

RESEARCHES CONCERNING THE INFLUENCE OF ORGANIC FERTILIZERS OVER PRODUCTION AND QUALITY AT *ECHINACEA PURPUREA* MOENCH. (L.)

Adina- Cătălina DRUȚU¹, Alexandra LEONTE¹, Simona POCHIȘCANU¹,
Alexandra-Andreea BUBURUZ²

e-mail: iadina2001@yahoo.com

Abstract

Echinacea purpurea (L.) Moench, is a perennial plant, native to North America, from which there are used in therapeutic purpose the aerial parts and the roots. The aerial parts contains flavonoides, polyphenolic acid, coffee acid, volatile oil etc. The medicine prepared from this plant determines the rise of self defense system of the human body (by mobilizing the leukocytes and extending the phagocytosis activity and inhibits virus multiplication). Since on the international and national market is required that the vegetable raw material to be produced in ecological system without chemical fertilizers, the research conducted at A.R.D.S. Secuieni, during 2008 – 2011 aimed to establish the optimum doses of muck at *Echinacea purpurea* L. (Moench.) species and track its impact on the herba production but also on the content in active principles. As a result of the performed determinations it was found that the on average over the three years of experimentation the highest height (91.74 cm) and weight (108.26 g) was at the plants from the variant fertilized with fermented muck in dose of 40 t/ha. In this variant the dry herba production obtained during three vegetation years were 39.14 g/ha in the second year, 72.13 g/ha in the third year and 74.02 q/ha in the fourth year. The highest coffee acid content (79.7 mg/100ml tincture) was determined in leaves from plants fertilized with 30t/ha muck and the highest content in polyphenolic acid (2.657 g chlorogenic acid) was determined in flowers from plants fertilized with 40t/ha muck.

Keywords: organic fertilizer, echinacea, herba, production

Echinacea purpurea (L.) Moench. is a perennial species, native from North America from that it is used the air part in therapeutic purposes and at the fourth year plants may be used also the roots (Muntean L. and col., 1990). The air part contains flavonosids, polysaccharides, caffeic acid derivatives, volatile oil, etc.(Muntean, L.S. and col., 2007). The medicinal products from this plant helps to increase the body's defense system (by mobilizing the leukocytes and increased the phagocytosis activity) and inhibit the multiplication of viruses (Vârban D., 2001). The pharmaceutical extracts and forms with extracts are used in urology, gynecology and internal medicine. The *Echinacea* herba is the raw material for the production of antiviral, antitumor, immunostimulant, healing, anti – inflammatory and diuretic products (Oniga I.,1997).

MATERIAL AND METHOD

Because the domestic and international market demands that the vegetal raw material to be obtained in ecological conditions without applying chemical fertilizers, herbicides and pesticides, at ARDS Secuieni during 2003-2006 it

was monitored the influence of some doses of natural fertilizers on the production of herba and on the content in active principle of the *Echinacea purpurea* (L.) Moench. Species. The experience was with a single factor and was located on a typical cambic chernozem soil after the randomized block method in four repetitions. The experimental variants were:

- V₁ – unfertilized (control)
- V₂ - 20 t/ha;
- V₃ - 30 t/ha;
- V₄ - 40 t/ha.

The seed used in the experiments was received from SCDPMA Fundulea, with 85% germination and 97% purity. The sowing was done manually, at a distance of 50 cm between rows and 15 cm between nests on row at the 1.5 cm depth. The maintenance works consisted in manual hoeings and weeding on row and the plants harvesting was done manually starting from the second year of vegetation, at the full flowering by cutting the planta t soil level. The manure was applied in autumn before sowing and was embedded with the disc harrow. The observations were made of plants in vegetation, and the biometric measurements were performed after the plants harvest when samples were taken for analysis.

The analyses regarding the content in active principles were performed at C.C.B. „Stejarul” Piatra Neamt and were conducted on various types of extracts obtained from dried plants of *Echinacea purpurea* (L.) Moench. For the chemical quality study was used the thin layer chromatography for three major groups of active principles pharmacologically important: flavonoids, phenolic acids and triterpene compounds.

RESULTS AND DISCUSSIONS

On average for the three experimental years the vegetation period at the *Echinacea purpurea* (L.) Moench. was of 155 days. The vegetation phenophases represented on average: starting in vegetation – leaf rosette formation 48 days (31%), leaf rosette formation – issuing floriferous stem – 16 days (10%), issuing floriferous stem – buds - 17 days (11%), buds – blooming – 26 days (17%) and blooming – seed ripening 48 days (31%) (Tab. 1). The average height was higher at the plants from the fertilized variant with manure at the dose of 40t/ha and it was of 85.2 cm at the plants in the second year of vegetation, of 87.0 cm at the plants in the third year and of 103.0 cm at the plants in the fourth year (Tab. 2). The plants weight was higher at the plants from the fertilized variant with manure at dose of 40t/ha, averaged over the three years it was of 108.26 g (Tab. 3).

The dose of manure influenced also the production of dry herba, so the highest yield was obtained from the fertilized variant with 40t/ha manure and it was of 39.14 q/ha in the second year of cultivation, of 72.13 q/ha in the third year and of 74.02 q/ha in the fourth year. On average in the three years of experimentation the highest average production was obtained in the same variant this being of 61.77 q/ha (Fig. 1).

Regarding the content in active principles, the polyphenolic compounds determined from the *Echinacea purpurea* (L.) Moench. tinctures were: chlorogenic acid, caffeic acid, rutoside and hyperoside. The chlorogenic acid was present only in the root and had the highest value (6.764 mg/100 ml tincture) at the fertilized variant with 40t/ha manure. The highest caffeic acid content (79.700 mg/100 ml tincture) was obtained from the leaves of *Echinacea purpurea* (L.) Moench. at the fertilized variant with 30t/ha manure. The highest content in rutozide (6.766 mg/100 ml tincture) was obtained from the flowers at the fertilized variant with 20t/ha, and the highest content in hyperoside

(0.721 mg/100 ml tincture) was obtained from the leaves at the control variant - unfertilized (Tab. 4).

The content in polyphenolic acids was determined from several types of extracts, respectively methanolic exhausted, 70% methanolic exhausted and tincture but the 70% methanolic exhausted made a more complete extraction. Thus, the highest content of 2.657 g chlorogenic acid/100g drug was obtained in the *Echinacea purpurea* (L.) Moench. flowers from the fertilized variant with 40 t/ha manure (Tab. 5).

The flavonoids were determined from the same type of extracts, the highest content of 1.1428 g rutoside/ 100 g drug was obtained in flowers from the fertilized variant with 30 t/ha manure (Tab. 6).

Since, finally from the vegetable product harvested from *Echinacea purpurea* (L.) Moench., whether it is the flowering aerial plant or the root are obtained hydroalcoholic extracts which are processed in drug forms or as food supplements, was followed their extractability in compliance with Pharmacopoeia requirements relating to tinctures. It was found that the extractability is different for polyphenolic acids, flavonoids respectively, as shown in table 7. Following the extractability of polyphenolic acids from the vegetable material compared to that of the flavonoid, has been found that it is limited to the first group of components, both leaves and at flowers, it fits between 6.420 and 9.033%. compared to the stems and roots, where the extraction level is between 11.009 and 14.041%, these are very small. The flavonoids on the other hand, were extracted much better especially from the leaves and flowers (38.145 and 55.451%). This aspect is important for any companies that wish to process the vegetable material, because it binds to a number of economic parameters; if the extractability of an active principle is reduced so it remains significantly higher in the raw material processed, the process is less economical.

Table 1

***Echinacea purpurea* (L) Moench. phenological phases**

| Vegetation years | Phenophase | Starting in vegetation – leaf rosette formation | Leaf rosette formation – issuing floriferous stem | Issuing floriferous stem – buds | Buds – blooming | Blooming – seed ripening | TOTAL |
|------------------|-------------|---|---|---------------------------------|-----------------|--------------------------|-------|
| II year | Date | 4.04.-15.05. | 16.05-29.05 | 30.05-16.06 | 17.06-24.07 | 25.07-10.09 | 147 |
| | No. of days | 31 | 14 | 18 | 38 | 46 | |
| | % | 21 | 9 | 14 | 25 | 31 | |
| III year | Date | 20.03.-22.05 | 23.05-5.06 | 6.06-25.06 | 26.06-17.07 | 18.07-1.09 | 162 |
| | No. of days | 61 | 14 | 20 | 22 | 45 | |
| | % | 38 | 9 | 12 | 13 | 28 | |
| IV year | Date | 1.04.-23.05 | 24.05-10.06 | 11.06-24.06 | 25.06-11.07 | 12.07-3.09 | 156 |
| | No. of days | 53 | 18 | 14 | 17 | 54 | |
| | % | 34 | 12 | 9 | 11 | 34 | |
| Average | No. of days | 48 | 16 | 17 | 26 | 48 | 155 |
| | % | 31 | 10 | 11 | 17 | 31 | 100 |

Table 2

Influence of organic fertilizers on plant height at *Echinacea purpurea* (L) Moench.

| Variant | PLANTS AVERAGE HEIGHT (cm) | | | AVERAGE |
|-------------------------------------|----------------------------|----------|---------|---------|
| | II year | III year | IV year | |
| V1- unfertilized | 83,2 | 82,0 | 96,0 | 87,07 |
| V2 – fertilized with 20 t/ha manure | 84,1 | 85,5 | 100,0 | 89,87 |
| V2 – fertilized with 30 t/ha manure | 84,8 | 86,0 | 102,0 | 90,94 |
| V2 – fertilized with 40 t/ha manure | 85,2 | 87,0 | 103,0 | 91,74 |

Table 3

Influence of organic fertilizers on plant weight at *Echinacea purpurea* (L) Moench.

| Variant | PLANTS AVERAGE WEIGHT (g) | | | AVERAGE |
|-------------------------------------|---------------------------|----------|---------|---------|
| | II year | III year | IV year | |
| V1- unfertilized | 92,18 | 95,22 | 107,89 | 98,43 |
| V2 – fertilized with 20 t/ha manure | 98,86 | 97,47 | 110,54 | 102,29 |
| V2 – fertilized with 30 t/ha manure | 96,12 | 99,16 | 111,62 | 102,30 |
| V2 – fertilized with 40 t/ha manure | 98,88 | 107,16 | 118,73 | 108,26 |

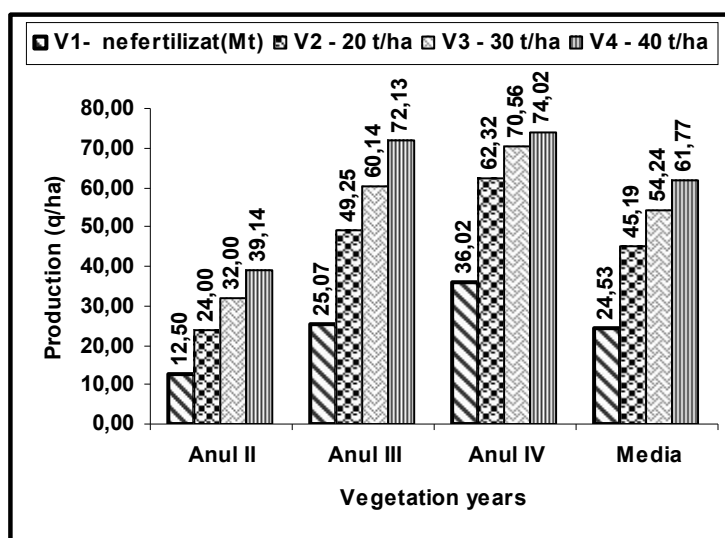


Figure 1 Influence of organic fertilizers doses on the production of dried herba at *Echinacea purpurea* (L) Moench. (2004-2006)

Table 4

Semi – quantitative determination (by HPLC) of some polyphenolic compounds from *Echinacea purpurea* (L) Moench. tinctures

| Variant | Mg/100 ml tincture | | | |
|----------------------------|--------------------|--------------|----------|------------|
| | Chlorogenic acid | Caffeic acid | Rutoside | Hyperoside |
| Leaves | | | | |
| V1- unfertilized (control) | - | 48.566 | 1.348 | 0.721 |
| V2 – 20 t/ha | - | 54.959 | 1.739 | 0.561 |
| V3 – 30 t/ha | - | 79.700 | 1.363 | 0.477 |
| V4 – 40 t/ha | - | 49.665 | 0.945 | 0.369 |
| Flowers | | | | |
| V1- unfertilized (control) | - | 36.061 | 5.597 | 0.388 |
| V2 – 20 t/ha | - | 37.467 | 6.766 | 0.528 |
| V3 – 30 t/ha | - | 38.050 | 6.696 | 0.607 |
| V4 – 40 t/ha | - | 34.918 | 6.118 | 0.549 |
| Stems | | | | |
| V1- unfertilized (control) | - | 7.482 | 0.486 | 0.581 |
| V2 – 20 t/ha | - | 8.010 | 0.538 | 0.591 |
| V3 – 30 t/ha | - | 6.817 | 0.240 | 0.249 |
| V4 – 40 t/ha | - | 4.785 | 0.343 | 0.251 |
| Roots | | | | |
| V1- unfertilized (control) | 2.119 | 14.468 | - | - |
| V2 – 20 t/ha | 2.133 | 16.692 | - | - |
| V3 – 30 t/ha | 3.073 | 10.083 | - | - |
| V4 – 40 t/ha | 6.764 | 16.768 | - | - |

Table 5

Quantitative determination of polyphenols acids (spectrophotometry) from *Echinacea purpurea* (L) Moench. samples

| Type of extract | Expressed in | Polyphenolic acids (g chlorogenic acid) | | | |
|--------------------------|-----------------|---|-------------|--------------|--------------|
| | | V1- nfertilized | V2 – 0 t/ha | V3 – 30 t/ha | V4 – 40 t/ha |
| Leaves | | | | | |
| Methanolic exhausted | 100 g drug | 1.168 | 1.440 | 1.064 | 0.834 |
| MeOH 70% exhausted | 100 g drug | 2.422 | 2.648 | 2.636 | 2.313 |
| Tincture (RDE=1:10 g/ml) | 100 ml tincture | 0.016 | 0.0173 | 0.017 | 0.017 |
| Flowers | | | | | |
| Methanolic exhausted | 100 g drug | 1.332 | 1.544 | 1.328 | 0.730 |
| MeOH 70% exhausted | 100 g drug | 2.192 | 2.544 | 2.475 | 2.657 |
| Tincture (RDE=1:10 g/ml) | 100 ml tincture | 0.016 | 0.018 | 0.016 | 0.024 |
| Stems | | | | | |
| Methanolic exhausted | 100 g drug | 0.568 | 0.848 | 0.761 | 0.521 |
| MeOH 70% exhausted | 100 g drug | 1.415 | 1.447 | 1.635 | 1.272 |
| Tincture (RDE=1:10 g/ml) | 100 ml tincture | 0.018 | 0.020 | 0.018 | 0.017 |
| Roots | | | | | |
| Methanolic exhausted | 100 g drug | 0.560 | 0.638 | 0.699 | 0.448 |
| MeOH 70% exhausted | 100 g drug | 1.282 | 1.457 | 1.352 | 1.683 |
| Tincture (RDE=1:10 g/ml) | 100 ml tincture | 0.018 | 0.018 | 0.017 | 0.020 |

Table 6

Quantitative determination of flavonoids (spectrophotometry) from *Echinacea purpurea* (L) Moench. samples

| Type of extract | Expressed in | Flavonoids (g rutoside) | | | |
|--------------------------|-----------------|-------------------------|-------------|--------------|--------------|
| | | V1- unfertilized | V2 – 20t/ha | V3 – 30 t/ha | V4 – 40 t/ha |
| Leaves | | | | | |
| Methanolic exhausted | 100 g drog | 0.3538 | 0.3311 | 0.3709 | 0.4740 |
| MeOH 70% exhausted | 100 g drog | 0.8547 | 1.0600 | 1.0081 | 0.8449 |
| Tincture (RDE=1:10 g/ml) | 100 ml tinctura | 0.0396 | 0.0457 | 0.0559 | 0.0445 |
| Flowers | | | | | |
| Methanolic exhausted | 100 g drog | 0.4756 | 0.5560 | 0.6339 | 0.8950 |
| MeOH 70% exhausted | 100 g drog | 0.8603 | 1.1168 | 1.1428 | 0.8950 |
| Tincture (RDE=1:10 g/ml) | 100 ml tinctura | 0.0357 | 0.0426 | 0.0457 | 0.0422 |
| Stems | | | | | |
| Methanolic exhausted | 100 g drog | 0.4875 | 0.4075 | 0.1625 | 0.3250 |
| MeOH 70% exhausted | 100 g drog | 0.5700 | 0.4875 | 0.4875 | 0.4875 |
| Tincture (RDE=1:10 g/ml) | 100 ml tinctura | 0.0179 | 0.0211 | 0.0146 | 0.0130 |
| Roots | | | | | |
| Methanolic exhausted | 100 g drog | 0.3250 | 0.3250 | 0.2450 | 0.3250 |
| MeOH 70% exhausted | 100 g drog | 0.4075 | 0.4075 | 0.3250 | 0.4075 |
| Tincture (RDE=1:10 g/ml) | 100 ml tinctura | 0.0114 | 0.0146 | 0.0097 | 0.0130 |

Table 7

| Polyphenols extractability from <i>Echinacea purpurea</i> (L) Moench. organs | | |
|--|--------------------|------------|
| Variant | Extractability (%) | |
| | Polyphenolic acids | Flavonoids |
| Leaves | | |
| V1- unfertilized (control) | 6.612 | 46.332 |
| V2 – 20 t/ha | 6.420 | 43.113 |
| V3 – 30t/ha | 6.449 | 55.451 |
| V4 – 40 t/ha | 7.350 | 52.669 |
| Flowers | | |
| V1- unfertilized (control) | 7.299 | 41.497 |
| V2 – 20 t/ha | 7.075 | 38.145 |
| V3 – 30t/ha | 6.465 | 39.989 |
| V4 – 40 t/ha | 9.033 | 47.151 |
| Stems | | |
| V1- unfertilized (control) | 12.721 | 31.404 |
| V2 – 20 t/ha | 13.822 | 43.282 |
| V3 – 30t/ha | 11.009 | 29.949 |
| V4 – 40 t/ha | 13.365 | 26.667 |
| Roots | | |
| V1- unfertilized (control) | 14.041 | 27.975 |
| V2 – 20 t/ha | 12.354 | 35.828 |
| V3 – 30t/ha | 12.574 | 29.846 |
| V4 – 40 t/ha | 11.884 | 31.902 |

CONCLUSIONS

1. In A.R.D.S. Secuieni conditions the organic fertilizers had a positive influence on plants growth and development and also on the herba production and content in active principles at the *Echinacea purpurea* (L.) Moench. species.

2. As a result of the performed measurements it was found that on average over the three years of experimentation the highest height (91.74 cm) and weight (108.26 g) was in the fertilized variant with manure in dose of 40t/ha.

3. The manure dose influenced the production of dry herba, thus the highest yield was obtained from the fertilized variant with 40t/ha manure and it was of 39.14 q/ha in II second year of cultivation, of 72.13 q/ha in III year and of 74.02 q/ha in IV year.

4. Regarding the content in polyphenolic compounds the chlorogenic acid was present only in the root and had the highest value (6.764 mg/100 ml tincture) at the fertilized variant with 40t/ha manure, and the highest content in caffeic acid (79.700 mg/100 ml tincture) was obtained from the leaves of *Echinacea purpurea* (L) Moench. at the fertilized variant with 30t/ha manure.

5. The highest content in rutoside (6.766 mg/100 ml tincture) was obtained in flowers from the fertilized variant with 20t/ha, and the highest content in hyperoside (0.721 mg/100 ml tincture) was obtained in leaves from the control variant - unfertilized.

6. The highest concentration in polyphenolic acids of 2.657 g chlorogenic acid/100g drug was obtained in the flowers of *Echinacea purpurea* (L) Moench. from the fertilized variant with 40 t/ha manure, and the highest content in flavonoids of 1.1428 g rutoside/ 100 g drug was obtained also in the flowers from the fertilized variant with 30 t/ha manure.

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