

Effects of growing methods and plant age on the yield, and on the content of flavonoids and phenolic acids in *Echinacea purpurea* (L.) Moench.

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ABSTRACT

The effect of three different growing methods, and plant age on the yield of purple coneflower (*Echinacea purpurea* L.) Moench., Asteraceae are described. The weight of fresh and dry mass of rhizomes increased significantly with the age of the plants. Independently of the age, the highest yield of rhizomes (1779 g/m² FW – 589 g/m² DW in the third year) was found in the plants grown from root cuttings and the smallest (385 g/m² FW – 108 g/m² DW in the third year) from seeds sown directly into the soil. A similar dependence was obtained in the yield of flower heads. No relation was noticed between the growing method and the flavonoids and polyphenol acids contents neither in the flower heads nor in the rhizomes. The polyphenol acids content was slightly higher in the flower heads (2.85% in the third year) than in the rhizomes (2.22% in the third year). The results demonstrate that the experimental factors can stimulate increased yield of rhizomes and flower heads of *E. purpurea*.

Keywords: *Echinacea purpurea*; experimental factors; yield; flavonoids; polyphenol acids

Echinacea purpurea is native to the Atlantic drainage area of the United States of America and Canada, but not Mexico (McGregor 1968). It has been introduced as a cultivated medicinal plant in parts of north and eastern Africa and in Europe (Iwu 2001).

Echinacea purpurea is herbaceous perennial with branched, fibrous root. Stem is erect, stout, branched, hirsute or glabrous, 60–180 cm high. As a member of the Asteraceae family, each “flower” or daisy-like head unit is actually a conglomeration of many tiny florets. The inner (disc) florets end in spines, and are surrounded by droopy outer (ray) florets with teeth at their ends. *Echinacea* is characterized by spiny flowering heads, with an elevated receptacle that forms the “cone”. Color of the disc florets is red-brown and the ray florets petals are purplish (Awang and Kindack 1991, Houghton 1994).

During the last 20 years *Echinacea* research has focused on its immune-stimulating properties.

Immunostimulatory, anti-inflammatory and antioxidant activities are the main properties described, which make *Echinacea* useful in the treatment or the prevention of different pathological conditions (Percival 2000). *Echinacea* is used today in dozens of preparations in European phytomedicine.

As *Echinacea* preparations may vary in chemical composition, their therapeutic effectiveness may be inconsistent. Factors that may influence the chemical composition of *Echinacea* preparations include the species of *Echinacea* used (*E. purpurea*, *E. pallida* or *E. angustifolia*), the part of the plant used (leaves, flowers, stems or roots), growing, drying and storage conditions and method of extraction (Schulthess et al. 1991, Percival 2000, Perry et al. 2001). Freshly harvested *Echinacea* is likely to be more effective than preparations that have been stored for a long period of time since the prolonged storage may result in a loss or damage of beneficial active constituents (Perry et al. 2001). The fingerprint of *E. purpurea* is characterized by

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cichoric acid as the main component (Pietta et al. 1998, Sloley et al. 2001) and cichoric acid is commonly used as a quality indicator for this species (Bergeron et al. 2000, Gray et al. 2003).

As the cultivation of *Echinacea* for use in therapeutic preparations increases, the industry is developing a need to further understand the effects of growing methods and plant age in order to increase the efficiency and quality while maximizing production. This study was conducted to examine the effects of three different growing methods for three consecutive growing seasons on quantity and quality of the raw material.

MATERIAL AND METHODS

Plant material

The experimental material was *Echinacea purpurea* (L.) Moench. There were used three different growing methods: 1. from seeds sown directly into the soil, 2. from seedlings, 3. from vegetatively obtained planting material (root cuttings). The plant material was collected in the first three years of vegetation and the yield of rhizomes and flower heads and content of flavonoids and phenolic acids was analyzed.

The plants were cultivated in the locality Nitra-Krškany in the years 1999–2001. The locality is characterized by brown soil. The climatic conditions were as follows: the average precipitations: 1999 – 574.1 mm, 2000 – 454.3 mm, 2001 – 445 mm; the average temperatures: 1999 – 10.54°C, 2000 – 11.35°C, 2001 – 9.89°C. These data were collected by the climatic station located in Nitra-Veľké Janíkovce. The field experiments were based on blocks in four repetitions. The area of the fields was 10 m². There were 80 plants on each field. The seeds were sown into the soil every year in the middle of April. The seedlings were grown in a greenhouse and transplanted into the soil with the root cuttings in the middle of May. The flower heads were collected in the optimal developmental phase – fully in bloom – in August. Entire roots were harvested at the end of October and rhizomes were thoroughly cleaned with water. Three replicate samples (15 plants) were taken for each growing method. All samples were weighed. The plant material (rhizomes and flower heads) was dried at 40°C for approximately 3 days. After drying, all samples were weighed, ground to powder in a hammer mill, and stored frozen (–20°C) prior to HPLC analysis.

Extraction of phenolic acids

The samples were extracted and analyzed in duplicate for cichoric acid, chlorogenic acid and caftaric acid. Approximately 50 mg of the powdered drug was extracted three times with 20 ml methanol using sonication (5 min), centrifugation and collection of the subsequent supernatant. 100 µl of the supernatant was dissolved in 400 µl of distilled water. 20 µl of sample of this material was applied directly to the HPLC apparatus.

Extraction of flavonoids

The samples were extracted and analyzed in duplicate for kaempferol, quercetin and rutin. Approximately 300 mg of the powdered drug was sonicated (5 min) with 50 ml ethanol and 20 ml distilled water. Using the fume hood 8 ml of concentrated hydrochloric acid and boiling chips were added. Extract was refluxed at moderate temperature for 2.25 hours and cooled to room temperature. The extract volume was adjusted to 100 ml, and filtered through a 0.45 µm PTFE membrane prior to injection of 10 µl into HPLC system.

HPLC analysis. The analytical equipment consisted of a Hitachi L-7100 gradient HPLC pump equipped with a Hitachi L-7400 UV detector (284 nm for phenolic acids; 270 nm for flavonoids). Chromatographic separation of constituents was accomplished by gradient elution on a Hypersil 5 µm (BDS) 250 × 4.6 mm reversed-phase column. Mobile phases were filtered and degassed under vacuum and pumped at 1 ml/min. Modifications of methods described by Gray et al. (2003) and by Bauer and Wagner (1991) were used for detection of the phenolic acids. The mobile phase was a binary gradient of A: 1% acetic acid – acetonitrile (9:1) and B: acetonitrile. The gradient used was as follows: 100% A from 0–15 minutes, to 80% A from 15–16 minutes, to 100% A from 16–25 minutes (linear). The mobile phase for the flavonoids consisted of methanol:0.5% phosphoric acid (50:50).

Standard solutions

For peak determination and concentration the standards for caffeic acid were used (Fluka, Praha, Czech Republic, purity 95% HPLC), chlorogenic acid (Fluka, Praha, Czech Republic, purity 97% HPLC)

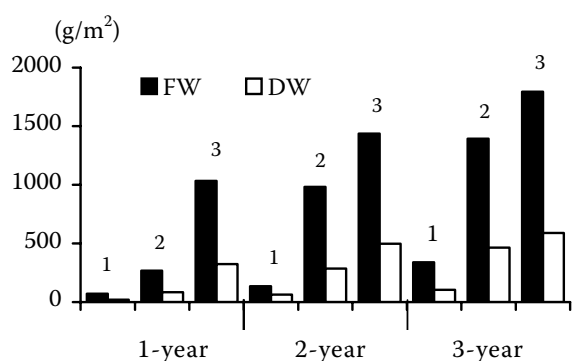


Figure 1. Fresh and dry weight of rhizomes of *Echinacea purpurea* (L.) Moench. (1 – direct sowing, 2 – seedlings, 3 – root cuttings)

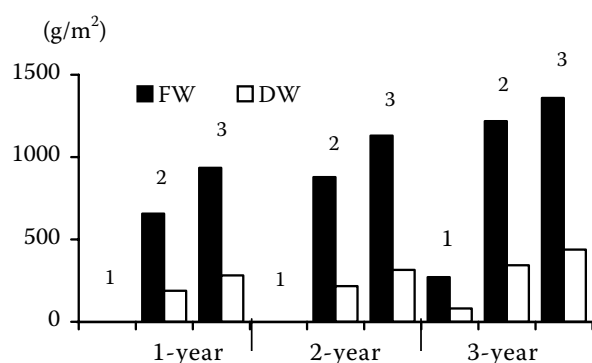


Figure 2. Fresh and dry weight of flower heads of *Echinacea purpurea* (L.) Moench., (1 – direct sowing, 2 – seedlings, 3 – root cuttings)

cichoric acid (Addipharma, Hamburg, Germany, purity 98% HPLC), kaempferol (Fluka, Praha, Czech Republic, purity 96% HPLC), quercetin (Fluka, Praha, Czech Republic, purity 98% HPLC) and rutin trihydrate (Fluka, Praha, Czech Republic, purity 90% HPLC).

Statistical analysis

Data were shown as means \pm standard errors of the mean (SEM). Statistical significance of the differences between parameters was evaluated by means of Student's test. $P < 0.05$ was chosen as a criterion of statistical significance.

RESULTS AND DISCUSSION

The underground part of *E. purpurea* consists of the root and rhizomes. The samples used in this work were only the rhizomes. Fresh mass and dry mass production of rhizomes are significantly dependent on the growing methods. The worst results were obtained from plants planted from seeds sown directly into the soil. The highest yield of rhizomes was found in plants growing from vegetatively obtained planting material, especially in 2-year and 3-year old plants (Figure 1). These observations prove that the seeds germinate unevenly and grow slowly in the initial phases of development. The results confirm the knowledge of other authors, who stated that *Echinacea* plants are resilient and drought resistant, but grow slowly (Houghton 1994). The controlled drought stress can stimulate increased root dry weight in *E. purpurea*. Plant developmental stage and growing conditions

can significantly influence the components that determine raw material quality (phytochemical content and dry matter accumulation) (Gray et al. 2003). Similar results were obtained by observing the fresh and dry mass of the flower heads. In contradistinction to the rhizomes the 1-year old plants planted from seeds sown directly into the soil and from seedlings did not grow to the flowers (Figure 2).

Our results also demonstrate a close connection between the age of the plants and the yield of the rhizomes and the flower heads. The highest yield of rhizomes and flower heads was found in 3-year old plants (Figures 1 and 2). Dry mass production of rhizomes of 2-year and 3-year old plants was considerably higher in comparison with the 1-year old plants for all three growing methods (Figure 1). Gray et al. (2003) showed that root dry weight increased significantly by an average of 70.0% for drought-stressed plants from two to three years of age, compared to an increase of well-watered controls.

The effect of different growing conditions and the age of plantation on the content of flavonoids is summarized in Table 1. No relation was found between the growing methods, age of the plants

Table 1. The content of flavonoids in flower heads of *Echinacea purpurea* (%)

Growing conditions	Age of the plants		
	1-year	2-year	3-year
Direct sowing	0	0.74	0.74
Seedlings	0	0.81	0.76
Root cuttings	0.75	0.74	0.80

Table 2. The content of polyphenolic acids in flower heads and rhizomes of *Echinacea purpurea* (%)

Growing conditions	Age of the plants					
	1-year		2-year		3-year	
	flower heads	rhizomes	flower heads	rhizomes	flower heads	rhizomes
Direct sowing	0	2.22	2.87	2.21	2.85	2.22
Seedlings	0	2.16	2.83	2.18	2.85	2.18
Root cuttings	2.86	2.18	2.86	2.17	2.84	2.18

and the flavonoid content in the flower heads. According to our results the polyphenol acids content neither depended on different growing methods neither on the age of the plants (Table 2). The polyphenol acids content was slightly higher in the flower heads than in the rhizomes. Our investigations correspond with the results of Binns et al. (2002), who found that *E. purpurea* flower heads from 2-year-old cultivated transplants contain about the same amount of cichoric acid as the young roots of that species. Results of Letchamo et al. (1999) indicate that the cichoric acid and isobutylamide content of *E. purpurea* is strongly influenced by the floral developmental stage. According to Binns et al. (2002) caffeic acid derivatives in *Echinacea* contain many closely related compounds at concentrations that vary throughout the growth of the plants. The phenolics content of *E. purpurea* roots decreased with age. Gray et al. (2003) observed that the acute periods of drought stress controversially increased total phenolic acids content in *E. purpurea* roots from two to three years of age. The authors determined that the drought stress during the period of initial flowering over two seasons significantly increases the content of root cichoric acid. Concerning cichoric acid stability, it has been shown recently that enzymatic degradation by polyphenol oxidases occurs in aqueous extracts of fresh *E. purpurea* (Kreis et al. 2000, Nüsslein et al. 2000). It is possible that an enzymatic degradation, of cichoric acid in particular, might have occurred in the material used in this investigation; however the effects are likely to have been minimized due to the drying of the plant material immediately after harvest, frozen storage of the samples, and extraction with 100% methanol prior to analysis.

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