MILK THISTLE (*SILYBUM MARIANUM* [L.] GAERTN.) CULTIVATED IN POLYFUNCTIONAL CROP ROTATION AND ITS EVALUATION

Miroslav HABÁN, Marta HABÁNOVÁ, Pavol OTEPKA, Ľubomír KOBIDA

Slovak Agricultural University in Nitra, Slovak Republic
Slovak Agricultural University in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic,
Corresponding author: Miroslav.Haban@uniag.sk

Abstract: *A polyfactorial field experiment was established and investigated during vegetation periods from 2004 to 2009 at the Dolna Malanta locality (Nitra district, Slovakia). The following parameters were measured: (1) yields of milk thistle (Silybum marianum [L.] Gaertn.) achenes, i.e. fruits, in 2004–2009; (2) content of silymarin in dry fruits of milk thistle in 2006–2009; and (3) total yields of silymarin per ha in 2006–2009. Factors of the experiment were as follows: (1) crop residues of cultivated pre-crop (no crop residues – K, with crop residues – R); (2) cultivation of freezing-out intercrop (no intercrop – B, with intercrop – M); (3) fertilization using artificial fertilizers (no fertilization – O, with fertilization – F); (4) year of cultivation (2004–2009). The highest yields of milk thistle fruits were recorded in 2006: from 1,426.5 kg/ha (RBO variant – incorporated crop residues without intercrop, no artificial fertilizers) to 1,832.0 kg/ha (KBF variant – without crop residues, without intercrop and with application of artificial fertilizers). The highest content of silymarin complex in dry fruits of milk thistle was measured in 2007: from 15.14 mg/kg (RMF – with crop residues, intercrop and fertilization) to 20.01 mg/kg (KBO – without crop residues, intercrop and fertilization). The highest total yield of silymarin per ha was recorded in investigated variants in 2006; in variant without crop residues it ranged from 16.45 kg/ha (KMF – with intercrop, with fertilizers) to 24.62 kg/ha (KMO – with intercrop, no fertilization).*

Key words*: *milk thistle, Silybum marianum [L.] Gaertn., quantitative-qualitative parameters, yield, technological quality

INTRODUCTION

Milk thistle (*Silybum marianum* [L.] Gaertn.) is a medicinal plant cultivated in agriculture. The achenes, i.e. fruits of the plant, are commonly used as a medicinal drug; they are the raw material for isolation of different substances with liver-protection activity. Production of high-quality milk thistle achenes depends on conditions of cultivation that directly influence the quality of final product. According to SPITZOVÁ and STARÝ (1985), the raised demand for the drug – *Silybi mariani semen* – caused a need of cultivation in cultural conditions. They also showed more issues and questions with respect to agronomic and physiology character of the plant. From the agronomical point of view, the forecrop value of milk thistle is significant as well. The milk thistle is recommended for incorporation into arable crop rotation as forecrop of maize, mainly maize cultivated for silage (MACÁK et al. 2007). The obtained yield depends most on managing of mechanization harvest (SCHUENKE 1992; GROMOVÁ 1997; HABÁN, OTEPKA 2007), because of non-uniform ripening time of the milk thistle fruits. Milk thistle belongs to the medicinal plants, and its introduction to the cultural growing conditions of Slovakia was successful (GROMOVÁ 1997; HABÁN 2005a). Recovery of milk thistle cultivation in the conditions of Slovakia nowadays is a result of long-term research of GROMOVÁ (1997) and introduction of food adjunct (*Anthemis* – food adjunct for the liver) to the production in 2005 (HABÁN 2005b). The food adjunct contains dry extract from milk thistle achenes in combination with the extract from Dyer's Chamomile (*Anthemis tinctoria* [L.] J. Gay).

The content of silymarin complex in natural milk thistle achenes is about 0.2–0.6%. Cultivar Silyb, which was bred in the early 80's is able to reach the content of about 2.0%

(INDRÁK, CHYTILOVÁ 1992). Silymarin complex usually contains 36.3% of silybin, 15.7% silychristin, 5.9% of silydianin, and 5.1% of isosilybin (ŠERŠEŇ et al. 2006). According to the Slovak Pharmaceutical Codex minimum content of silymarin complex is 1.0% (CPhS 1, 1997).

MATERIAL AND METHODS

The medicinal plant Milk thistle (*Silybum marianum* [L.] Gaertn.) belongs to the *Asteraceae* family. Different genera of *Silybum* sp. are widely cultivated in the agri-ecological conditions of Slovakia. Cultivar Silyb, originating from the Czech Republic, is the most cultivated one as well as the most used for the pharmaceutical processing. It provides the achenes production with appropriate quality. This cultivar was used for direct sewing on the plots of the Experimental Base in this work.

Experimental Base of Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra is situated in cadastre of Dolná Malanta village near Nitra, Slovakia (18°07'E, 48°19'N). Geographically, this locality is situated in the western part of the river Zitava upland. The experimental locality has flat character with little declination to south. The altitude is 177–180 m above sea level (HANES et al. 1993).

The experiment was realized in the framework of agri-climatic areas in the territory with the following features: Macro area: warm with the sum of temperature during days when t > 10°C in a range of 3,100–2,400°C; Area: predominantly warm with temperature t > 15°C in a range of 3,000–2,800°C; Sub area: very dry with climatic humidity factor for the months June–August $K_{VI-VIII}$ = 150 mm; Ward: predominantly mild winter with the average of absolute temperature minimum T_{min} = from – 18 to – 21°C. The average annual temperature in 2004 was 10.0°C, in 2005: 9.6°C, in 2006: 10.1°C, and in 2007: 11.4°C. The sum of annual precipitations were: in 2004: 514.5 mm, in 2005: 633.0 mm, in 2006: 507.0 mm, and in 2007: 606.4 mm. The average long-term (1961–1990) annual precipitation is 532.5 mm, for the vegetation period it is 309.4 mm (Table 1). The average long-term (1961–1990) annual temperature is 9.8°C and for the vegetation period it is 16.4°C (ŠPÁNIK et al. 1996).

Table 1.

Average air temperatures (T) and sums of precipitation (P) of the experimental locality in month intervals during the long-term period 1961–1990 (Špánik et al. 1996)

Month	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	Year	IV.–IX.
T (°C)	–1.7	0.6	5.0	10.4	15.1	18.0	19.8	19.3	15.6	10.3	4.5	0.2	9.8	16.4
P (mm)	31.2	31.8	29.6	38.5	57.7	64.0	51.4	57.7	40.1	35.8	54.8	39.9	532.5	309.4

Type of the soil is brown soil; selected soil properties were: proportional soil weight 2.60–2.63 t/m^3; content of humus in arable soil/topsoil 1.95–2.28%; soil reaction 5.03–5.69 (acidic, almost mild acidic). The experimental soil was created at the proluvial sediments. The soil profile of brown soil contains three genetic horizons (A_p, B_t, C). Their stratography is following: humus horizon (A_p) with the depth of 0–0.32 m; underneath is the main diagnostic luvisolic horizon (B_t), which was created as a result of alluvial accumulation of translocated colloids, and whose depth is from 0.33 to 0.65 m; then, there is a transitional horizon (B_t/C) with the depth from 0.66 to 0.85 m followed continually with the soil forming substrate up to the depth of 1.5 m. The studied brown soil is clayey in its sub-layer and in its topsoil is mildly firm. Humus is of a humo-phulvate type (HANES et al. 1993).

Milk thistle is an annual crop. Therefore it was fully integrated to four-cycle crop rotation with following order of crops: 1. Common pea; 2. Winter wheat; 3. Milk thistle; 4. Maize cultivated for grains (MACÁK et al. 2006, 2007). Description of variants: (1) Crop residues of cultivated pre-crop: first year maize, than according to the crop rotation winter wheat (no crop residues – K, with crop residues – R); (2) Cultivation of white mustard (Sinapis alba L.) as a freezing-out intercrop (no intercrop – B, with intercrop – M); (3) Fertilization using artificial fertilizers (no fertilization – O, with fertilization – F); (4) Year of experiment (2004–2009). Following doses of nutrients used in the fertilized variant were calculated according to KUBÍNEK (1987): 20 kg/ha N, 20 kg/ha P and 80 kg/ha K.

Polyfactorial field experiment was established and experimentally controlled during the vegetation period of the years 2004–2009. The experiment was arranged in one independent block. Plant material was harvested in the ontogenetic stage of the achenes ripening. Harvesting was done with adapted combine harvester. The yield data of milk thistle achenes were taken from randomly selected areas (3×1 m^2, i.e. three replications in each variant) and calculated to the yield in kg/ha. There was a qualitative parameter of milk thistle yield – content of active ingredients in sylimarine: silychristin, silydianin, silybin and isosilybin were determined according to the adapted method (SPITZOVÁ, PLACR 1988; INDRÁK, CHYTILOVÁ 1992; QUAGLIA et al. 1999; KVASNIČKA et al. 2003) using the HPLC analytic system.

Sample preparation: 0.5 g softly grinded achenes sample was mixed with 2 ml 2% tartaric acid. Suspension remained steady for at least 1 hour (INDRÁK, CHYTILOVÁ 1992). Then 23 ml of acetone was added and the mixture was treated for 30 minutes by ultrasonic (BANDELIN DT 100, Germany). Extraction was finished and the mixture was separated using centrifuge in 3,500 g (Hettich 320, Germany) during 10 min. Continuously 1 ml of solution was pipetted to 1.5 ml microtubes and was given to centrifuge by 21,000 g. Solution was then injected to HPLC (Spitzová, Placr 1988). The HPLC system consisted of In-Line Degasser AF, Waters 1525 Binary HPLC Pump and Waters 2487 Dual λ Absorbance Detector. The column was Waters SPHERISORB ODS 2 250 × 4.6 mm, 5 µm. Mobile phase consisted of methanol:water:acetic acid; 40:50:5; v/v/v with flowing of 1 ml/min. Detection was measured at 288 nm. The content of silymarin complex was recorded in comparison to silybin standard (INA Method 115.000).

The obtained data were evaluated statistically using the Statgraphics software with the analysis of variance (ANOVA); minimum significant differences were calculated by the Tukey test.

RESULTS AND DISCUSSIONS

The yields of milk thistle (*Silybum marianum* [L.] Gaertn.) achenes recorded in the investigated variants in 2004 were as follows: variant without crop residues from 588.6 kg/ha (KMO – with intercrop, no fertilizers) to 794.5 kg/ha (KBF – no intercrop, with fertilization) as it is shown in Table 2. In the variants with crop residues, the obtained yields ranged from 232.9 kg/ha (RMF – with intercrop and fertilization) to 580.3 kg/ha (RBO – without intercrop and no fertilization). In the second experimental year (2005), yields found in the variants without crop residues were from 1,005 kg/ha (KBO – no intercrop, no fertilization) to 1,314 kg/ha (KBF – without intercrop, with fertilization). In the variants with the crop residues ploughed under, the yields were measured from 554 kg/ha (RMO – with intercrop, no fertilization) to 1,480 kg/ha (RBO – without intercrop, no fertilizers). In the third year of experiment (2006) the yields varied from 1,426.5 kg/ha (RBO variant: – incorporated crop residues without intercrop, no artificial fertilizers) to 1,832.0 kg/ha (KBF variant – without crop residues, without intercrop and with application of artificial fertilizers). In the fourth year of experiment (2007) the yields varied from 413 kg/ha (KMO variant: – without crop residues with intercrop, no artificial fertilizers) to 886 kg/ha (KBF variant – without crop residues, without intercrop and with application of artificial fertilizers). Statistical differences were found in average yields of milk thistle between years of cultivation; the highest data were measured in 2006. The best variant for production of milk thistle achenes in this experiment was KBF (without crop residues, without intercrop and with application of artificial fertilizers), although it was not statistically significant.

Table 2.

Average yields (kg/ha) of milk thistle (*Silybum marianum* [L.] Gaertn.) at the standard humidity level (14%) in 2004–2009 with their statistical analysis

Variants			Yield/Years					
Crop residues	Intercrop	Fertilization	2004 DE	2005 B	2006 A	2007 CD	2008 E	2009 C
No crop residues (K)	no intercrop (B)	no fertilization (O)	644.8	1,005.0	1,699.0	677.5	344.5	519.0
		with fertilization (F)	794.5	1,314.0	1,832.0	886.0	353.0	876.5
	with intercrop (M)	no fertilization (O)	588.6	1,063.0	1,763.5	413.0	308.5	655.5
		with fertilization (F)	689.7	1,294.0	1,790.5	477.5	418.0	882.0
With crop residues (R)	no intercrop (B)	no fertilization (O)	580.3	1,480.0	1,426.5	647.5	457.0	853.0
		with fertilization (F)	328.6	1,317.0	1,697.0	794.0	457.0	793.5
	with intercrop (M)	no fertilization (O)	295.3	554.0	1,572.0	532.0	432.5	586.5
		with fertilization (F)	232.9	1,071.0	1,660.0	672.0	472.0	673.0

A, B, ... – significance levels of ANOVA (Tukey test) at probability $P \leq 0.01$

The average content of an active ingredient in milk thistle (*Silybum marianum* [L.] Gaertn.) achenes, i.e. silymarin complex, was measured in yields harvested in 2006 – 2009. The content recorded in investigated variants in 2006 was related to variant. In variant without crop residues silymarin content ranged from 9.19 mg/kg (KMF – with intercrop, with fertilizers) to 13.96 mg/kg (KMO – with intercrop, no fertilization), as it is shown in Table 3; in variant with crop residues, the obtained values were between 8.40 mg/kg (RMO – with intercrop, no fertilization) and 13.50 mg/kg (RBF – without intercrop, with fertilization). The content of silymarin complex in 2007 ranged from 15.14 mg/kg (RMF – with crop residues, intercrop and fertilization) to 20.01 mg/kg (KBO – without crop residues, intercrop and fertilization). Statistical differences in the average content of silymarin in dry fruits of milk thistle were obtained between the years of cultivation, with the highest values measured in 2007. The best variants for production of silymarin complex in this experiment were KBO and KMO (without crop residues, with or without intercrop and with no application of artificial fertilizers); the results were however not statistically significant.

The total yield of silymarin complex in milk thistle was calculated from yields of achenes and from the content of this active ingredient in samples from 2006 – 2009. The total yield recorded in investigated variants in 2006 varied as follows: without crop residues from 16.45 kg/ha (KMF – with intercrop, with fertilizers) to 24.62 kg/ha (KMO – with intercrop, no fertilization) (Table 3); with crop residues from 13.20 kg/ha (RMO – with intercrop, no fertilization) to 22.91 kg/ha (RBF – without intercrop, with fertilization). The total yield of silymarin complex in 2007 ranged from 8.26 kg/ha (KMO – no crop residues, with intercrop and no fertilization) to 16.02 kg/ha (KBF – without crop residues and intercrop, with fertilization). Statistical differences in total yield of silymarin per area in dry fruits of milk thistle were obtained between years of cultivation, with the highest data measured in 2006. The best variants for production of silymarin complex per area unit in this experiment were those without intercrop, as it was statistically determined as it is given in Table 3.

DANIM and YOM-TOV (1990) described the accumulation of yield potential of aboveground biomass and yields of achenes in milk thistle as a medicinal plant. These authors characterized the yields of milk thistle achenes as dependent mostly on applied artificial fertilizers. It is possible to consider the yield of milk thistle of about 0.75 t/ha as an average; however, in optimal growing conditions it can be even more than 1.5 t/ha (Kubínek 1987). The milk thistle yields recorded in the agri-ecological conditions of south Slovakia were from 0.5 to

1.7 t/ha (GROMOVÁ 1997; HABÁN 2004). Yields of this medicinal plant obtained in the experiment within evaluation of influence of crop residues, intercrop, and fertilization correspond to the results of these authors. ANDRZEJEWSKA and SKINDER (2006) found that yields of milk thistle grown in monoculture were about 40% lower than the yields obtained in crop rotation; such decrease of yield is caused mainly due to damage of plant roots by pests. In crop rotation, these authors found higher fruit yields when the earlier sowing date was applied. In contrast, the delay in the sowing date resulted in an increase of the content of silymarin complex in fruits of about 0.3 to 0.5%. It is possible to agree with Omidbaigi and Nobakht (2001), ANDRZEJEWSKA and SKINDER (2006), and Andrzejewska and SADOWSKA (2007) that the content of silymarin is mostly correlated with the weather conditions during vegetation period in comparison to the other factors.

Table 3.

Average content of silymarin in dry fruits (mg/kg) of milk thistle (*Silybum marianum* [L.] Gaertn.) and total yields of silymarin (kg/ha) in 2006–2009 with their statistical analysis

Variants			Content/Years				Yield of silymarin			
Crop residues	Intercrop	Fertilization	2006 B	2007 A	2008 B	2009 C	2006 A	2007 B	2008 C	2009 C
No crop residues (K)	no intercrop (B)	no fertilization (O)	13.74	20.01	5.14	4.43	23.34 a	13.56 a	1.78	2.27
		with fertilization (F)	10.02	18.08	10.65	4.96	18.36 b	16.02 a	3.87	4.39
	with intercrop (M)	no fertilization (O)	13.96	19.99	11.82	5.83	24.62 a	8.26 b	3.67	3.76
		with fertilization (F)	9.19	19.05	13.96	6.15	16.45 b	9.10 b	5.90	5.43
With crop residues (R)	no intercrop (B)	no fertilization (O)	12.23	17.62	13.07	5.67	17.43 a	11.41 a	5.99	4.94
		with fertilization (F)	13.50	16.60	9.67	5.79	22.91 a	13.18 a	4.24	4.59
	with intercrop (M)	no fertilization (O)	8.40	17.32	14.01	6.03	13.20 b	9.21 b	6.07	3.60
		with fertilization (F)	8.90	15.14	11.13	5.63	14.77 b	10.17 b	5.80	3.70

a, b – significance levels of ANOVA (Tukey test) at probability $p \leq 0.05$

A, B ... – significance levels of ANOVA (Tukey test) at probability $P \leq 0.01$

CONCLUSION

Selected quantitative and qualitative parameters of milk thistle (*Silybum marianum* [L.] Gaertn.) yields were analyzed during 2004–2009 growing seasons. The highest yields of milk thistle fruits were recorded in 2006, which was statistically determined. The highest content of silymarin complex in dry fruits of milk thistle was recorded in 2007. However, the highest total yield of silymarin per ha was recorded in investigated variants in 2006. The obtained yield of silymarin per ha was more influenced by the level of milk thistle fruits yield in 2006 than the content of silymarin in dry fruits in 2007. The highest yield of silymarin complex per area unit in this experiment, with respect to the intercrop, was obtained in 2007, in variant without intercrop, as it was statistically determined. Based on the six-year results, it is recommended to continue the research of the production parameters of milk thistle yields in following growing seasons.

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BIBLIOGRAPHY

1. ANDRZEJEWSKA J., SADOWSKA K., 2007. Content and composition of silymarin from the fruit of milk thistle (Silybum marianum [L.] Gaertn.) under moderate climate conditions. In: 1st

International Scientific Conference on Medicinal, Aromatic and Spice Plants (Book of Scientific Papers and Abstracts). Nitra, Slovak University of Agriculture: 94–97.
2. ANDRZEJEWSKA J., SKINDER Z., 2006. Yield and quality of raw material of milk thistle (Silybum marianum [L.] Gaertn.) grown in monoculture and in crop rotation. Herba Polonica, 52: 11–17.
3. CODEX PHARMACEUTICUS SLOVACUS**.** Editio prima, 1997. Bratislava, Ministry of Health of Slovak Republic, Herba: 159–161.
4. DANIM A., YOM-TOV Y., 1990. Ant nests as primary habitants of Silybum marianum (Compositae). Plant Systematics and Evolution, 169: 209–216.
5. GROMOVÁ Z., 1997. Pestovanie pestreca mariánskeho (Silybum marianum [L.] Gaertn.) na Slovensku. In: Pestovanie liečivých rastlín a korenín. [Zborník príspevkov.] Nitra, ARVI: 24–28.
6. HABÁN M., 2004. Pestovanie a využitie liečivých, aromatických a koreninových rastlín (8). Pestrec mariánsky (Silybum marianum [L.] Gaertn.) – ostropestřec mariánský. Liečivé rastliny – Léčivé rostliny, 41: 54–57.
7. HABÁN M., 2005a. Hodnotenie úrody pestreca mariánskeho [Silybum marianum [L.] Gaertn.] pestovaného v agroekologických podmienkach Žitavskej pahorkatiny /The yield evaluation of milk thistle [Silybum marianum [L.] Gaertn.] cultivated at Zitavska pahorkatina/. In: MATI R., HECL J. (eds.), Realizáciou poznatkov vedy a výskumu k trvalo udržateľnému poľnohospodárstvu. Michalovce, VÚRV OU: 236–240.
8. HABÁN M., 2005b. Pestovanie a využitie liečivých, aromatických a koreninových rastlín (17). Rumanovec farbiarsky (Cota tinctoria L.) – rmen barvírsky. In: Liečivé rastliny – Léčivé rostliny, 42: 194–200.
9. HABÁN M., OTEPKA P., 2007. Production and quality of milk thistle (Silybum marianum [L.] Gaertn.) cultivated in cultural conditions of warm agri-climatic macroregion. Scientific Papers of Faculty of Agriculture, Part I, 39: 89–92.
10. HANES J., MUCHA V., SISÁK P., SLOVÍK R., 1993. Charakteristika hnedozemnej pôdy na výskumno-experimentálnej báze AF VŠP Nitra, Dolná Malanta. Nitra, VES VŠP: 49.
11. INA METHOD 115.000. Silymarins in Milk Thistle by HPLC. Available at http://www.nsf.org/business/ina/milkthistle.asp?program=INA.
12. INDRÁK P., CHYTILOVÁ D., 1992. K problematice stanovení silybinu v droze ostropestřece mariánského. Zahradnictví, 22: 309–313.
13. KUBÍNEK J., 1987. Ostropestřec mariánský – metodika pěstování. České Budějovice, MZVž ČSR: 25.
14. KVASNIČKA F., BÍBA B., ŠEVČÍK R., VOLDŘICH M., KRÁTKÁ J., 2003. Analysis of the active components of sylimarin. Journal of Chromatography A, 990: 239–245.
15. MACÁK M., SMATANA J., GOLISOVÁ A., DEMJANOVÁ E., 2006. The influence of crop and management of crop residues on basal soil biological properties. In: Lucrari stiintifice Universitatea de Ştiințe Agronomice sii Medicina Veterinara. – Timisoara: Red. Revistelor Agricole, 38: 155–160.
16. MACÁK M., DEMJANOVÁ E., HUNKOVÁ E., 2007. Forecrop value of milk thistle (Silybum marianum [L.] Gaertn.) in sustainable crop rotation. In: 1st International Scientific Conference on Medicinal, Aromatic and Spice Plants (Book of Scientific Papers and Abstracts). Nitra, Slovak University of Agriculture: 102–104.
17. OMIDBAIGI R., NOBAKHT A., 2001. Nitrogen fertilizer affecting growth, seed yield and active substances of milk thistle (Silybum marianum). Pakistan Journal of Biological Sciences, 11: 1345–1349.
18. QUAGLIA M.G., BOSSÙ E., DONATI E., MAZZANTI G., BRANDT A., 1999. Determination of silymarine in the extract from the dried Silybum marianum fruits by high performance liquid chromatography and capillary electrophoresis. Journal of Pharmaceutical and Biomedical Analysis, 19: 435–442.
19. SCHUENKE U., 1992. Mariendistel. Erste Erfahrungen bei Anbau und Ernte. Landtechnik (Germany), 11: 548–550.
20. SPITZOVÁ I., PLACR M., 1988. Metodika udržovacího šlechtění silybinové chemovariety ostropestřece mariánského Silybum marianum [L.] Gaertn. Praha, Výzkumný ústav pro farmacii a biochemii: 21.

21. SPITZOVÁ I., STARÝ F., 1985. Obsah a lokalizace flavonolignanů u ostropestřece mariánského (Silybum marianum [L.] Gaertn.) v průběhu ontogeneze. Zahradnictví, 15: 301–307.
22. ŠERŠEŇ F., VENCEL T., ANNUS J., 2006. Silymarin and its components scavenge phenylglyoxylic ketyl radicals. Fitoterapia, 77: 525–529.
23. ŠPÁNIK F., REPA Š., ŠIŠKA B., 1996. Klimatické a fenologické pomery Nitry (1961–1990). Nitra, Vydavateľské a edičné stredisko Slovenskej poľnohospodárskej univerzity: 62.