

## EFFECT OF *SILYBUM MARIANUM* (L.) GAERTN. ON GERMINATION, EARLY GROWTH AND NUTRIENT UPTAKE OF *ZEA MAYS* L.

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**Abstract.** In our experiment, the effect of water extract of *Silybum marianum* (L.) Gaertn. on germination and early growth of *Zea mays* L. was examined in laboratory circumstances. The examined concentrations (m/v%), originated from dried *S. marianum* water extract were the following: 1%, 5%, 7.5% and 10%. The fresh and dry weight of maize, the length of coleoptile and radicle and the nutrient element contents were measured. The results demonstrate that the 1% milk thistle water extract treatment has a significant positive effect on all of the measured parameters of maize. In the case of higher concentrations, the fresh and dry weight of coleoptile significantly decreased compared to the control. The same tendency can be observed for the radicle. N, K, Ca, Mg and Zn concentration of radicle and K, Ca and Zn concentration of coleoptile increased when maize was treated with milk thistle extract, at all kind of concentration levels. The Cu and Fe content of maize seedlings decreased at all applied concentrations. Our results demonstrate the importance of *S. marianum*'s appearance in field because it has very strong allelopathic effects. Further examination is needed to determine if the remaining plant residues have an effect on germination and growth of crops.

**Keywords:** *biomass production, corn, milk thistle, growth inhibition*

### Introduction

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals, to plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. Allelochemicals are a subset of secondary metabolites not required for metabolism (growth and development) of the allelopathic effects and are important part of plant defence against herbivory (Fraenkel, 1959; Stamp, 2003).

Commonly cited effects of allelopathy include reduced seed germination and seedling growth (Lehoczky et al., 2011a, b, 2014). Inhibition due to allelopathy is complex and can involve the interaction of different chemical classes, like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids

with different compound mixtures sometimes having higher allelopathic effect than individual compounds alone (Györi et al., 2014; Lehoczky et al., 2016; Nwaichi and Ayalogu, 2010).

The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds (Lehoczky and Gólya, 2013). To demonstrate the phenomenon and mechanism of allelopathy, plant origin, production, and identification of allelochemicals have to be established as well as persistence in the environment over time in concentrations sufficient to affect different plant species. Interactions among allelopathic plants, host crops, and other non-target organisms must also be considered (Ferguson and Rathinasabapathi, 2012).

Many researchers have documented the allelopathic effect of milk thistle on mustard, cucumber, wheat and sorghum (Isam and Hussain, 1988), but there is little knowledge on the effect on maize. Qasem and Foy (2008) established, that milk thistle have the potential to be used in solving problems of other weed species and represents an excellent source of natural chemicals that may be involved in developing natural herbicides.

*S. marianum* is by far the most widely known species. Milk thistle, also known as the Marian, St. Mary's or Our Lady's thistle, is native to the Mediterranean region of Europe and is grown commercially as a medical plant in Egypt, Tunisia, China and Argentina (Anonymous, 1995; Penksza and Szerdahelyi, 2009) but it has been reported as a noxious weed in many other countries. Otherwise, milk thistle is a serious weed in many areas of North and South America, Africa, Australia and the Middle East (Holm et al., 1997).

Weeds are an important variable in crop production (Bilalis et al., 2010; Eftimiadou et al., 2009; Karkanis et al., 2010; Lehoczky et al., 2013, 2015). Weed science will be advantageously positioned in the future if research focuses on research – decision process, weed biology and ecology, weed control and management practices, herbicide resistance, issues related to transgenic plants, environmental issues, and potential benefits of weeds (Hall et al., 2000).

Weeds have serious effect on crop production. Nowadays, the milk thistle is one of the most prevalent weeds found in the fields in North Africa. This research will be positioned for the future, focusing on interaction, weed biology and ecology, weed control and management practices, herbicide resistance.

The basic aim of the experiment was to make bioassay examination which assesses the allelopathic effects of *Silybum marianum* (L.) Gaertn. on maize seedlings in laboratory circumstances. The test plant's (maize) element composition, germination and growth inhibition were also investigated at the end of the experiment.

## Materials and methods

The *Silybum marianum* (L.) Gaertn. leaves were dried on 45 °C. After drying, the sample was chopped than milled. The plant material of *S. marianum* was collected from agricultural area. The element content of milk thistle is shown in *Table 1*.

The first 150 ml of the 10% concentration water extract was made by soaking 15 g powdered plant sample in 150 ml tap water. The mixture was kept at room temperature for 24 h and was mixed occasionally. After 24 h, the extract was filtered through MN 619 G ¼ filter paper. The 10% concentration water extract was diluted to obtain the

concentrations of 1%, 5% and 7.5%. Tap water was used as a control ( $\emptyset$ ). Twenty maize seeds (*Zea mays* L.) were placed separately into 18 cm diameter Petri dishes on MN 640 m filter paper. 15 ml of each concentration of water extracts were added to the Petri dishes in four replications. All of them were placed in a Binder APT Line KBW plant growing chamber at  $21 \pm 1$  °C for 7 days.

**Table 1.** Element content of *Silybum marianum* L. Gaertn. leaves ( $\text{mg kg}^{-1}$ , N (%))  $N = 2 \pm SD$

N	P	K	Ca	Mg
$2.42 \pm 0.09$	$1764 \pm 42$	$34165 \pm 54$	$35432 \pm 2917$	$5156 \pm 59$
Fe	Mn	Cu	Zn	Mo
$321 \pm 86$	$30.4 \pm 2.4$	$14.8 \pm 0.7$	$43.5 \pm 0.5$	$1.07 \pm 0.15$

The used experimental design and method have already been published by Lehoczky et al., (2011a, b, 2017).

Seven days after the start of the experiment, the shoots and roots of every seedling were measured with millimetre paper. The fresh weight of the shoots and roots was determined at the same time. After drying, the dry weight was also measured.

Determination of the seedling nutrient concentrations for P, K, Ca, Mg, Fe, Mn, Cu, Zn and Mo was carried out with ICP-OES method after microwave Teflon bomb digestion with cc.  $\text{HNO}_3 + \text{H}_2\text{O}_2$ . The nitrogen concentration of samples was determined using the Kjeldahl method.

The substances with allelopathic effects of the *Silybum marianum* L. Gaertn. sample have been investigated by HPLC. According to the methanol extract, 1 g plant sample contained 63  $\mu\text{g}$  rutin and 194  $\mu\text{g}$  salicylic acid. Both rutin and salicylic acid have inhibitory effects on germination (Basile et al., 2000; Szepesi, 2009; Szabó, 1997).

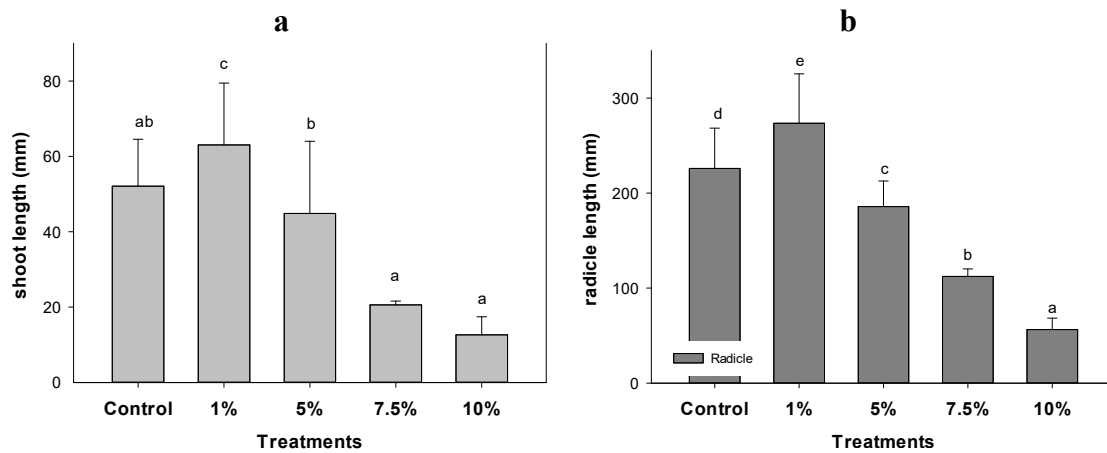
ANOVA (Analysis of Variance) was used to evaluate the significant differences. On the figures the error bars indicate the standard deviation and the a–e indicate significant differences within treatments at the 5% level of probability according to Duncan's test. For the statistical analysis Sigma Plot 12.0 was used. Examinations were carried out in three replications and in different cases with four replications. The experiments were conducted in controlled environmental conditions, so there was no difference between the results of three biological replications. The data of three repetitions were summarized and used for statistical analysis – results were calculated from twelve data.

## Results

Usually, the effects of allelopathy include reduced seed germination and seedling growth. All maize seeds started to germinate by the end of the seventh day, hence the germination percentage was 100% in all treatments. However, the number of seeds with a length of a few mm shoot have increased in parallel with increasing extract concentration (Fig. 1a, b).

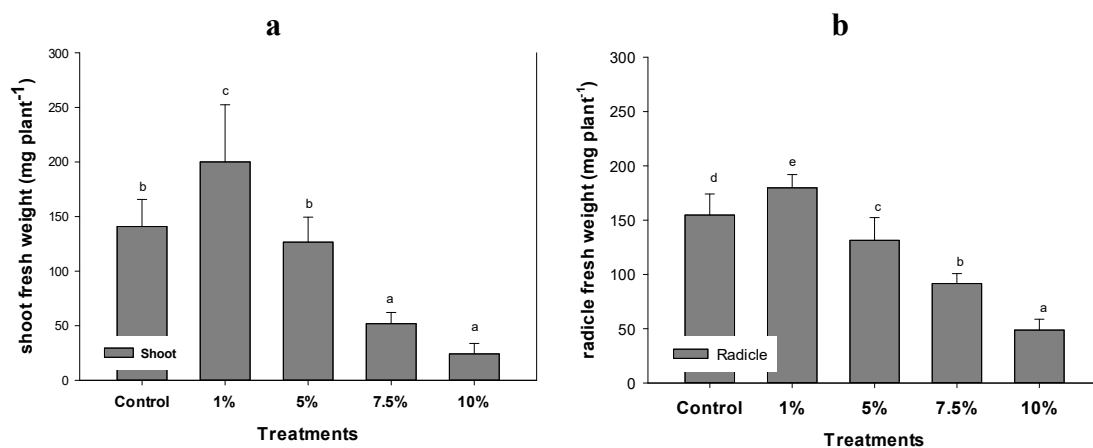
Decrease of shoot and radicle length was observed at the 5, 7.5 and 10% extract treatments. The length of shoot increased by 18% in the 1% extract treatment. The increase is significant compared to the control. The length of radicle increased by 18%

in the 1% milk thistle extract treatment. The decrease is parallel with the increasing concentration of milk thistle extracts.



**Figure 1.** The effect of *Silybum marianum* L. Gaertn. water extracts (a) on the length of shoot of maize ( $LSD_{5\%}$  12.36)  $N = 240 \pm SD$  and (b) on the length of radicle of maize ( $LSD_{5\%}$  49.80)  $N = 240 \pm SD$

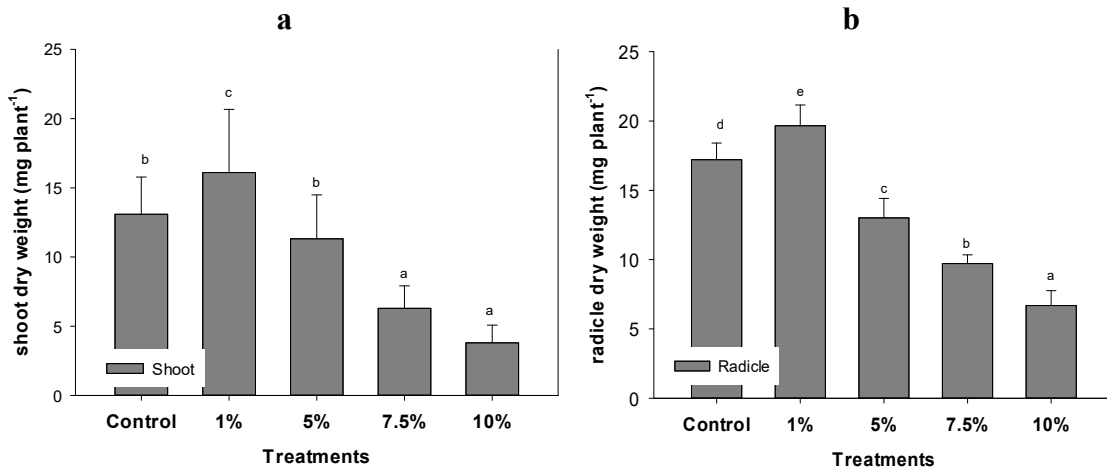
Additionally, the effect of *S. marianum* was observed on the fresh weight of shoot (coleoptile) and root (radicle) of maize (Fig. 2a, b).



**Figure 2.** The effect of *Silybum marianum* L. Gaertn. water extracts (a) on the fresh shoot weight of maize ( $LSD_{5\%}$  35.11)  $N = 12 \pm SD$  and (b) on the fresh radicle weight of maize ( $LSD_{5\%}$  18.86)  $N = 12 \pm SD$

The positive effect was observed when 1% extract was applied. The fresh weight of coleoptile was significantly increased by 30% in the 1% treatment. At the higher concentrations, the effect was negative on fresh weight. The fresh weight of shoot decreased by 11%, 64% and 83% in the 5%, 7.5% and 10% treatment, respectively. In the case of the highest concentration the decrease was significant compared to the control. The same tendency can be observed in the case of the fresh weight of radicle (Fig. 2b). The fresh weight of radicle increased by 14% in the 1% extract treatments.

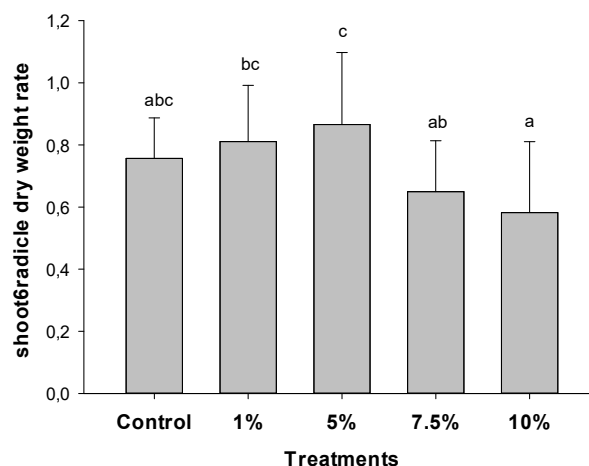
Decrease was observed at the higher concentration. The highest decrease was in the case of the 10% extract treatment. The dry weight of shoots and radicles also were measured (Fig. 3a, b).



**Figure 3.** The effect of *Silybum marianum* L. Gaertn. water extracts (a) on the dry weight of shoot of maize ( $LSD_{5\%} 4.06$ )  $N = 12 \pm SD$  and (b) on the dry weight of radicle of maize ( $LSD_{5\%} 1.72$ )  $N = 12 \pm SD$

The 1% extract treatment had less positive effect on dry weight than on fresh weight of shoot. The dry weight of shoot increased with 19%. The decrease at the higher extract concentrations also had less negative effect on the dry weight of shoots as well as on the fresh weight of shoot. The decrease was mathematically proven in both cases.

Figure 4 shows the different extract treatments on the shoot/root dry weight rate of maize. The shoot/root dry weight ratio of seedlings was higher in the case of 1 and 5% concentrations compared to the control (0.76), which is a trend that is not statistically significant.



**Figure 4.** The effect of *Silybum marianum* L. Gaertn. water extracts on the shoot/root dry weight rate of maize ( $LSD_{5\%} 0.33$ )  $N = 12 \pm SD$

In the case of the 1% extract treatment, the mass of the root and shoot both increased, but the increase of shoot weight was greater than of root weight. Similar results were observed at 5% concentration level regarding the ratio, however the root and shoot growth was also inhibited (Fig. 3a, b). The inhibitory effect was more strongly manifested in the case of roots, which explains the growth of shoot/root dry weight ratio shown in Figure 4. In the case of the 7.5 and 10% extract treatments the shoot/root ratio was significantly lower compared to the treatment with 5% concentration. The background in both cases is the strong growth inhibiting effect of the extracts, in which the shoot growth inhibition was stronger than the root growth inhibition, thus the significant reduction of the ratio can be explained.

Tables 2 and 3 show the element concentrations of shoot and radicle of maize. The concentration of N is significantly decreased in shoot in 1%, and increased in 5, 7.5 and 10% treatment in radicle. The concentration of P significantly decreased by 22% in the 10% extract treatment in shoot compared to the control, but there was no significant difference detected in radicle between *S. marianum* treatments and control. The concentration of K is significantly increased in both shoot and radicle in 5%, 7.5% and 10% water extracted milk thistle treatments. The increment is 36%, 32% and 36% in shoot, and 53%, 54% and 56% in radicle, respectively. The concentration of K was not changed significantly parallel with the increasing water extract concentration. The content of calcium significantly increased in all extract treatments; the highest increase could be observed at 7.5% water extract concentration in shoot and at 5% in radicle treatment compared to the control. In order to protect roots against the adverse effects of high concentrations of various other cations in the soil solution, the  $\text{Ca}^{2+}$  concentrations required for optimal growth have to be substantially higher in soil solution than in balanced flowing nutrient solutions (Asher and Edwards, 1983). Also increment was determined in the Mg content in the radicle of maize when maize was treated with milk thistle extract. Mg is also required for optimal plant growth. At permanently insufficient root supply, remobilization of Mg from mature leaves reduces their longevity (Lange et al., 1987). The content of Cu decreased parallel with the increasing extract concentration. The Fe content decreased in the all treatments. The concentration of molybdenum in shoot was changed significantly in the 10% treatments, while no significant changes were observed in radicle compared to the control.

## Discussion

Allelopathic effect of milk thistle on different crops has been documented sporadically, but there is no literature on its effect on maize.

Therefore, a laboratory based allelopathic extracts bioassay was conducted. During our experimental period the allelopathic effects of donor species on germination and seedling growth of maize was observed. Results has been shown, *Silybum marianum* L. extract did not have any effect on the germination of maize. The maize seedlings growth was inhibited by 5, 7.5 and 10% extract of *Silybum marianum* L. but growth stimulation effect was observed by 1% concentration extract.

Sultana and Asaduzzaman (2012) also observed inhibition effect on the above-mentioned parameters in case of canola and ryegrass. They established that the extracts from fresh water at 100% was more toxic to receiver species followed by 50% concentration of fresh and 100% from hot water extracts. This concentration reduced the root, shoot growth of ryegrass and canola by 84.97%, 84.27% and 89.90%,

respectively. The result also revealed that the allelopathic pattern of hot water extracts was the same; however, it was less toxic to both receiver species.

**Table 2.** Element concentrations of shoot of maize treated with 1, 5, 7.5 and 10% milk thistle water extract treatments ( $\text{mg kg}^{-1}$ , N (%))  $N = 2 \pm \text{SD}$

	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>
<b>Control</b>	5.7 ± 0.2 b	9752 ± 412 b	24458 ± 4353 a	210 ± 42 a	1805 ± 97 a
<b>1%</b>	5.1 ± 0.2 a	9789 ± 209 b	27259 ± 1410 a	623 ± 8 b	1834 ± 21 a
<b>5%</b>	5.6 ± 0.0 b	9551 ± 245 b	38087 ± 1979 b	763 ± 51 b	1834 ± 1 a
<b>7.5%</b>	5.8 ± 0.1 b	9382 ± 345 b	35635 ± 707 b	779 ± 129 b	1881 ± 96 a
<b>10%</b>	5.7 ± 0.1 b	7636 ± 624 a	38043 ± 5139 b	762 ± 196 b	1962 ± 4 a
<b>Mean</b>	5.6	9222	32696	627	1863
<b>LSD5%</b>	0.4	1056	8136	304	192
	<b>Fe</b>	<b>Mn</b>	<b>Cu</b>	<b>Zn</b>	<b>Mo</b>
<b>Control</b>	84.4 ± 10.7 d	9.4 ± 0.1 b	7.5 ± 1.5 b	35.4 ± 6.3 a	0.43 ± 0.04 b
<b>1%</b>	76.9 ± 11.7cd	9.5 ± 0.8 b	6.7 ± 0.9 ab	35.2 ± 1.7 a	0.38 ± 0.03 b
<b>5%</b>	63.5 ± 0.1 bc	9.1 ± 0.5 b	5.3 ± 0.2 a	45.8 ± 2.3 ab	0.41 ± 0.01 b
<b>7.5%</b>	51.1 ± 5.9 ab	8.5 ± 0.9 b	4.7 ± 0.4 a	45.8 ± 3.4 ab	0.42 ± 0.03 b
<b>10%</b>	39.9 ± 1.4 a	6.1 ± 0.3 a	4.8 ± 0.0 a	51.5 ± 4.5 b	0.23 ± 0.09 a
<b>Mean</b>	63.1	8.5	5.8	42.8	0.37
<b>LSD5%</b>	23.0	2.0	1.7	5.1	0.15

Note: different letters indicate significant difference ( $P < 0.05$ )

**Table 3.** Element concentrations of radicle of maize treated with 1, 5, 7.5 and 10% milk thistle water extract treatments ( $\text{mg kg}^{-1}$ , N (%))  $N = 2 \pm \text{SD}$

	<b>N</b>	<b>P</b>	<b>K</b>	<b>Ca</b>	<b>Mg</b>
<b>Control</b>	3.6 ± 0.1 a	6894 ± 413 a	22883 ± 2593 a	425 ± 67 a	1362 ± 162 a
<b>1%</b>	3.5 ± 0.2 a	6830 ± 361 a	27693 ± 1699 a	2967 ± 749 b	1908 ± 100 a
<b>5%</b>	4.2 ± 0.2 b	7348 ± 31 a	48743 ± 3604 b	5116 ± 337 c	3972 ± 441 b
<b>7.5%</b>	4.6 ± 0.1 b	7358 ± 270 a	50157 ± 4391 b	4736 ± 671 c	4027 ± 276 b
<b>10%</b>	5.6 ± 0.3 c	7271 ± 332 a	51724 ± 2579 b	3104 ± 864 b	3794 ± 429 b
<b>Mean</b>	4.3	7140	40240	3270	3013
<b>LSD5%</b>	0.5	898	9538	1787	620
	<b>Fe</b>	<b>Mn</b>	<b>Cu</b>	<b>Zn</b>	<b>Mo</b>
<b>Control</b>	57.3 ± 2.8 c	6.0 ± 2.1 a	24.1 ± 5.1 c	34.6 ± 5.2 ab	0.25 ± 0.06 ab
<b>1%</b>	54.8 ± 1.5 c	5.1 ± 0.7 a	15.9 ± 1.8 b	32.4 ± 3.5 a	0.36 ± 0.06 ab
<b>5%</b>	52.2 ± 2.2 bc	5.4 ± 0.5 a	13.3 ± 0.1 ab	40.1 ± 1.6 ab	0.39 ± 0.02 b
<b>7.5%</b>	47.9 ± 3.4 ab	5.1 ± 0.3 a	11.2 ± 1.6 ab	42.2 ± 3.4 b	0.29 ± 0.12 ab
<b>10%</b>	42.4 ± 2.1 a	5.2 ± 0.4 a	8.8 ± 0.2 a	51.2 ± 1.7 c	0.19 ± 0.02 a
<b>Mean</b>	50.9	5.3	14.7	40.1	0.29
<b>LSD5%</b>	2.0	2.8	7.2	8.9	0.20

Note: different letters indicate significant difference ( $P < 0.05$ )

The allelopathic potential of *S. marianum* was investigated by Khan et al. (2011) on kidney bean (*Phaseolus vulgaris* L.), mung bean (*Vigna radiate* L.), chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* L.). The results show that by increasing the leaves extract concentration of *S. marianum*, a significant decrease was noted in the germination percentage, mean germination time, germination index and seed vigor index of test species. The tolerance order of the mung bean and soybean against the extract concentration of *S. marianum* was higher than the other two species, while the chickpea was more susceptible to the toxicity of milk thistle.

Milk thistle is a major weed in sugar beet, wheat and canola causing large yield reductions (Shimi et al., 2006). However, research has documented the allelopathic effect of milk thistle on mustard, cucumber, wheat and sorghum (Inam and Hussain, 1988; Lehoczy et al., 2017).

In our experiment, the uptaken elements, e.g. K, Ca and Zn, were increased both in the shoot and root when maize was treated with *S. marianum*, but the Cu and Fe content decreased as a result of the milk thistle extract treatment.

In summary, the microelement concentrations decreased by the application of milk thistle water extracts. The micronutrients have prominent effect on dry matter, grain and straw yield (Aller et al., 1990). Fe plays role e.g. in nitrogen fixation (Römheld and Marschner, 1991) and it is very essential for normal plant growth (Fageria, 2007). Mn is utilized in enzyme activation (Burnell, 1988), Zn is important to membrane integrity and protein synthesis (Zhao et al., 2012). So, the adequate uptake of micronutrients has an effect on plant growth and development. Otherwise, plant will suffer from growth inhibition.

Seed germination is affected by the nutritional status of the mother plant and therefore by nutrient concentration in the seeds (*Table 1*). P and K concentrations of below 0.3% in seeds negatively affect their total germination (Hrdlicková et al., 2011). Seed germination and the time required for the 50% of the seeds to germinate are significantly affected by nitrogen (Hejzman et al., 2012). There was no detected inhibited germination in our experiment.

In our experiment the inhibition effect of milk thistle on the measured parameters of maize was also observed. Significant decrease was observed at increasing milk thistle extract concentration in the fresh and dry weight of shoot and radicles of maize, length of shoot and radicle. Compared to the stimulating 1% treatment, the 5, 7.5 and 10% extract resulted in significant reduction of the fresh weight of coleoptile, which was 11, 6 and 83%, respectively. The length of shoot decreased by 12% in the 5% extract treatment, 60% in the 7.5 and 76% in the 10% milk thistle extract treatments. But a remarkable stimulating effect was observed in 1% extract treatment on the fresh weight (30%) and length (14%) of coleoptile.

Lehoczy et al. (2017) investigated the allelopathic effect of milk thistle on winter wheat. According to their results, the winter wheat is more sensitive for applied concentrations of *S. marianum* than the maize. The concentrations of 1%, 2%, 3%, 4% and 5% of water extract were used. They found that the allelopathic potential of milk thistle increased with increasing concentration. The fresh and the dry weight and the length of the shoot and radicle of wheat significantly decreased at 5% concentration.

Recent assessment of allelopathic effect of weed on weed has the goal of using naturally produced allelochemicals to reduce reliance on herbicides (Putman and Duke, 1978). Milk thistle is a worldwide known weed. It has a special allelopathic effect, therefore, there is a significant difference when we grow milk thistle as medicinal plant



or it acts as a weed. In the latter case, *S. marianum* may decrease the yield due to its allelopathic effect. So, special attention should be given to the plant residues of milk thistle to be worked in the soil after the vegetation period.

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