Sel'skokhozyaistvennaya Biologiya [Agricultural Biology], 2010, № 5, p. 98-105.
*UDK* 633.8:581.144:577.175.19:58.056

# GROWTH AND BIOSYNTHESIS OF ECDYSTEROIDS IN *Rhaponticum carthamoides* (Willd.) Iljin UNDER THE INFLUENCE OF EDAPHIC FACTORS

***N.P. Timofeev***
*Research - Production Enterprise "CF BIO",*
*Koryazhma 165650, Arkhangel'sk province, Russia*
*e-mail: timfbio@atnet.ru*

*Received March 11, 2009*

Summary

**The growth processes in *Rhaponticum carthamoides* (Willd.) Iljin in the conditions of Arkhangel'skaya oblast' and their link with a biosynthesis ecdysteroids are investigated. The environmental factors limiting growth of propagules (a range of growth responses to light exposure, temperature and humidity), resistance to the low and negative temperatures, and influence of stressful factors on synthesis and accumulation of ecdysteroids in leaves organs are established.**

**Key words: *Rhaponticum carthamoides*, *Leuzea*, environmental factors, growth of propagules, stress, biosynthesis ecdysteroids.**

*Rhaponticum carthamoides* (Willd.) Iljin (*Leu-zea*, Maral root) is the plant widely used in pharmaceuticals and biologically active food supplements aimed at treatment of cardiovascular diseases, cancer, immune disorders, life support and health protection in extreme conditions, as well as in agriculture (1-3). Ecdysteroids isolated from this plant are used as objects and instruments of research in cell biology and molecular genetics (ecdysone-induced gene expression systems), in professional sports (preparations of Ekdisten group and their ecdysterone-based analogues) and in development of ecologically safe insecticides (4-7).

It has been shown that the primary synthesis of phytoecdysteroids (plant ecodysteroids, PES) and their accumulation occurs in adult leaves, and then PES move into intensively growing tissues and organs (8-9), which proceeds constantly (10). Environmental conditions (temperature, daylight duration, illumination, stress at drought and frost) can alter biosynthesis and accumulation of target compounds in pharmaceutical herbal raw by affecting the growth and direction of metabolic processes in plants. The controlling factors of PES formation have not been studied for a long time (except the impact of light spectrum on the content of ecdysteroids in leaves of plants the genera *Rhaponticum* and *Lychnis* under phytotron conditions) (11).

The purpose of this work was studying the role of main edaphic factors (illumination, high and low air temperatures, air humidity and water stress) in regulation of growth processes and biosynthesis of ecdysteroids in *Rhaponticum carthamoides.*

*Technique*. The objects of study were two agropopulations of *R. carthamoides* including plants of different ages (areas - 1 and 4 ha) located in the middle taiga subzone of the European North-East region (62° N, 47° E ; Kotlas-Koryazhma city, Arkhangelsk province). Detailed characteristics of the object and climatic conditions were described in the earlier author's publications (10, 12). In general, the climate of the subzone is moderately cool, the average temperature in January is equal to -14,3 °C, in July - +17,4 °C, the sum of temperatures above +5 / +10 °C - 1936/1577 °C, above +15 ° C - 911 ° C. The daylight duration in May and June is 16-20 h; precipitation-evaporation coefficient - 1,5 (13). Soil of the plots - loamy sod-podzol on water-glacial two-layer sediments, medium term of cultivation (pH 6,4-6,5; humus - 1,5-3,6%; $K_2O$ - 7,1-12,3; $P_2O_5$ - 18,0-31,2 mg/100 g; MgO - 1,0-1,4 mg-eqv/100 g).

Seeds were sown at the rate of 3 kg/ha (seeds of own production), without use of any mineral or organic fertilizers and chemical pesticides. Air temperature and relative air humidity were measured by portable digital devices (PDT 300, "COMARK Div. of Fluke Electronics", USA), as well as the illumination in the level of herbage (illuminometer ELVOS LM 1010, "Elvos GmbH", Germany; measurement range 0-200 thousand lux). Soil moisture was determined in the root zone of 1-25 cm depth (averaged soil samples were taken in 6-10 points along the diagonal of each plot, brought to air-dry state and then finally dried at 130 °C for 30 min.).

Average daily increase in length of shoots was measured in 15-20 typical randomly chosen plants. Growth dynamics of each individual was accounted by the height of its most developed shoots - both vegetative (leaf rosettes with short internodes) and generative (long reproductive). Hourly growth rates of shoots were determined in six plants per each microallotment within 9 days during regrowth and budding; the obtained data were correlated with conditions of lightening, temperature and air humidity.

Variability of PES content was monitored in adult rosette leaves of vegetative shoots. The samples were taken every 3-6 days (two leaves from an individual, 20-25 plants), dried at room temperature and then formed an average sample. The reversed-phase high performance liquid chromatography (HPLC) of PES was performed in the biochemical laboratory of the Botanical Garden of the Institute of Biology Komi SC RAS (Syktyvkar) (14). The content of PES was calculated into the absolutely dry substance.

Mathematical processing of data was performed by standard statistical methods. To assess the results, the authors used an arithmetic mean ($x$), correlation coefficient ($r$) and their confidence levels ($\rho$), as well as the quartic smoothed (approximated) curves of experimental data with determined reliability of approximation ($R^2$ on the figures).

*Results. Development of R. carthamoides in different climatic zones.* The data on cultivation of *Leuzea* in different regions indicate that heat supply and soil fertility don't affect the formation of plant productivity. For example, at latitudes 52° (Baltic), 55-57° (Tomsk and Novosibirsk provinces) and 61-62° (Karelia and Komi republic), the sum of active temperatures above 10 °C differed in 1,5-1,8 times ( from 2100-2300 to 1400-1500 °C), humus content – in 2-3 times (from 5-9% to 2-3%) (15), while the phytomass production of 3-4-years-old plants grown in these areas didn't differ and varied from 55-60 to 81-86 g for aboveground parts and from 58-60 to 76-91 g - for underground organs (1, 12).

The author cultivated *R. carthamoides* during 4 years in central Poland (53 ° N) and in the European North-Eastern part of Russia (62 ° N) using a single technique. The comparative study of these two agropopulations revealed the identity of growth processes and development of plants in these two climatic zones – individuals of equal ages had similar average number of shoots and their height, as well as width of leaf blades 16).

In Poland, the weight of dry aboveground phytomass by the 1st, 2nd, 3rd and 4th years of life amounted to, respectively, 0,23; 7,00; 17,50 and 47,80 g; the close values were obtained in the European North of Russia - respectively, 0,26; 6,20; 16,40 and 56,80 g.

The phytomass of underground organs (roots and rhizomes) on the 3rd and 4th years were, respectively, 16,80-29,50 and 11,90 -38,20 g. The content of PES in adult leaves averaged to 0,036% in immature plants grown in Poland and 0,18% - in virginile plants; in the European North these values were, respectively, - 0,17-0,19 and 0,22%.

*Growth responses to temperature, light and soil moisture. Resistance to negative temperatures.* The mass regrowth of *Leuzea* vegetative shoots was observed in the period between April 17 and May 12 depending on weather conditions of the year (1990-2008), and a visible growth of generative shoots started in 5-7 days later. In this period, cold weather often returns with snowfall and repeated freezing. *R. carthamoides* withstanded negative temperatures (up to –5 °C) without visible damage. At – 8... – 10 °C, apical growth zones of leaves (top of leaf blade - 1,5½2,0 cm) got hurt; 4-5 days later, the damaged areas were restored with newly formed tissue. The short-term night frosts in April-May (–7 ... –10 °C) caused an irreversible lesion of apexes of generative shoots: inflorescences blackened and died off. Autumn frosts (–2 ... – 3 °C) in September-October didn't harm leaf rosettes.

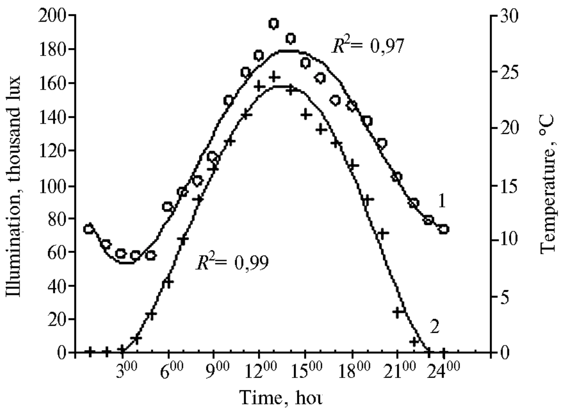


**Fig.1 The diurnal dynamics of temperature** (1) **and light** (2) **conditions during the regrowth of *Rhaponticum carthamoides*.** $R^2$ — reliability of approximation (Arkhangelsk province, subzone of middle taiga, 2003-2004).

*Diurnal cycle of environmental factors*. Growth processes are very sensitive to fluctuations of light, temperature and relative air humidity (17). The dynamics of hourly growth rate of shoots depending on daily cycle of environmental factors was studied in plants aged 3 and 9 years, during their regrowth (II decade of May, daylight length 17-18 h). In this period, the absolute absence of light wasn't detected even at night in cloudless weather. The minimum level of scattered light (10-20 lux) was established at $1^{00}$-$2^{00}$; from $3^{00}$-$4^{00}$ to $12^{00}$-$14^{00}$, illumination was raising to a maximum (156-164 thousand lux ) and then decreased by $21^{00}$-$22^{00}$ to 24,7 thousand lux (Fig. 1). When nebulosity was slight or medium, illumination in the afternoon reduced in 1,5-2,0 times (up to 110-70 thousand lux), as well as in the morning and in the evening - in 3,0-4,0 times (up to 30-40 thousand lux in $9^{00}$ and $18^{00}$).

Temperature pattern showed its minimum (6-10 °C) in early morning ($3^{00}$-$5^{00}$), and its maximum (28-30 °C in a herbage) - at $13^{00}$-$14^{00}$ with a gradual decrease in afternoon (to 20 °C in $19^{00}$, up to 13-11 °C - in $22^{00}$-$24^{00}$). The temperature curve followed the illumination curve at a small inertial shift to the right (see Fig. 1). The correlation between temperature and illumination was negative at night, from $5^{00}$-$6^{00}$ - positive, and during the day ($9^{00}$-$10^{00}$ AM) it was linear (r = 0,94-0,97; ρ = 0,999).

Relative air humidity was changing controversially to dynamics of light and temperature. The correlation between them was negative and high: (ρ = 0,999) — *r* = –0,74… –0,82 for air humidity and illumination, *r* = –0,85… –0,90 for air humidity and temperature.

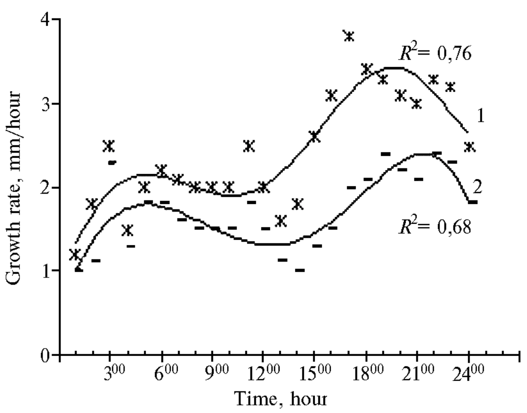


**Fig. 2. The diurnal growth dynamics of vegetative shoots of *Rhaponticum carthamoides* individuals aged 3** (1) **and 9** (2) **years of life.**
$R^2$ — reliability of approximation (Arkhangelsk province, subzone of middle taiga, 2003-2004).

Maximum air humidity (88-90%) was observed in the early morning ($2^{00}$-$8^{00}$), minimum (31-33%) – in afternoon - $14^{00}$-$18^{00}$. In the period from $9^{00}$ to $12^{00}$, relative air humidity gradually decreased from 80-83% to 40-42%, and from $19^{00}$ to $23^{00}$ it was rising from 35 to 80%.

*Hourly growth rates of shoots in a diurnal cycle***.** Growth patterns of vegetative shoots (Fig. 2) had similar trend in plants of different ages, but levels of absolute indices in young 3-years-old (virginile) plants were 1,5-1,7 times smaller than that in 9-years-old late-generative individuals. When air humidity was high, growth processes didn't stop even at night: the increase of about 1 mm/h was recorded in 3-years-old plants, and 1,2-1,8 mm/h - in 9-years-old. Under the scattered light in twilight (2 thousand lux at $3^{00}$), these parameters increased twice, and then tended to slow down under the bright light at $9^{00}$-$10^{00}$ (see Fig. 2). Extreme solar radiation (150-160 thousand lux) and air temperature (28-30 °C) at $13^{00}$-$14^{00}$ inhibited growth of shoots up to the levels comparable to night (1,0-1,1 mm/h in 3-year-old plants and 1,6-1,8 mm/h in 9-year-old).

The greatest increase (2,0-2,6 and 3,0-3,8 mm/h, respectively, for 3 - and 9-years-old plants) was observed in the evening - from $16^{00}$-$17^{00}$ to $22^{00}$-$23^{00}$, when illumination reduced from 120 thousand to 7 thousand lux, air temperature – from 25 to 10-12 °C and relative air humidity increased from 30 to 70-80%.

In general, the obtained data are consistent with results of model experiments performed by T.K. Golovko et al. (18), who have established the point of light compensation (the balance between photosynthesis and $CO_2$-respiration) for *R. carthamoides* equal to 4,4-6,7 W/m$^2$ and the adaptive radiation - 30-50 W/m$^2$, or about 5-10% of a maximum insolation.

The curve of approximated values of correlation between growth rates of shoots and environmental factors revealed that 3-years-old plants manifested higher need in light than the 9-years-old ones ($R^2$ = 0,84 vs. $R^2$ = 0,54 in the diurnal cycle). For the older individuals, realization of their potential depended primarily on optimum air humidity and temperature (respectively, $R^2$ = 0,96 and $R^2$ = 0,92), which may be connected with greater importance of root system for life support in later ages (12).

*Average daily growth rate of shoots*. The development of rosette shoots depending on air temperature and humidity was studied upon mature generative plants aged 6 years (Table 1). At the start of regrowth, significant temperature differences were observed - from 15-18 °C during the day to 2-7 °C in the evening and at night. The relative air humidity was within 56-87%. In this period, daily growth of vegetative shoots averaged to 2,1 cm, insignificantly reducing (up to 1,7 cm/day) at low daytime temperatures (7-10 °C) while the slight oscillations of air humidity were observed (62-73%).

**1. Average daily growth rates of *Rhaponticum carthamoides* vegetative shoots during a vegetation season depending on air temperature and relative air humidity** (Arkhangelsk province, 6-years-old plants, 1995)

| Parameter | Date | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | April, 24 | May, 14 | May, 18 | May, 28 | June, 06 | June, 12 | June, 18 | June, 23 |
| Air temperature (daytime), °C | 15-18 | 0-5[a] | 7-10 | 20-25 | 7-10 | 15-18 | 20-25 | 23-30 |
| Relative air humidity, % | 56-87 | 78-93 | 62-73 | 50-65 | 46-58 | 42-48 | 30-35 | 23-26 |
| Duration of vegetation, days | 7th | 27th | 31st | 41st | 50th | 56th | 62nd | 67th |
| Observation interval, days | 7 | 20 | 4 | 10 | 9 | 6 | 6 | 5 |
| Growth rate of shoots, cm/day | 2,1 | 0,5 | 1,7 | 5,1 | 1,5 | 0,5 | 0,4 | 0,1 |

Note: a - snowfall and ground frost (−2…−6 °C).

The largest increase (5,1 cm/day during the 10-day interval) was recoded at 20-25 °C and air humidity of 50-65%. Under extreme conditions (snowfall and repetitive ground frosts up to – 2 … – 6 °C), growth of shoots didn't stop completely: they elongated about 0,5 cm/day during a short-term daytime warming (3-5 °C). The next period of growth slowdown was II-III decade of June owing to decrease in air humidity from 78-93 % to 30-35% and the resulting moisture deficit in root layer of soil, while air temperature was within the optimum (20-25 °C).

For the fitted curves of dependence "growth of shoots - air humidity", $R^2$ amounted to 0,99, and "air humidity – temperature" $R^2 = 1,00$. However, these positive correlations were reliable only within the limits: air temperature ≤ 25 °C and relative air humidity ≥ 40% ($r =$ 0,93-0,73; $\rho = 0,95$).

Thus, average growth rate of *R. carthamoides* rosette shoots declined more than 10 times (from 5,1 up to 0,4-0,5 cm/day) with decline in relative air humidity and soil moisture reserves during a growing season. Similar results were obtained in Belarus: 4-years-old plants demonstrated growth rates of vegetative shoots in the II decade of May amounted to 4,4 cm/day, and in early June - 0,8 cm/day (19).

*The impact of stressors on PES accumulation.* In stressful situations, medicinal plants use to demonstrate ambiguous changes in contents of biologically active substances in the phytomass (20). During the regrowth of *R. carthamoides*, significant temperature shifts in diurnal cycle (daytime drop from 12-15 to 5 °C, night and morning frosts up to −6 °C) has led to inhibition of growth: dry weight of the leaf aged 9 and 12 days remained almost constant (respectively, 0,12 and 0,14 mg) (Table 2), whereas the total content of PES increased in 1,33 times (from 0,36 to 0,48 mg).

After cessation of frosts, the pool of PES increased 2,06 times (from 48 to 99 mg), and dry weight of leaf - 2,28 times. On the 30th-37th days of vegetation, rapid temperature decline (with short-term night frosts up to −7 ... −10 ° C) affected the production of PES once again: its content increased from 0,31 to 0,43% and total yield - 1,61 times (dry weight of leaf grew only 1,16 times).

**2. The dynamics of phytoecdistheroids (PES) accumulation parameters in *Rhaponticum carthamoides* mature rosette leaves depending on environmental conditions** (Arkhangelsk province, 7-years-old plants, 1996)

| Parameter | Time of vegetation by phenophases, days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | regrowth | | | budding | | | flowering | | fruiting |
| | 2nd | 9th | 12th | 22nd | 30th | 37th | 41st | 57th | 77th |
| Air-dry mass of a leaf, g | 0,02 | 0,12 | 0,14 | 0,32 | 0,37 | 0,49 | 0,60 | 1,43 | 2,11 |
| PES content, % | 0,25 | 0,30 | 0,34 | 0,31 | 0,43 | 0,33 | 0,28 | 0,11 | 0,07 |
| PES total yield, mg/leaf | 0,05 | 0,36 | 0,48 | 0,99 | 1,59 | 1,62 | 1,68 | 1,57 | 1,48 |
| Growth coefficient: | | | | | | | | | |
| - dry mass of a leaf | – | 6,00 | 1,17 | 2,28 | 1,16 | 1,32 | 1,22 | 2,38 | 1,47 |
| - PES pool | – | 7,20 | 1,33 | 2,06 | 1,61 | 1,02 | 1,04 | 0,93 | 0,94 |
| Air temperature, °C | 8-10 | 12-15 | 0-4[a] | 3-10 | 0-5[b] | 15-25 | 20-30 | 25-35[c] | 20-25 |
| Relative air humidity, % | 65-90 | 56-87 | 78-93 | 56-78 | 62-73 | 46-58 | 38-48 | 27-32 | 22-28 |
| Soil moisture, % | 23,4 | 17,3 | 16,4 | 14,7 | 12,8 | 12,7 | 9,4 | 2,5 | 6,3 |

Note: a – repetitive frosts up to −6…−8 °C; b — night frosts up to −7…−10 °C; c — draught with g temperature up to 48-52 °C on soil surface; dashes – observation of the process wasn't performed.

Daytime heat (37th and 41st days of vegetation) inhibited accumulation of PES (see Table 2.). During the draught accompanied by water stress (days 57th - 77th: air temperature reached 35 °C and relative air humidity reduced to 23-27%) (see Table 2), young rosette leaves lost turgor, apexes of generative shoots got yellow and withered. In this period, moisture content in soil reduced to 2,5-3,0% of its dry mass – the level close to the minimum moisture capacity with rupture of capillaries in arable layer of sandy soil (21).

In contrast to low temperature, soil moisture deficit didn't stimulate the raise of ecdysteroids content in leaves: PES content sharply decreased to 0,11% (57th day of vegetation), and then to 0,07% (77th day). The negative trend of PES accumulation (coefficient - 0,93-0,94) and PES total content in leaves were observed. It has been found a reliable ($\rho$= 0,99) negative correlation between the accumulation of PES and soil moisture on the 57th -77th days of vegetation ($r =$ − 0,88 ... −0,92).

Similar data on multi-directional effects of temperature and water stress in alkaloid-synthesizing plants were obtained by other authors (20). In field experiments on plume poppy (*Macleaya cordata*), the total content of active substances (sanguinarine and heleritrin) in leaves didn't reduce after repetitive November compared with the level of September, while yellow hornpoppy (*Glaucium flavum*) demonstrated 16% decrease in the content of claucine after the short-term reduce of soil moisture in 2,5 times (up to 40% of total water capacity) occurred in 7 days before the harvest.

According to S.S. Shahin, the prolonged exposure of Alexandrian Senna (*Cassia acutifolia*) to water stress (20-days drought; the VILAR experiment station, Shymkent region of Kazakhstan) resulted in decrease of content of anthracene derivatives by 55% (20).

So, heat supply and soil fertility in the region of cultivation *Leuzea* didn't cause a significant impact on productivity and biosynthesis of phytoecdysteroids (PES) – the phytomass of plants grown on poor podzolic soil in the cool climate of European North didn't yield to those cultivated on rich soil of southern regions. In early spring with low temperature and optimal humidity, growth rate of shoots was greater than that in summer and growth processes continued even at night. The greatest increase was observed in the evening (from $17^{00}$ to $22^{00}$-$23^{00}$). Extreme illumination (150-160 thousand lux) and temperature conditions (25-30 °C and above) inhibited growth of shoots. Optimum combination of air temperature, relative air humidity, illumination and soil moisture were, respectively, 12-25 °C, 40-80%, 7-120 thousand lux, and 9-16%. Growth rates of plants aged 9 years were 1,5-1,7 times higher than that of 3-years-old. Young plants demand more light, while the mature generative individuals depend on optimal soil moisture - this fact necessitates the study of root system role in the species' potential of PES biosynthesis. Univariate effect of low and negative temperatures was manifested as slowdown of shoot growth, as well as the raise of PES content and PES total yield in leaves (in 1,3-2,1 times). Multi-factorial effects of water stress (accompanied with high air temperature at poor soil moisture and low air humidity) caused an opposite result - biosynthesis cessation and PES outflow from aboveground organs.

*The author thanks the senior staff scientist of biochemical laboratory of the Botanical garden of the Institute of Biology Komi SC UB RAS, Cand. Sci. (Chem.) Punegov V.V. for his assistance in HPLP of phytoecdysteroids.*

REFERENCES

1. Timofeev N.P. Achievements and Problems in Investigation of Biology in Medicinal Herbs of *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L., *S.-kh. biol*., 2007, no. 3, pp. 3-17.
2. Maslov L.N. and Guzarova N.V., Cardiotropic and Antiarrhythmic Properties of Preparations from *Leuzea carthamoides*, *Aralia mandshurica*, *Eleutherococcus senticosus, Eksp. i klin. farmakol.,* 2007, vol. 70, no. 6, pp. 48-54.
3. Gaube F., Wolfl S., Pusch L., Werner U., Kroll T.C., Schrenk D., Hartmann R.W. and Hamburger M., Effects of *Leuzea carthamoides* on Human Breast Adenocarcinoma MCF-7 Cells Determined by Gene Expression Profiling and Functional Assays, *Planta Med*., 2008, vol. 74, pp. 14, pp. 1701-1708.
4. Bathori M. and Pongracz Z., Phytoecdysteroids — from Isolation to Their Effects on Humans, *Cur. Med. Chem*., 2005, no. 12, pp. 153-172.
5. Dinan L. and Lafont R., Effects and Applications of Arthropod Steroid Hormones (Ecdysteroids) in Mammals, *J. Endocrinol*., 2006, vol. 191, pp. 1-8.
6. Timoveef N.P., Achievements and Problems in Investigation, Application and Prognostication of Biological Activity of Ecdysteroids, *Butlerovskie soobscheniya,* 2006, vol. 8, no. 2, pp. 7-34.
7. Gorelick-Feldman J., Maclean D., Ilic N., Poulev A., Lila M.A., Cheng D. and Raskin I., Phytoecdysteroids Increase Protein Synthesis in Skeletal Muscle Cells, *J. Agric. Food Chem*., 2008, vol. 56, pp. 10, pp. 3532-3537.
8. Timofeev N.P., Volodin V.V. and Frolov Yu.M., Distribution of 20-hydroecdyzone in the Aboveground Biomass of *Rhaponticum carthamoides* (Willd.) Iljin, *Rastitel'nye resursy*, 1998, vol. 34, no. 3, pp. 63-69.
9. Bakrim A., Maria A., Sayah F., Lafont R. and Takvorian N., Ecdysteroids in Spinach (*Spinacia oleracea* L.): Biosynthesis, Transport and Regulation of Levels, *Plant Physiol. Biochem.,* 2008, vol. 46, no. 10, pp. 844-854.
10. Timofeev N.P., Accumulation and Variability of the Ecdysteroids Content in Medicinal Raw Material of *Rhaponticum carthamoides*, *S.-kh. biol.,* 2009, no. 1, pp. 106-117.
11. Karnachuk R.A., Protasova N.N., Dobrovol'skii M.V., Revina T.A. and Nichiporovich A.A., Physiological Adaptation of Leuzea Leaves to Light Spectrum, *Fiziol. rast*., 1987, vol. 34, no. 1, pp. 51-59.
12. Timofeev N.P., Productivity and Dynamics of Phytoecdysteroids Content in Agropopulations of *Rhaponticum carthamoides* and *Serratula coronata* (*Asteraceae*) in European North, *Rastitel'nye resursy*, 2006, vol. 42, no. 2, pp. 17-36.
13. *Prirodno-klimaticheskii ocherk Kotlasskogo rajona Arkhangel'skoj oblasti* (Essay on Nature and Climate of Kotlas Region of Arkhangelsk Province), Moscow, 1994.
14. Punegov V.V. and Savinovskaya N.S., The Method of Internal Standard for Determining Ecdysteroids in Plant Raw Material and in Pharmaceuticals Using HPLC, *Plant Resources,* 2001, vol. 37, no. 1, pp. 97-102.
15. *Spravochnik agronoma Nechernozemnoi zony* (Reference Manual for Agromist of the Non-Chernozem Zone), Gulyaev G.V., Ed., Moscow, 1990.
16. Timoveev N.P., Kowalski P. and Krywuch J., Comparative Results of Cultivation *Rhaponticum carthamoides* (Willd.) Iljin during 4 Years in Conditions of Poland and the European North of Russia, in *Lekarstvennye rastenija i biologicheski aktivnye veschestva: fitoterapiya, farmatsiya i farmakologiya* (Medicinal Plants and Biologically Active Substances: Phytotherapy, Pharmacy and Pharmacology. Compilation of Sci. Works), Belgorod, 2008, pp. 264-267.
17. Shevelukha V.S., *Rost rastenii i ego regulyatsiya v ontogenese* (Plant Growth and Its Regulation in Ontogeny), Moscow, 1992.
18. Golovko T.K., Garmash E.V., Kurenkova S.V., Tabalenkova G.N. and Frolov Yu.M., *Rapontik saflorovidnyj v kul'ture na evropejskom Severe-Vostoke (jekologo-fiziologicheskie issledovanija)* (Cultivation of *Rhaponticum carthamoides* in the European North-East: Ecological and Physiological Studies), Syktyvkar, 1996.
19. Boreisha M.S., Semenov B.Ya. and Chekalinskaya I.I., *Maralij koren'(Raponticum saflorovidnyi)* (Maral Root *Rhaponticum carthamoides)*, Minsk, 1985.
20. Shain S.S., *Bioregulyatsia produktivnosti rastenii* (Bioregulation of Plant Productivity), Moscow, 2005.
21. Kulakovskaya T.N., *Optimizatsiya agrokhimicheskoi sistemy pochvennogo pitaniya rastenii* (Optimization of Plants' Agrochemical Soil Nutrition System), Moscow, 1990.