

Technology Improvement for Seedling Growing Method of *Echinacea Purpurea* Under Drip Irrigation

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1. Abstract

Development of new technological methods of cultivation of traditional medicinal crops and improvement of existing ones are the main ways to increase production of pharmaceutical raw materials for pharmacy uses.

Today, the main limiting factor in the increasing in the *Echinacea purpurea* production under the conditions of Ukrainian Forest-Steppe zone is lack of moisture during spring and the start of culture vegetation. This lack is caused by global climate changes. Another deterrent is formed by plant diseases leading to losses in yield of raw materials and seeds. Diseases also can cause decrease in of biologically active substances (BAA) content and sometimes even full loss of crops.

2. Introduction

The damage of purple coneflower plants by viral diseases is significantly reduced when used in the technology of elements of organic farming, when the soil humus content is over 5.2% [1]. Optimization of phytosanitary status of medicinal plants for diseases is possible only with on-time complex of protective measures of good quality. The basis of such complexes is the use agro technical method [2].

Development and improvement of existing technologies using new agro technical methods can reduce the cost of raw materials cultivation due to innovations. It also significantly increases the yield and quality of raw materials and plant resistance to phytophages and pathogens of different nature.

During the last decade, research on growing medicinal crops under drip irrigation was conducted in Ukraine. This method of irrigation has proved to be the most progressive. It is confirmed by numerous research works in vegetable growing and cultivation of certain medicinal plants, in particular garden valerian, wild majoran, melissa, peppermint [3-6], yacon [7] etc.

High soil moisture is maintained for vegetables and medicinal crops growing during the vegetation. The result of this is increase in humidity of the surface layer. It causes favorable microclimate

for the development of diseases [1, 5].

The introduction of optimal quantities of active substances with complex of mineral fertilizers enhances the process of photosynthesis and activates the work of many vitamins and enzymes involved in nitrogen and carbohydrate metabolism, redox processes [2, 8, 9], which increases the resistance of plants against diseases [2, 10, 11].

Echinacea purpurea (L.) Moench. is a medicinal culture that responds well to irrigation and fertilization. Due to high demand for purple coneflower raw materials - rhizomes with roots, grass and inflorescences, the area occupied by this culture expands. *Echinacea purpurea* takes one of the leading roles as component of meds for treatment human diseases. It is also an important source of various biologically active substances [12]. Improvement of water-nutrient regime and methods of cultivation of *Echinacea* allows providing optimal conditions for formation of high yield of corresponding quality during one year of cultivation. Therefore, the purpose of the work was to improve the technology of seedlings growing under drip irrigation conditions.

2.1. Research Methodology: The studies were conducted on the research fields of the Department of Medicinal Cultivation Technology of the Research Station of Medicinal Plants of the Institute of Agroecology and Environmental Management of the National

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Academy of Sciences of Ukraine (Poltava region, Ukraine) in 2016 and 2017.

The impact of agro technical measures on the development of *Echinacea purpurea* varieties of 'Charivnitsya' and its diseases was determined by monitoring of diseases spreading and development. This monitoring was conducted in the experiments of research the drip irrigation and fertilizer application effectiveness.

The disease spreading was determined by systematic accounting of plant damage after the first signs of the disease. For this purpose method of two stepped diagonals was applied. 10 areas in one linear meter on the same distance from each other on the plantation were selected for counting of healthy and damaged plants calculation. The spread of the disease was defined as the percentage of affected plants by the following formula [11]:

$$P = \frac{N \times 100}{n},$$

P – disease speeding, %; N – number of plants; n – number of plants damaged by diseases.

Ступінь ураження рослин визначались за площею ураженої поверхні органів та інтенсивністю прояву інших ознак захворювання.

The degree of plant damage was determined by the affected area of the organ surface and the intensity of other signs of the disease.

The intensity of damage was rated by 6-point scale.

0 – no signs of damage;

0,1 – the damage is non-intense, there are few small spots on single leaves, in general less than 1% of leaf surface is affected;

1 – the damage is non-intense, but lower leaves are affected by chlorosis, small spots take up to 10% of the leaf surface;

2 – approximately 25% of the leaf surface of lower leaves and 15% of middle and upper leaves are covered with spots;

3 – there are numerous spots, that cover about 50% of the leaf surface of lower and up to 30% of the middle and upper leaves;

4 – the whole plant is affected by disease, the leaves are covered with numerous spots that merge on 75-100% of the leaf surface. The leaves turn yellow and crumble.

Disease development was determined by the following formula:

$$R = \frac{\sum b}{n \times k} \times 100,$$

a – number of damaged plants; b – rate of damage (1 – 6); n – number of plants in sample; k – the highest score on the accounting scale.

3. Results

The research of the development and improvement of seedling method growing technology elements under drip irrigation for purple echinacea was carried out according to the scheme, which included the following steps:

- seedlings planting with study of their pre-sowing seed preparation, timing of sowing into cassettes, seedlings care and wintering monitoring;
- cultivation of plants in the field conditions – preparation of soil for planting seedlings, timing of planting seedlings, care of plants, maintaining of soil moisture 75 - 80% of the lowest moisture content, harvesting timing, the raw materials quality determination;
- the drip irrigation and mineral fertilizers influence on the spread and development of diseases of establishment.

Seed material of own reproduction was used for experiments., laboratory studies were conducted before sowing seeds into cassettes in order to find optimal ways of increasing germination energy and seed germination. The seeds were treated with stimulants – solutions of Gibberellin, Emistim and Nanomix of different concentrations and kept for 24 hours. The seeds were dried and plated into Petri dishes after treatment. Germination energy was determined on 4th day, germination – on the 14th or 15th day.

The highest efficiency was shown with the use of Gibberellin, with a concentration of working solution 0.005%. Its use increased the germination of seeds of purple coneflower by 17 – 19%. The effectiveness of Emistim and Nanomix was 8% and 10%, respectively.

Seedlings were grown in a specialized nursery. For this purpose, the seed were sown in boxes, on the prepared soil bed using ridges (ridge method) and cassettes (cassette method).

Seeds were sown in cells in the amount of 3-4 pieces. The number of sown seeds was determined experimentally using previous laboratory results. These laboratory results showed the ability to germinate depending on the accuracy (from 1 to 10 seeds in one cell of the cassette).

Before sowing in cassettes, the seeds were treated with Gibberellin solution with concentration of 0.005%. Sowing was carried out in the first decade of September. The seeds were sown with a row spacing of 15 cm to a depth of 1.0 cm when the ridges method of

seedling cultivation was applied.

Before sowing, the cartridges were filled with soil mixture; the seeds were sown on a prepared soil bed in cassette cells (manufactured by A green).

In order to protect the crop from sharp changes in air temperature and to reduce soil moisture loss by evaporation, the cassette boxes were covered with a 30 g/m² agro fiber (manufactured by Premium – Agro).

In the breeding nursery, during the autumn period of the vegetation, soil moisture was maintained in the range of 80-90% of the lowest moisture content, and plant growth and development were monitored. The beginning of seedling of purple coneflower in cassettes was at the 10th day, in ridges – at the 14th day.

Echinacea purpurea formed 1-2 true leaves before the persistent frosts. The nursery was covered with winter wheat straw with a 10-15 cm layer in order to improve the wintering of seedlings.

Plant care was to maintain optimum soil moisture and manual weeding during the spring period. Restoration of the purple coneflower plants vegetation began in the first decade of April.

The wintering of plants was recorded in the second decade of April. The purple coneflower plants in the cassettes had 2-3 true leaves, those planted in ridges – 1-2 true leaves.

Planting under field conditions was carried out on two backgrounds - without fertilizer (control) and with the introduction of pre-planting cultivation of mineral fertilizer (Nitroamophoska) at a dose of N₄₅P₄₅K₄₅ in terms of the active substance. The purple coneflower was planted with a density of 42, 56 and 83 thousand plants per hectare in order to determine the optimal area of plant nutrition on the plantation using the seedling method of cultivation under drip irrigation conditions.

The planting of seedlings *Echinacea purpurea* cv. 'Charivnitsya' was

carried out in the second decade of May. Seedlings, in both cultivation methods, were in the phase of 3 true leaves.

A drip irrigation system was used after planting in open ground to keep the plants moist. The system was completed with the EU-RODRIP Eolos irrigation pipeline (Greece) with a diameter of 16 mm, the distance between the water vents (emitters) - 20 cm. For 1 linear meter of watering pipeline water usage was 6,2l/h, when water pressure inside pipeline was 1 atm. The irrigation pipelines were placed near the line, 2-3 cm away from the plants.

During the first two weeks, irrigation was performed at a moisture content of the root soil layer up to 90% of the lowest moisture content. Then irrigation was used to reduce soil moisture to 80% of the lowest moisture content. The magnitude of irrigation norm, frequency and duration of irrigation of inter-irrigation period depended on the phase of plant development, planting scheme, weather conditions and arrangement of irrigation pipelines.

In the conditions of the Left-bank Ukraine of Forest Steppe zone, 24 - 26 irrigations with the norm of 90 - 130 m³ were conducted to maintain the humidity of the active soil layer at the level of 80%. The depth of the watering area was determined by the phase of plant development: for the first period (from the beginning of planting to the formation of a developed rosette) the specified soil moisture level was maintained in a layer of 20 - 25 cm, in the second period – 40- 50 cm.

Control of soil moisture was determined using tension meters placed at a depth of 15-30 cm to determine the timing of irrigation.

За всіма біометричними параметрами рослини ехінацеї пурпурової були більш розвинуті у варіантах закладених розсадою з касет (табл.1). According to all biometric parameters, *Echinacea purpurea* plants were more developed when laid by seedlings from cassettes (Table 1).

Table 1: Growing seedlings methods, mineral fertilizers and micro fertilizers effects on the growth and development of *Echinacea purpurea* (average for 2016-2017).

Variants	Leaves number, quantity/plant	Plants height, cm	Root length, cm	Root weight, g/unit.
Cassette without fertilizers (control)	17.2±2.1	27.6±1.8	17.3±0.9	26.3±3.5
Cassette without fertilizers (control)+ microfertilizers	20.5±1.9	48,3±1.5	19.3±1.1	31.2±3.1
Cassette, application of fertilizers N ₄₅ P ₄₅ K ₄₅ before planting + microfertilizers	23.1±2.5	53,1±2.6	22.6±0.8	47.7±2.5
Cassette, application of fertilizers N ₄₅ P ₄₅ K ₄₅ before planting	26.5±3.1	39.8±2.9	18.2±1.2	41.2±2.9

Ridges, without fertilizers (control)	22.8±1.8	26.3±2.2	16.6±1.3	21.3±3.2
Ridges, without fertilizers + microfertilizers	26.3±1.6	35.2±1.9	17.3±0.9	26.6±2.2
Ridges, application of fertilizers $N_{45}P_{45}K_{45}$ before planting + microfertilizers	31.9±2.2	33.5±2.3	19.6±1.4	41.5±2.6
Ridges, application of fertilizers $N_{45}P_{45}K_{45}$ before planting	32.2±2.8	39.2±1.7	18.1±1.2	38.2±2.3

The obtained data indicate that the use of micro fertilizers caused more intensive growth and development of *Echinacea* plants compared to the control group. The plants had more powerful root system weighing 26.6 – 31.2 g/eq. exceeding the control group by 4.9 – 5.3 g/eq. Pre-planting fertilizer in combination with the use of

micro fertilizers has been the most favorable conditions for plant growth and development. Under these conditions, the weight of the roots was 41.5 – 47.7 g/eq., and the height of the aboveground mass was 33.5 – 53.1 cm (Figure 1), the length of the roots was in the range 19.6 – 22.6 cm.



Figure 1 *Echinacea purpurea* (L.) Moenh cv. 'Charivnitsya' in phase of developed rosette.

It was established that *Echinacea purpurea* for seedlings growing under irrigation conditions had a yield of raw materials on average:

grasses within 4.3 – 6.6 t/ha, roots 1.3 – 2.2 t/ha (Table 2).

Table 2: Effect of growing seedlings methods, mineral and micro fertilizers on the purple coneflower yield under drip irrigation conditions.

Variants	Yield of dry grass, t/ha			Yield of dry roots with rhizomes, t/ha		
	2016	2017	average	2016	2017	average
Cassette without fertilizers (control)	4.4	4.2	4.3	1.4	1.2	1.3
Cassette without fertilizers (control)+ microfertilizers	6.1	5.5	5.8	1.9	1.8	1.9
Cassette, application of fertilizers $N_{45}P_{45}K_{45}$ before planting+ microfertilizers	6.8	6.4	6.6	2.2	2.1	2.2
Cassette, application of fertilizers $N_{45}P_{45}K_{45}$ before planting	6.9	6.1	6.5	2	1.9	2
Ridges, without fertilizers (control)	6.3	3.7	5	1.4	1.1	1.3
Ridges, without fertilizers + microfertilizers	6.7	4.8	5.8	1.4	1.3	1.4
Ridges, application of fertilizers $N_{45}P_{45}K_{45}$ before planting + microfertilizers	7.3	5.4	6.4	1.5	1.7	1.6
Ridges, application of fertilizers $N_{45}P_{45}K_{45}$ before planting	7.7	5.1	6.4	1.8	1.5	1.7
HIP ₀₅	0.5	0.3		0.2	0.2	

The highest yields of both grasses and roots were for the variants where mineral fertilizers based on $N_{45}P_{45}K_{45}$ were applied before planting. At the same time, the use of micro fertilizers on the background of mineral fertilizers increased the yield of *Echinacea* grass by 1.4 - 2.3 t/ha, roots - 0.3 - 0.9 t/ha. So, effectiveness of irrigation

and combination of nitroxin and nitrogen treatment on *E. purpurea* cultivation is shown [13].

(Table 3) shows the yield data of *Echinacea* roots by years of research depending on the area of plant nutrition:

Table 3: The yield of *Echinacea purpurea* cv. 'Charivnitsya' depending on the area of plant nutrition using the seedling method of growing under drip irrigation conditions.

Variants	Yield of dry grass, t/ha			Yield of dry roots with rhizomes, t/ha		
	2016	2017	average	2016	2017	average
42 thousand plants per 1 ha (control)	5.2	4.7	5	1.3	1.1	1.2
56 thousand plants per 1 ha	6.1	5.4	5.8	1.7	1.6	1.7
83 thousand plants per 1 ha	7.3	6.2	6.8	2.2	2	2.1
110 thousand plants per 1 ha	6.8	5.9	6.4	2.1	1.8	2
HIP ₀₅	0.4	0.5		0.3	0.3	

The highest yields of both grass and roots, on average, 6.8 t/ha and 2.1 t/ha, respectively, were obtained, with the number of plants per hectare - 83 thousand. The obtained results show that the seedling cultivation of purple coneflower gives the opportunity to cultivate it as a one-year crop, since the yield of dry grass is on average 5.0 – 6.4 t/ha, dry rhizomes with roots – 1.2 – 2.1 t/ha.

Pinto et al. revealed that *Echinacea pallida* plants growth under sub irrigation treatment showed increased seedling quality: with more biomass (14%), better nitrogen use efficiency (13%), greater nitrogen content (N; 11%), more height (15%), and lower mortality compared with overhead-irrigated seedlings [14].

It is known that *Echinacea purpurea* plants are affected with agents of different etiology in Ukraine. Most often they are fungi and viruses. Damaging by them causes reduce of yield and content of biologically active substances and increase concentration of some heavy metals in plants [15-17]. It should be mentioned that new reports on infection of purple coneflower by fungal and viral pathogens are still recorded in different countries. For the first time, *Rhizoctonia solani* in Italy [18], *Phoma herbarum* [19] and *Cucumber mosaic*

virus in Iran [20] are revealed on purple coneflower. *Cercospora* sp. and *Phymatotrichum omnivorum* were the most common fungal diseases in American purple coneflower farms [21]. Leaf spot diseases caused by *Cercospora* sp., *Alternaria* sp., *Septoria* sp. and/or *Pseudomonas cichorii* were registered on purple coneflower plants in China under conditions of high humidity and poorly ventilated area [22]. Research of the impact of irrigation regimes on the spread and development of plant diseases receives a lot of attention nowadays, due to their impact on several aspects of different plant disease cycles, such as the sporulation, survival of pathogen, their transmission to new hosts, germination and infection. Thus, irrigation is the most important practice in the management of plant diseases.

In addition to determining the effectiveness of agro-measures, we conducted a research of the effects of fertilizers and irrigation on the spread and development of cercosporosis (*Cercospora rudbeckia* Sacc.) on the purple coneflower. It was found that in 2016 and 2017, soil moisturizing using drip irrigation contributed to the spread of the disease causing *Cercospora rudbeckia* Sacc (Table 4).

Table 4: Spreading and development of *Cercospora rudbeckia* Sacc on purple coneflower depending on irrigation and fertilizer application (2016- 2017).

Factors		Disease spreading, %		Disease development, %	
Moisturizing (A)	Fertilizers application (B)				
		2016	2017	2016	2017
Control (without moisturizing)	Without fertilizers	65.3	69	8.7	22.4
	Application of fertilizers NPK ₄₅ before planting	69.6	52.9	8.9	16.1
Drip irrigation	Without fertilizers	89	89.9	9.2	24.7
	Application of fertilizers NPK ₄₅ before planting	87.4	82.5	8.5	21.4
HIP ₀₅ for factor A		13.9	13.2	F _f < F _t	F _f < F _t
HIP ₀₅ for factor B		F _f < F _t	F _f < F _t	F _f < F _t	F _f < F _t

The tendency of increasing of number of damaged plants was shown. There were 89.0-89.9% damaged plants when irrigation was applied and 65.3-69.0% - when there was no irrigation (control). The development of the disease has not changed significantly. Fertilizer application before planting did not change phytosanitary state inside the coenosis. The tendency of higher damaging of *Echinacea* with disease in the variant with irrigation persisted.

Experiments with potato showed that drip-irrigation use reduced the incidence of *Sclerotium rolfii* disease at harvest (13 to 23% on tubers) comparing to sprinkler plots (56 to 62%). The low incidence of disease was associated with relatively dry surface soil [23]. Such different results can be explained by the fact, that one fungus species requires more water for dispersion, so the type of irrigation has a strong effect on such group of pathogens. The size and amount of the water drops may affect capacity of spore dispersion, since smaller drops are unlikely to dislocate and disseminate spore from one spot to another.

The response of plant pathogens (fungi, oomycetes, bacteria, nematodes, viruses) to the range of irrigations methods varies widely. That's why irrigation method (furrow, overhead sprinkler, micro sprinkler, and drip irrigation) must be selected for each particular plant-pathogen system because it may strongly affect propagule dispersion, induction of germination, bio film formation, penetration and survival of each specific group of pathogens. «For the oomycetes and bacteria associated to aerial plant organs, due to their strong dependency on free water and high humidity, drip irrigation might be the appropriate choice. Among the true fungi, the effects of the irrigation system and management differ, and species of dry and wet spores respond distinctly to each individual method» [24]. Nematodes and oomycetes need free water in the soil for their active distribution. Thus, for oomycetes, bacteria and fungi associated with plant roots, the best method to avoid their reproduction and spreading can be using of sprinklers; for nematodes on the root – drip irrigation and sprinklers. Viruses transmitting with their vectors, can be controlled by sprinkle irrigation, which disrupts the contact of the insect with the plant [24]. The knowledge of the causal agent is important for deciding the irrigation method and is the key to obtain high quality yields.

4. Conclusions

It was established that the best way of growing seedlings of purple echinacea is cassette way, which provides the highest yield of both grasses and roots of purple echinacea. In this case, seedlings grown in cassettes are aligned in size, which is especially important when planting with the use of seedling machines.

During the research, it was found out that high efficiency of seedling cultivation and drip irrigation in combination with mineral fertilizers significantly affect crop yield. However, no statistically significant differences were observed in the development of the diseases. Fertilizer application did not generally affect the phytosanitary condition in agrocenosis, and the tendency of higher coneflower disease in the variant with irrigation remained.

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