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EFFECTS OF FOLIAR FERTILIZER CONCENTRATION ON THE BIOMASS ACCUMULATION AND NITRATE ASSIMILATION RATE OF MILK THISTLE (*SILYBUM MARIANUM* L)

Ira Stancheva*, Maria Geneva, Grigor Zehirov, Georgy Georgiev

Department of Mineral Nutrition and Water Regime, Acad M.Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Block 21, Sofia 1113, Bulgaria.

Corresponding author:

E-mail: ira_stancheva@abv.bg

ABSTRACT . The milk thistle plants (Silybum marianum L.) were grown for 21 days under glasshouse conditions in a 0,8 plastic pot (4 plants/pot) contained ½ strength Hellriegel's solution at the following variants: (1) control plants, without application of foliar fertilizer; (2) plants, grown with application of 0.3% foliar fertilizer; (3) plants, grown with application of 0.5% foliar fertilizer. The plants grown with addition of foliar nutrition have shown increased leaf number and dry biomass accumulation, reduced rosette diameter, enhanced rates of nitrogen assimilatory enzymes. A favorable effect of foliar feeding on the protein content, tissue nitrogen and potassium concentration have been also established.

KEY WORDS: Milk thistle (*Silybum marianum* L.), foliar fertilizer, dry biomass, nitrate reductase activity, glutamine synthetase activity.

INTRODUCTION

During development, the milk thistle (*Silybum marianum*) seeds accumulate flavonolignans (often referred to as silymarin complex). Therapeutic efficacy of silymarin in treating liver disorders is well known. However little is known about milk thistle nutrient and growth conditions requirement. In our previous study low nitrogen requirements of milk thistle seedlings was reported (Stancheva et al., 2004a). There is some evidence indicating that elevated P concentration in the growth medium positively influenced some of the morphological parameters of the 21 day

old milk thistle plants (shoot:root dry weight ratio and rosette diameter), root N and P content and leaf protein content (Zehirov et al., 2005).

The foliar application of mineral nutrients offers a method of supplying nutrients to higher plants more rapidly than methods involving root application. It was established that foliar fertilizer applied at early vegetative stages increased yields and enhanced quality in soybean (Mallarino and UI-Hag, 1998) and garden been (Stancheva et al., 2004b). Plant response is dependent on species, fertilizer form, concentration, and frequency of application, as well as the stage of plant growth (Faulkner, 1999; Eddy, 2000).

The paper is aimed to study the effect of different foliar fertilizer concentrations on the plant growth and nitrogen assimilation rate of milk thistle.

Material and methods

The milk thistle seeds were surfaced sterilized with 80% ethanol and germinated in dark at 25^{o}C. The five-old-day seedlings were transferred to a 0.8 L plastic pot (4 plants/pot) contained ½ strength Hellriegel's solution and completed with micronutrients after the Hoagland-Arnon. The foliar fertilizer Agroleaf® total (Scotts Company, Ohio, USA) N:P:K=20:20:20 + all important microelements was applied with each nutrient solution replacement starting from the 10th day. Chelated microelements are present in unique Scotts M77 formula: 0.1% Fe, 0.06% Mn, 0.06% Cu, 0.06% Zn, 0.02% B. Agroleaf® was applied with spraying under high pressure.

The following variants were tested: (1) control plants, without application of foliar fertilizer; (2) plants, grown with application of 0.3% (0,120 g/40ml) foliar fertilizer; (3) plants, grown with application of 0.5% foliar fertilizer

Nitrate reductase (NR: NADH, EC 1.6.6.2) activity was assayed by Hageman and Reed (1980). Glutamine sinthetase (GS: EC 6.3.1.2) activity was determined by a biosynthetic assay based on γ-glutamil hydroxamate synthesis (O'Neal and Joy, 1973). Protein content was determined according to Bradford (1976) with BSA as a standard. The total N content was measured after Kjeldahl and determined with automatic analyzer „Contiflo" (Hungary). Total tissue P content was determined according to Lowry and Lopez (1946) with some modifications and total K content was measured by the flame-emission spectroscopy.

Results represented the means collected from three separate experiments and every parameter was measured in triplicate. Data are subjected to a standard statistical procedure.

RESULTS AND DISCUSSION

Foliar feeding seems to be one of the easiest ways of increasing growth speed, yield and quality. Foliar fertilizer in both applied concentrations (0,3 and 0,5%) combined with the root mineral nutrition resulted in rosette diameter (Fig. 1) reduction but increased the leaves numbers (Fig. 1) in comparison with the root fed plants only. The lower size of plant rosette diameter enables to increase plant density and therefore the yield from unit area. The similar stimulating effect of foliar fertilizer application has been observed regarding the plant biomass accumulation (Fig. 1). Thus in the variants with foliar fertilization dry biomass is about two times higher compared to the variants with root nutrition. A favorable effect of foliar

fertilization on the plant growth and development was established by Peuke et al. (1998). In our results there is no significant difference between the two tested concentrations regarding plant biomass. The absence of considerable difference in shoot and root pea dry biomass after treatment with elevated concentrations of Agroleaf was observed on our previous study (Hristozkova et al., 2005). The lack of the effect on the biomass and yields of soybeans treated with high rates of foliar fertilizer was shown by Mallarino and UI-Haq (1998).

Foliar application of inorganic nutrients, including nitrogen, can be additional to insufficient as well as normal root supply of nutrients and could resulted in enhanced N uptake and assimilation (Peuke et al. 1998). We also observed increased levels of nitrate reductase and glutamine synthetase activities in the variants with foliar application, which is in correspondence with the higher protein levels in these variants (Fig. 2). A positive effect of Agroleaf treatment on the nitrate reductase and glutamine synthetase activities in pea plants has been observed in our previous work (unpublished data). Some differences between the two applied foliar fertilizer concentrations have been observed regarding NR activity only. It could due to the inhibitory effect of higher NH_4^+ ions content supplied with the higher foliar fertilizer concentration.

Despite the suggestion of Tejada and Gonzales (2003) that foliar fertilization increased leaf concentration of N, K, Fe, Mn and Zn, the content of main macronutrients N, P and K in milk thistle is higher in the roots than the shoots and did not depend on the way of nutrient application (Fig. 3). As a result of foliar application the content of nitrogen slightly increased only in the roots while the K content became higher both in the shoots and roots. The definite dependency regarding P content as a result of foliar application has not been observed. Milk thistle plants grown under conditions of root and combined root and foliar nutrition are shown on figure 4.

In conclusion application of additional foliar nutrition to milk thistle plants resulted in reduction of rosette diameter, increased leaf number and dry biomass accumulation, enhanced rates of nitrogen assimilatory enzymes. A favorable effect of foliar feeding on the protein content, tissue root nitrogen and potassium shoot and root concentration have been also established. A lack of differences regarding the effects of both applied foliar fertilizer concentration allow us to propose the lower one for the treatments of milk thistle plants.

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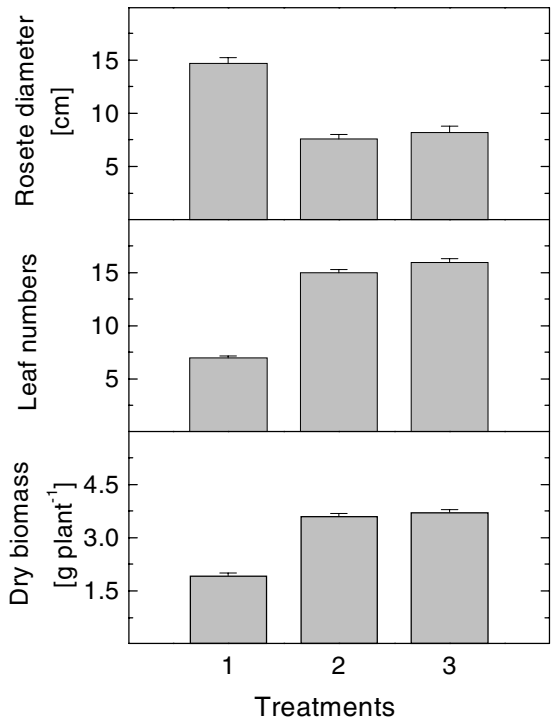

Figure 1. *Effect of foliar fertilizer on the rosete diameter, leave number and dry biomass of milk thistle plants: 1. Control plants, without application of foliar fertizer; 2. Plants grown with application of 0.3% foliar fertilizer; 3. Plants grown with application of 0.5% foliar fertilizer.*

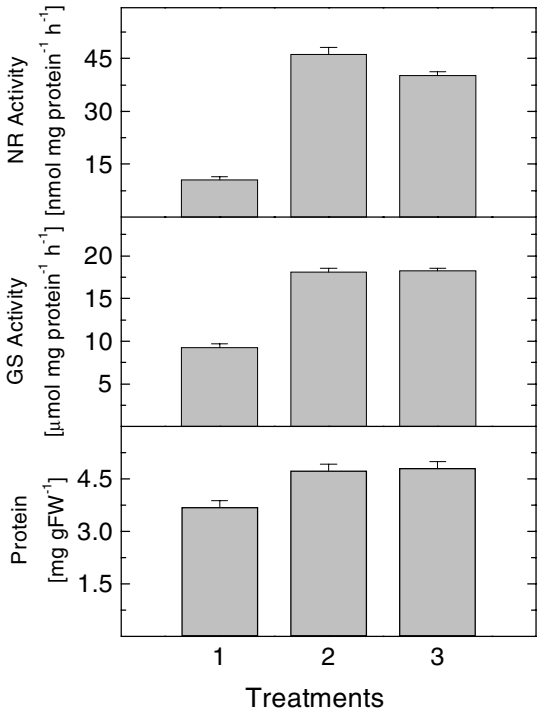

Figure 2. *Effect of foliar fertilizer on the activity of nitrate reductase, glutamine synthetase and protein content of milk thistle plants: 1. Control plants, without application of foliar fertizer; 2. Plants grown with application of 0.3% foliar fertilizer; 3. Plants grown with application of 0.5% foliar fertilizer.*

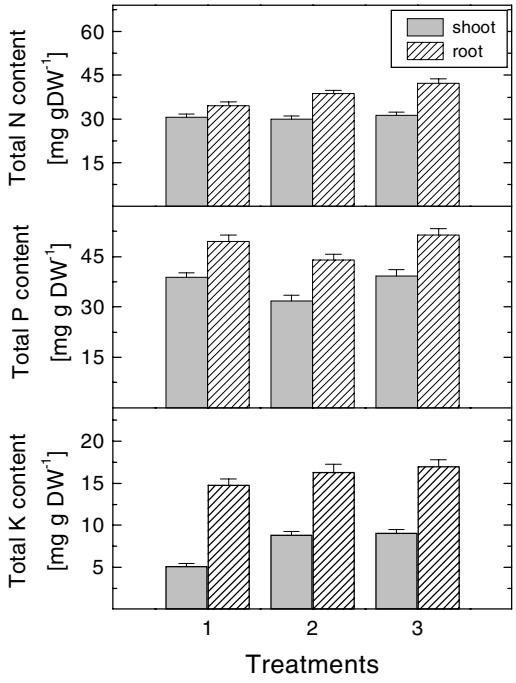

Figure 3. *Effect of foliar fertilizer on the nitrogen, phosphorous and potassium total content in milk thistle plants: 1. Control plants, without application of foliar fertizer; 2. Plants grown with application of 0.3% foliar fertilizer; 3. Plants grown with application of 0.5% foliar fertilizer.*

Figure 4. *Milk thistle plants grown under conditions of root and combined (root and foliar) nutrition*.