manure, started flowering stage by receiving less heat units while using chemical fertilizer and integrated systems of mycorrhiza and vermicompost postponed the start of elongating and flowering stages, respectively which might be associated with the availability of higher N during crop growth, improving plant nutrition and expansion of the vegetative stage.

Acknowledgement

This study was financially supported by Ferdowsi University of Mashhad, Iran.

References

Akbari P, Ghalavand A, Modarres Sanavy SAM, 2009. Effects of different nutrition systems and biofertilizer (PGPR) on phenology period yield and yield components of sunflower (*Helianthus annuus* L.). Electronic Journal of Crop Plants 2, 119–134.

Bakht J, Shafi M, Yousaf M, Shah HU, 2010. Physiology, phenology and yield of sunflower (autumn) as affected by NPK fertilizer and hybrids. Pakistan Journal of Botany 42 (3), 1909–1922.

Barea JM, Azcón-Aguilar C, 1982. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. Applied and Environmental Microbiology 43 (4), 810–813.

Chen J, 2006. The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In: International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, Bangkok, Thailand, October 16–20, pp. 16–20.

Clarke JM, Simpson GM, 1978. Growth analysis of *Brassica napus* cv. tower. Canadian Journal of Plant science 58 (3), 587–595.

Desclaux D, Roumet P, 1996. Impact of drought stress on the phenology of two soybean (*Glycine max* L. Merr) cultivars. Field Crops Research 46 (1), 61–70.

Ghavami N, Ramin AA, 2008. Grain yield and active substances of milk thistle as affected by soil salinity. Communications in Soil Science and Plant Analysis 39 (17-18), 2608–2618.

Guz NR, Stermitz FR, 2000. Synthesis and structures of regioisomeric hydnocarpin-type flavonolignans. Journal of Natural Products 63, 1140–1145.

Malakooti MG, Tehrani MM, 1993. The Role of Micro Nutrients in Crop Yield and Quality Improvement (Micro-elements with Grand Effect). Tarbiat Modares University, Tehran (In Persian).

McMaster GS, Wilhelm WW, 1997. Growing degree-days: one equation, two interpretations. Agricultural and Forest Meteorology 87 (4), 291–300.

Omer FA, Rafaat AM, Ahmad SS, 1990. Effect of spacing and fertilization on the yield and active constituents of milk thistle (*Silybum marianum*). Journal of Herbs, Spices, and Medicinal Plants 1, 17–23.

Omidbaigi R, 1995. Production Approaches and Processing of Medicinal Plants. Astan Quds Razavi, Mashhad, Iran (In Persian).

Omidbaigi R, Nobakht A, 2001. Nitrogen fertilizer affecting growth, seed yield and active substances of milk thistle (*Silybum marianum*). Pakistan Journal of Biological Science 4 (11), 1345–1349.

Shamsi K, 2009. Effect of planting date and density on the yield and yield components of milk thistle (*Silybum marianum* L.). Journal of Applied Biosciences 16, 862–863.

Tamayo C, Diamond S, 2007. Review of clinical trials evaluating safety and efficacy of milk thistle (*Silybum marianum* [L.] Gaertn.). Integrative Cancer Therapies 6 (2), 146–157.