

# Bioecological Characteristics and Natural Resources of *Rhaponticum integrifolium* in Uzbekistan

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## Abstract

The large life cycle *Rhaponticum integrifolium* in natural populations (Qashqadaryo, Uzbekistan) was studied. Duration of before generative, generative and senile periods of *Rhaponticum integrifolium* is 2 - 5, 8 - 10 and 2 - 4 years respectively. The beginning of phenophases depends on the height of the terrain above sea level: raising the terrain from 400 to 1500 m leads to a delay in the onset of phenophases. Age states of *Rhaponticum integrifolium* in different ecological and phylogenetic conditions were revealed. Updated distribution of detected participation of vegetation and the raw *Rhaponticum integrifolium* in Uzbekistan, describes the dynamics of the contents ecdysteroids in the periods of vegetation. At the same time, a map of the species area in Central Asia was made and the term of its phytomass recovery under different operating modes of thickets was determined.

## Keywords

*Rhaponticum integrifolium*, Ecdysteroids, Ontogenesis, Seed Production, Yield, Reserves in Uzbekistan

## 1. Introduction

In the world, more than 3000 kinds of natural remedies are used in the treatment of diseases that are dangerous to human health, and 35% of them are plant materials. In this regard, the large-scale use of ecdysteroids in modern medicine requires the identification of plant species containing their derivatives and an assessment of the state of their raw materials.

Today in the world ecdysteroid-containing plants are not grown on a large scale at a level that satisfies the needs of the pharmaceutical industry. Of the

numerous botanical, morphological and ecological studies in various climatic conditions, it was found that for the production of ecdysteroid-containing preparations, the introduction of species of the genus *Rhaponticum* containing phytoecdysteroids in large quantities has an economic value from an economic point of view [1].

In modern scientific medicine ecdysteroids with a natural composition are used for disorders of the cardiovascular, central nervous and reproductive systems. In addition, they are used for mental and physical fatigue as an encouraging and stimulant, for loss of working capacity, for impotence and for weakening the functions of various organs [2] [3].

95% of the representatives of the flora of the whole world have traces of ecdysteroids, while 4% - 6% of them are contained in 0.001% - 0.0001% of the amount, and only in a few species in certain developmental phases in the composition of some aboveground organs can the amount of ecdysteroids be 1% - 3% [4] [5] [6] [7].

The genus *Rhaponticum* includes 17 species, distributed in a narrow and almost continuous ribbon-like form in the Northern Hemisphere from the Atlantic Ocean to the shores of the Pacific Ocean, only 1 species is common in Australia (Camelyn, 1973). On the territory of Central Asia, there are 9 species of the genus *Rhaponticum* [8] [9]. Today, 9 types of the genus *Rhaponticum* are used in pharmacy and medicine [10] [11] [12].

In the countries of the former USSR, the only source of phytoecdysteroids based tonic preparations was (and remains) *Rhaponticum carthamoides* (Willd.) Iljin (*Leuzea carthamoides* (Willd.) DC.). Medicinal raw materials of this species, widespread in Russia in the mountains of Southern Siberia, are rhizomes with roots.

Eight species of the genus *Rhaponticum* grow in Central Asia, including *Rh. integrifolium* C. Winkl. [9], from the leaves, stems and baskets of which phytoecdysteroids  $\alpha$ -ecdysone, 20-hydroxy ecdysone, integristerone A and 24 (28)-dehydromaksisterone A were isolated [13] [14] [15], which have anabolic and immunostimulating properties [15] [16].

In this regard, for the Republic of Uzbekistan is the actual problem of the possibility of substitution of raw materials *Rh. carthamoides* for raw materials *Rh. integrifolium* as a source of phytoecdysteroids.

*Rh. integrifolium* is found in the Western Pamir-Alai mountains—in the Zeravshan, Gissar, Kartegin, Darvaz, Petr-I, Babatau, Fergana ranges and South Tajikistan low mountains. The aboveground plant organs contain 0.3% ecdysterone, 0.1% in stems and 0.1% in leaves [12] [17] [18] [19] [20]. Scientists of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan have proved the equivalent of ecdysterone isolated from the aerial part of *Rh. integrifolium* with ecdysterone *Rh. carthamoides* brought from Russia [15] [16].

In addition to the biochemical composition, resources and distribution in the CIS countries, breeding technologies and methods for growing *Rh* have also

been developed. *carthamoides* in different soil and climatic conditions. However, today bioecological features of *Rh. integrifolium* in Uzbekistan is not well understood. On this basis, the study of ontogenesis and seed productivity of a plant, the substantiation of the variability of the number of ecdysteroids in different parts of a plant, the assessment of the role of a plant in vegetation and natural resources acquire an important scientific and practical importance.

From the analysis of literary sources we can say that in addition to the biochemical composition, resources and distribution in the CIS countries, breeding technologies and methods of growing *Rh. carthamoides* are also perfectly developed in different soil and climatic conditions. However, to date, the bioecological features of *Rh. integrifolium* have not been sufficiently studied in Uzbekistan. Based on this, the study of ontogenesis and seed production of plants, the rationale for the variability of the number of ecdysteroids in different parts of the plant, the assessment of the role of plants in the vegetation and natural resources is practical importance.

## 2. Material and Methods

The work was carried out from 2005 to 2010. For the mapping of the range of *R. integrifolium*, materials from the Central Herbarium of Uzbekistan, hernia BIN them. V.L. Komarov, Moscow State University M. V. Lomonosov, the Institute of Botanists of Tajikistan and its own fees, carried out during the expeditionary work. On the territory of Uzbekistan (Kashkadarya region), we studied meeting, association with certain plant communities and the abundance of *R. integrifolium*. Determination of yields and stocks of the aerial parts was carried out according to generally accepted methods [21].

Complete ontogeny of *Rh. integrifolium* was studied under planting-grown conditions in the territory of Chodak (Namangan region) and Gilan (Kashkadarya region) in 2001-2018. When studying the complete ontogenesis of *Rh. integrifolium* used methods TA Rabotnova [22] [23]. The study of the rhythm of seasonal plant development was carried out by the methods of I.V. Borisova [24] and I.N. Beideman [25]. Seed productivity was identified based on the recommendations of I.V. Vainagius [26] and V.F. Ramanovich [27]. In determining the natural resources of the plant (Gilan), methods for studying the resources of medicinal and useful wild plants were applied [21] [28] [29] [30].

Phytomass of the aboveground part of one specimen was determined by the method of model specimens, dividing them in height into small (up to 60 cm), medium (60 to 100 cm) and large (120 to 150 cm) individuals. The calculation of the number and determination of the specific raw phytomass was carried out at key sites, where 10 accounting sites of 10 m<sup>2</sup> were laid. It weighed 25 individuals from each site. The obtained data were processed statistically [29].

Determination of the areas of thickets was carried out by the route method using cartographic material and data of forestry workers, the reserve and local residents.

To determine the timing of the recovery of phytomass after its harvesting, experiments were conducted on sites of 10 m<sup>2</sup> on the slopes of the southern macroslope of the Hissar ridge in the village Gilon. The aboveground part was cut at a height of 5 - 6 cm from the soil surface in the flowering phase. The experiments included several variants differing in the mode of operation: I—two years of “rest” after cutting shoots, II—one year of “rest” after cutting shoots, III—annual cutting shoots, control (without cutting shoots).

Quantitative determination of 20-hydroxyecdysone in the studied plant was carried out by HPLC. The material for the analysis was underground (root) and aboveground (stems and leaves) parts and fruits of plants growing in the vicinity of Gilon village of Kashkadarya region, as well as cultivated in Ferghana region (Chodak village), collected in the first decade of May-July 2007 in the second year of life [15].

Chromatography was carried out at room temperature for VEZH chromatograph Agilent 1100 brand. Detection at a wavelength of 247 nm (UV). Water and acetonitrile in different ratios were used as the mobile phase for HPLC. The analysis time of 45 min, flow Rate 0.5 ml/min, volume of injected sample 20 mkl. The measurements were carried out using a HPLC chromatograph equipped with a gradient pump and a variable-wave UV detector. Column-Zorbax Eclipse XDB-C18, inner diameter 3 mm, length 15 cm, sorbent dispersion 3.5 µm or similar; micro-particle 20 µl; laboratory scales of General purpose and standard according to GOST 24104; methanol for chromatography “Merck” or similar; acetonitrile for chromatography “Merck” or similar; ethyl rectification alcohol-technical according to GOST 18300; volumetric flasks with a capacity of 25 cm<sup>3</sup> according to GOST 23932.

### 3. Results and Discussion

#### 3.1. Ontogeny of *Rhaponticum integrifolium*

*Rhaponticum integrifolium* is a perennial often herbaceous plant. Stem 60 - 150 cm (**Figure 1**). Basket spherical 4.5 - 6.0 cm in diameter. Corollas pale yellowish, at the end of flowering pale orange. Achenes are inversely ovate, glabrous, milk-white. The bristles of the tuft with grooves exceeding the diameter of the bristle more than 3 - 4 times, cream. Length 0.8 - 1.0 cm, width 0.3 - 0.5 cm.

LATENT PERIOD. Seed sizes common in different geographical latitudes and longitudes of individuals *Rh. integrifolium* differ sharply from each other. (Chodak: length  $0.9 \pm 0.25$  cm, width  $0.25 \pm 0.11$  cm, weight of 1000 seeds  $22.5 \pm 0.67$  g; Gilan: length on average  $1.1 \pm 0.15$  cm, width  $0.37 \pm 0.13$  cm, weight of 1000 seeds  $23.5 \pm 0.94$  g) (**Table 1**).

In laboratory conditions, the temperature increase from 18°C to 28°C has a positive effect on the germination of *Rh. integrifolium* seeds. It was found that the germination of plant seeds is very sensitive to the process of stratification (27% - 33%). In the field, the maximum germination of *Rh. integrifolium* seeds is observed when planting in October-November in a mechanically processed,

refrigerated form (68% - 75%). In such conditions, the seed germinate 20 - 25 days earlier compared to untreated seeds in favorable weather conditions and forms the root system to increase air temperature and drainage of 10 - 20 cm of the soil horizon.

**VIRGINALLY PERIOD.** 1-year. *Stage sprout (p)*. The germination of *Rh. integrifolium* aboveground, from the seed germinates primary (main) root. After reaching the length of the root to 1.5 - 2 cm, rapidly developing cotyledon leaves rise to the soil surface. The length of the new cotyledon leaves 1.5 - 2 cm, width 0.6 - 0.7 cm.

From the first time, plants 20-25 April formed true leaves in the middle of cotyledon leaves. By this time, the root system of the seedling is formed and reaches 6 - 9 cm. In natural conditions, 2% - 3% of the seedlings are preserved. However, in the experimental plots with the help of appropriate agrotechnical measures, this figure is achieved up to 80% - 85%.



**Figure 1.** *Rhaponticum integrifolium*.

**Table 1.** Germination of *Rhaponticum integrifolium* seeds in different laboratory conditions.

Experience options	Temperature, t °C		
	18 - 20	20 - 24	24 - 28
No treatment	10.5 ± 0.33	11.5 ± 0.33	18.5 ± 0.62
Stored in the refrigerator (0°C - 5°C, 50 day)	10.5 ± 0.54	15.3 ± 0.59	27.2 ± 0.66
Stored in the freezer (-1°C - 3°C, 50 day)	25.3 ± 0.61	31.1 ± 1.10	33.4 ± 0.81
10 minutes treated with 1% H <sub>2</sub> SO <sub>4</sub> solution	8.90 ± 0.33	8.40 ± 0.61	9.10 ± 0.66
10 minutes treated with 1% HCl solution	9.60 ± 0.82	9.10 ± 0.84	13.2 ± 0.33
10 minutes treated with 1% HNO <sub>3</sub> solution	10.3 ± 0.66	12.6 ± 0.36	17.7 ± 0.81
Scarification	80.4 ± 0.75	90.5 ± 0.33	91.4 ± 0.33

*Juvenile stage (j)*. The plant passes to the juvenile stage in 20 - 24 days. This stage is justified by the formation of 6 - 10 basal leaves, the emergence of a new underground organ—the stem, the active formation of roots and the formation of dormant buds at the root. At the beginning of the stage, after 3 - 5 days after the formation of the first coming leaf, the second real leaves appear. Further formed leaves *Rh. integrifolium* compared with the previous leaves differ in the size of the petiole and leaf blade and accelerated development.

At the juvenile stage, one of the important morphological changes observed in the plant is the thickening of hypocotyl and the formation of additional roots formed in the internal tissues. At the same time, an underground organ is formed—a stem with a new functional structure. At the juvenile stage in the aboveground part of the stem, in the lower center of the leaf petioles 2 - 6 cone-shaped buds are formed-developing in the spring of next year.

Vegetation *Rh. integrifolium* continues from the end of July to the first decade of August and ends its vegetation with the formation of up to 6 - 7 basal leaves in each individual.

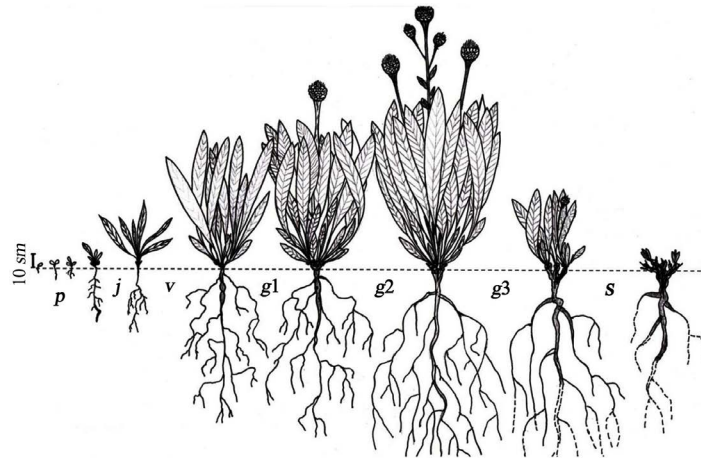
During the studies in the first year of vegetation *Rh. integrifolium* no signs characteristic of the immature stage were observed. In our opinion, the absence of the immature stage in the conditions of Uzbekistan in the first year of vegetation is associated with the manifestation of morpho-biological adaptations to arid conditions of the territory with the acceleration of the stages of ontogenesis of plants in hot weather conditions. Such morpho-biological adaptation is also manifested by the activity of above-ground and underground parts of plants growing in the conditions of Chodak (1000 - 1200 m above sea level) and Gilan (2200 - 2400 m above sea level) In particular, the plants growing in the experimental areas of Gilan are characterized by the active development of underground organs, and growing on the territory of Chodak—by the active development of above-ground organs—leaves, in addition to the number of regenerating buds.

*2-year. Virginile stage (v)*. In the great life cycle of *Rh. integrifolium*, *virginile* or mature stage lasts up to 3 years (**Table 2**). The stage is characterized by the complete formation of vegetative organs, increased biomass, the formation of these rosette leaves from the stem and the strong development of the root system (**Figure 2**).

The appearance of tuberous extensions 12 - 15 cm of the main root in the third year virgin stage is of particular importance. Tuberous part of the root remains till the end of the year virgin stage and 2-phase generative period disappears as a result of intensive development of the main root.

GENERATIVE PERIOD. *Young generative plants (g1)*. The first renal escape *Rh. integrifolium* passes to the generative period in the second year of vegetation. If we compare with the number of individuals, in the third year of vegetation 7% - 8% of plants pass to the generative period, in the fourth year 75% - 80%, 5-year 10% - 12% (**Figure 2, Table 2**).





**Figure 2.** Stages of ontogenesis of *Rhaponticum integrifolium*. p—stage sprout; j—juvenile stage; v—virginile stage; young (g1), middle (g2), old (g3) generative plants, s—senile period.

**Table 2.** The change in morphometric parameters *Rhaponticum integrifolium* in virginile (v), generative (g1, g2, g3) and senile (ss, s) periods, Gilan/Chodak.

Indicators	Unit	Years of vegetation						
		II	III	IV	V	VI-X	XI-XIV	XV-XVIII
<i>Stages of ontogenesis</i>		v	v-g1	v-g1	g1-g2	g2	g3-s	ss-s
<b>Aboveground part</b>								
Rosette leaves: diameter	cm	0.9/1.1	1.5/1.9	2.3/2.7	3.1/3.6	3.9/4.3	4.1/4.6	4.3/4.7
number of leaves	piece	12.4/12.1	15.7/18.3	25.8/37.8	26.2/31.7	35.4/38.3	18.5/26.4	4.3/4.7
Shoots. total	шт.	1.8/2.3	4.1/4.5	4.3/5.6	6.4/8.2	12.4/13.8	4.6/3.3	2.3/2.6
of these generative:	шт.	-/-	1/1	1/1	1.3/2.7	7.8/9.5	1.0/1.0	-/-
Stocking kidneys:	шт.	6.1/8.5	6.6/10.1	10.2/12.6	12.4/16.4	16.1/18.6	4.4/6.2	-/-
Vegetative shoot: height	cm	52.7/65.7	55.1/70.4	62.8/75.6	65.4/78.6	84.8/98.3	22.1/23.8	8.6/9.3
the diameter of the main part	cm	0.6/0.7	0.8/0.8	1.1/1.3	1.2/1.5	1.2/1.5	1.3/1.6	1.3/1.6
Generative shoot: height	cm	-/-	21.7/28.2	75.1/90.4	80.8/95.1	102.4/110.5	19.5/18.8	-/-
the diameter of the main part	cm	-/-	0.5/0.5	0.5/0.6	0.8/0.9	0.8/0.9	0.8/0.9	-/-
Leaf blade: width	cm	3.5/3.9	4.1/4.8	4.4/4.8	4.9/5.3	6.1/7.4	3.6/3.4	2.5/2.5
Above-ground dry mass	gr	37.2/48.8	76.4/85.1	91.1/112.4	124.0/156.5	220.5/310.0	31.3/35.5	-/-
<b>Root system</b>								
Main root: length	cm	39.4/26.7	58.3/41.6	96.6/76.4	122.7/95.4	131.4/110.6	111.9/98.1	65.3/34.8
the diameter of the main part	cm	0.5/0.4	0.7/0.5	0.9/0.7	1.3/1.1	2.6/2.1	2.8/2.4	3.0/2.5
Tuberous part of the root: length	cm	-/-	4.5/2.5	7.5/4.5	9.5/6.5	-/-	-/-	-/-
depth of visit	cm	-/-	15.6/12.1	16.5/13.8	17.3/14.0	-/-	-/-	-/-
Number of lateral roots (d > 1 mm)	piece	-/-	9.8/6.5	15.1/10.4	18.0/18.4	24.6/25.2	12.1/8.6	>10
length	cm	-/-	26.2/19.0	39.5/27.5	52.3/38.3	61.0/48.3	>8	>8
Number of partikula	piece	-/-	-/-	-/-	-/-	1.3/0.9	3.2/1.8	3.5/2.3
Underground dry mass	gr	3.5/2.0	22.2/14.8	34.5/26.1	48.5/33.2	66.6/51.1	53.6/46.4	>40

Most generative shoots formed in the third year of vegetation do not pass the full cycle of development. In most individuals, there is a weakening of the development of internodes in the early stages of generative shoots, which leads to drying of inflorescences. Only a small number of generative shoots 10% - 15% can reach the flowering phase. On experimental plots Gilan and Chodak for 3 - 4 years of vegetation in each plant is formed by 1 generative shoot. If the length of the generative shoot formed in the first year of vegetation is low (20 - 28 cm), then in subsequent years it exceeds (70 - 90 cm) the vegetative shoot (**Table 2**). One of the main features of the first generative stage is the increase in the number of storing kidneys. Their increase is observed, especially in the 4th year of vegetation, which ensures the transition of plants to the Mature generative stage (*g2*).

*Middle-aged generative plants (g2).* *Rh. integrifolium* from 5 years of vegetation goes to the generative mature stage and lasts up to 10 years of growth. A 5-year vegetation 40% - 45% of the plants, and 6 - 7 year growing season 100% of plants are move on to a full generative mature stage. At the same time, up to 6 - 10 generative shoots can be formed. In the 2 - 4 upper nodes of the Central generative shoot, the formation of 2 generative branches and the formation of additional inflorescences in them is observed. However, the inflorescences formed in the Central generative shoot differ in a smaller volume from the other inflorescences (**Table 2, Figure 2**). Despite the maximum value of all the morphological parameters of the *Rh. integrifolium*, at the mature generative stage, the formation of particulates begins, which determines the transition of the plant to the senile period. At the *g2* stage, 10% - 15% of plants form a particula.

*Old generative plants (g3).* To 9, and especially 10 - 11 year vegetation of *Rh. integrifolium*, weakens the formation of vegetative and generative organs. Reducing the number of rosette leaves, buds, generative shoots, reducing the volume of inflorescences and leaf blade and most importantly—the rotting of the root indicate the transition of the plant to the old generative stage. At this stage *Rh. integrifolium* begins its vegetation 15 - 20 days later in comparison with early vegetating plants and the formation of generative shoots is carried out at the expense of old generative polycyclic shoots. They number no more than 1 piece, and the height is much lower (18 - 20 cm) than vegetative shoots (**Table 2, Figure 2**).

In the old generative age, the formation of particulates is accelerated and is 2 - 3 pieces. In *Rh. integrifolium* the old generative stage lasts 3 - 4 years. Despite the weakening of all morphological features, plants at this stage are characterized by an increase in the diameter of the outlet from the aboveground part and the diameter of the base of the main root, in addition, this trend continues almost until the end of the senile period.

Senile period. The previous stage of the senile period(s)—the *subsenile stage (ss)* begins in 13 - 14 years of vegetation *Rh. integrifolium* and accelerated in the 15th year of vegetation. At the same time, the habitus of the plants is highly re-



duced, the flange is thickened at the expense of the partucula and the top of the main root is also being consolidated. Usually *Rh. integrifolium* finishes its vegetation in 17 - 18 years, but some individuals of the plant die in 20 - 21 years.

### 3.2. Seasonal Development and Seed Production of *Rhapticum integrifolium*

General vegetation *Rh. integrifolium* on the experimental plot Gilan equal 125 - 135 days, and on the experimental plot Chodak 100 - 115 days. For budding will require 10 - 15 days of the growing season, flowering is 24 - 26 days (Chodak 20 - 21 days), for the formation of seeds 30 - 35 days (the Chodak—25 - 30 days) for maturation of seeds 25 - 32 days (Chodak 20 - 22 days).

The beginning, end and duration of phenological phases of the plant are associated with air temperature, soil moisture and ontogenesis periods. Air temperature rise in relatively flat areas in 1000 - 1200 m above sea level affect the reduction of phenological phases (Chodak) or wet weather and relatively low temperatures of 2200 - 2400 m above sea level affect the duration of phenological phases (Gilan). In addition, with the approach to the middle age period of the plant there is a relatively early start of phenological phases and this is probably due to the characteristic biological characteristics of the plant.

The real seed productivity (RSP) *Rh. integrifolium* is 2 - 4 times less than the potential seed production (PSP), which indicates the ability to reproduce in adverse conditions. If we compare the plants in the experimental plots Chodak and Gilan (Table 3), we can see high rates of RSP in the g1 stage in plants growing on the Chodak, g2 stage in plants on the territory of Gilan.

Quality seed ripening *Rh. integrifolium* observed on site Gilan. This is due to the high coefficient of productivity in all generative stages compared with plants growing on the territory of Chodak (Table 2). The high coefficient of productivity of plant seeds in the territory of Gilan confirms that *Rh. integrifolium* grows in optimal conditions of its area.

**Table 3.** Indicators of seed production *Rhapticum integrifolium* (n = 25).

Indicators	Gilan	Chodak	Gilan	Chodak	Gilan	Chodak
	$g_1$		$g_2$		$g_3$	
Number of inflorescence, piece	1.3 ± 0.1	2.7 ± 0.1	9.9 ± 0.4	11.5 ± 0.4	0.9 ± 0.2	1.1 ± 0.1
Number of flowers, pieces	124.8 ± 2.8	141.4 ± 3.6	157.1 ± 7.5	185.5 ± 8.9	95.6 ± 4.5	110.3 ± 5.1
Number of seeds, pieces	44.2 ± 1.3	36.6 ± 1.5	61.7 ± 2.4	49.3 ± 1.5	22.6 ± 1.2	14.7 ± 0.8
... from them ripe, pieces	36.2 ± 0.9	29.3 ± 1.2	54.2 ± 1.6	41.4 ± 0.9	6.5 ± 0.2	2.9 ± 0.1
Weight of 1000 seeds, gr	21.6 ± 1.4	20.8 ± 1.1	23.5 ± 1.1	22.5 ± 1.4	10.8 ± 0.3	11.2 ± 0.2
PSP	162.2 ± 6.1	381.8 ± 11.4	1555.3 ± 32.1	2133.3 ± 28.2	86.0 ± 1.7	121.3 ± 2.1
RSP	47.1 ± 1.3	79.1 ± 2.7	536.6 ± 8.2	476.1 ± 8.5	5.9 ± 0.3	3.2 ± 0.3
Coefficient of productivity, %	29.0	20.7	34.5	22.3	6.7	2.6

### 3.3. The Value of *Rhaponticum integrifolium* in Plant Cover and Natural Resources

In Uzbekistan the location of *Rh. integrifolium* observed in Surkhandarinskaya region (mountain of Hojagurgurata), Boysun mountains (the village Gilon and Kul), on the slopes of the Gissar and Turkestan ranges (neighborhood villages of Urgut and Madm), in Tajikistan—on the slopes of the Gissar (lane Anzob, Khodjdobigarm, a neighborhood of village Tagob, surrounds the cities of Kulyab, Shurabad) and Darvaz ranges (neighborhood villages Tavildar, Kildare and Sagirdasht). In Kyrgyzstan, the *Rh. integrifolium* habitat occupies the North-Western part of the Fergana ridge and the slopes of mount Baubashata (gorge of the rivers Kugart and Shaydansay).

Sh.G. Ganiev (1980) for the first time described only two associations involving *Rh. integrifolium* in the North-East of the area (Kyrgyzstan) and identified reserves of the aboveground part of the plant on an area of 25 hectares, which were: leaves—23 centner, inflorescences—5, roots—10 centner.

We studied the role of *Rh. integrifolium* in the composition of vegetation on the slopes of Gissar mountain ridge, within the Kashkadarya region; described 11 associations in which boljshegruzy Manchurian participates as edificatory or sub edificatory in the composition of the formations of the *Acer turkestanicum*, *Amygdalis bucharica* and *Rhaponticum integrifolium*:

I ACER TURKESTANICUM FORMATION. Associations: 1.1. *Acer turkestanica* + *Agropyron trichophorum* + *Rhaponticum integrifolium* + *Prangos pabularia*; 1.2. *Acer turkestanica* + *Rhaponticum integrifolium* + *Eremurus regelii* + *Rheum maximowiczii*; 1.3. *Acer turkestanica* + *Rhaponticum integrifolium*.

II AMYGDALIS BUCARICA FORMATION. Associations: 2.1. *Amygdalis bucharica* + *Prangos pabularia* + *Rhaponticum integrifolium* + *Ferula kuhistanica*; 2.2. *Amygdalis bucharica* + *Eremurus regelii* + *Rhaponticum integrifolium* + *Bromus inermis*; 2.3. *Amygdalus bucharica* + *Agropyron trichophorum* + *Rheum cordatum* + *Rhaponticum integrifolium*; 2.4. *Amygdalus bucharica* + *Poa nemoralis* + *Strigosella trichocarpa* + *Rhaponticum integrifolium*.

III RHAPONTIUM INTEGRIFOLIUM FORMATION. Associations: 3.1. *Rhapontium integrifolium* + *Rumex paulsenianus* + *Carex pachystylis* + *Allium suworowii*; 3.2. *Rhaponticum integrifolium* + *Rheum cordatum* + *Eremurus robustus* + *Rosa kokanica*; 3.3. *Rhaponticum integrifolium* + *Inula grans* + *Prangos pobularia* + *Bromus oxyodon*; 3.4. *Rhaponticum integrifolium* + *Cerasus mahaleb* + *Ferula kuhistanica* + *Poa bulbosa*.

Observations show that a significant part of the territory of the *Rh. integrifolium* formation is used by the local population, mainly for the preparation of hayfields for cattle. For this reason, before the end of July, *i.e.* before the harvest of hayfields and small horned cattle here are not available. As a result, there is a possibility of self-seeding walleye. In our opinion, perhaps this contri-

buted to the emergence of optimal conditions for germination of this plant. This, on the one hand, testifies to the positive role of anthropogenic impact in the preservation of *Rh. integrifolium* formation communities.

Site Gilan, Kizilemgac, Kul, Hisarak located in the vicinity of Tanhazdarya river in the foothills of the Hissar mountains dominate the area and natural resources of plants. In other words, 98% of the total *Rh. integrifolium* distribution in the republic and 88% of the total natural resource plants is located in the vicinity of these geographical points.

The yield of the aboveground part in the associations that make up the price complex *Rh. integrifolium* ranges from 210 to 950 kg/ha (**Table 4**).

The studied massifs with the participation of *Rh. integrifolium* were divided into three categories depending on the yield, the degree of remoteness from settlements and roads. The massifs of the 1st category include thickets that are easily accessible for the collection of raw materials and convenient for its transportation due to the proximity to roads. Massifs of thickets of the 2nd category are farther (on 8 - 10 km) from highways in comparison with the first category and on higher and less accessible slopes. Massifs of the 3rd category are removed from highways on considerable distance—12 - 20 km and more. Delivery of raw materials to cars is possible only with the help of pack transport.

The results of determining the areas of arrays and reserves of raw materials *Rh. integrifolium* in the Hissar range within Uzbekistan are shown in **Table 5**. As can be seen from the table, the 1<sup>st</sup> category of thickets occupies a leading position in the total area (125 hectares, or 52% of the area of all surveyed arrays). The share of less productive and less suitable for harvesting raw materials (category 3) thickets is slightly more than 22%. The most promising for the development are investigated in the thickets surrounding villages Gilan, Khisorak and Kizilimgak.

The results of experiments to determine the rate of recovery of above-ground phytomass *Rh. integrifolium* using single-factor analysis of variance [29] are presented in **Table 6**. In the first year after cutting, the recovery of phytomass occurs due to dormant kidneys. In this generative shoots appear only in the second year of vegetation. Annual cutting of shoots *Rh. integrifolium* (option III) showed that the number of shoots and their height decreased by more than 69%, a strong change was observed in the yield of phytomass, which decreased to 72% at the end of the experiment. In the experiment with a break in the workpiece in one year (option II) *Rh. integrifolium* oppression is also clearly expressed, the number of shoots and their weight on the test sites were lower than in the control. Yields during the experiment fell by 20% (was 50.3, was 40.2 g/10m<sup>2</sup>). In the variant of the experiment with “rest” in two years after cutting (option I), the recovery of shoots was 91, and phytomass—88%.

Comparison of the results of the experience in the restoration of the above-ground parts of the plant in different variants allows us to conclude that the mode of operation of the plant thickets with a break of three years provides a

complete restoration of the raw phytomass *Rh. integrifolium*. However, we recommend that this period be extended by another year to guarantee a reliable recovery of the phytomass, *i.e.* the raw material in the same array should be harvested only four years after the previous one. Breaks in the blanks *Rh. integrifolium* in the same array should be at least five years, and the volume of annual blanks should not exceed 20% of its operational reserve.

To determine the optimal timing of preparation of medicinal raw materials as a source of ecdysteroids (20-hydroxyecdysone) is necessary to know the dynamics of their accumulation in individual plant organs by phases of development. The obtained data on the quantitative content of 20-hydroxyecdysone in different parts of plants cultivated in the Fergana region and in the vicinity of Gilon village of Kashkadarya region are given in **Table 6**. As can be seen from the above data, depending on the phase of development and part of the plant, the content of 20-hydroxyecdysone varied from 0.21% to 1.42%. The maximum value recorded in the fruit—1.42%, in the roots early in the growing season—89%, and in the aerial part at the flowering stage—0.98% (Chodak, Fergana region). In the vicinity of the village Gilon Kashkadarya region the content of 20-hydroxyecdysone was from 0.06% to 2.0%, the maximum was fixed in the roots—2.0% and in the above-ground part in the flowering phase—0.9% (**Table 7**).

**Table 4.** Community *Rhaponticum integrifolium* and the yield of aboveground parts (Kashkadarya region, Gilan).

Formations	Associations	Number of plants, specimen/100 m <sup>2</sup>	The aboveground parts yield, kg/ha
Acer turkestanicum	<i>Acer turkestanica</i> + <i>Agropyron trichophorum</i> + <i>Rhaponticum integrifolium</i> + <i>Prangos pabularia</i>	31 ± 2.8	850 ± 5.0
	<i>Acer turkestanica</i> + <i>Rhaponticum integrifolium</i> + <i>Eremurus regelii</i> + <i>Rheum maximowiczii</i>	11 ± 1.0	450 ± 4.0
	<i>Acer turkestanica</i> + <i>Rhaponticum integrifolium</i>	27 ± 2.5	850 ± 5.5
	<i>Amygdalis bucharica</i> + <i>Prangos pabularia</i> + <i>Rhaponticum integrifolium</i> + <i>Ferula kuhistanica</i>	13 ± 1.3	210 ± 2.0
Amygdalis bucharicum	<i>Amygdalis bucharica</i> + <i>Eremurus regelii</i> + <i>Rhaponticum integrifolium</i> + <i>Bromus inermis</i>	20.5 ± 2.0	470 ± 3.0
	<i>Amygdalus bucharica</i> + <i>Agropyron trichophorum</i> + <i>Rheum cordatum</i> + <i>Rhaponticum integrifolium</i>	1 ± 1.2	320 ± 4.0
	<i>Amygdalus bucharica</i> + <i>Poa nemoralis</i> + <i>Strigosella trichocarpa</i> + <i>Rhaponticum integrifolium</i>	19 ± 1.8	620 ± 3.0
Rhapontium integrifolium	<i>Rhapontium integrifolium</i> + <i>Rumex paulsenianus</i> + <i>Carex pachystylis</i> + <i>Allium suworowii</i>	27 ± 2.5	820 ± 3.0
	<i>Rhaponticum integrifolium</i> + <i>Rheum cordatum</i> + <i>Eremurus robustus</i> + <i>Rosa kokanica</i>	31 ± 1.5	950 ± 5.0
	<i>Rhaponticum integrifolium</i> + <i>Inula grans</i> + <i>Prangos pobularia</i> + <i>Bromus oxyodon</i>	31 ± 3.0	950 ± 5.0
	<i>Rhaponticum integrifolium</i> + <i>Cerasus mahaleb</i> + <i>Ferula kuhistanica</i> + <i>Poa bulbosa</i>	18 ± 1.7	570 ± 3.0

**Table 5.** Areas of thickets and reserves of the aboveground part of *Rhaponticum integrifolium* in the Hissar range.

Location	Overgrown area, ha	Category thickets	Average number of plants on the area of 10 m <sup>2</sup>	The aerial parts yield, kg/10 m <sup>2</sup>	Stock of air-dry raw materials, ton		The amount of possible annual harvesting a ton
					biological	operational	
Gilan							
Tashkurgan	38	I	10.4	1.6 ± 0.2	6.8 ± 0.8	4.9 ± 0.5	1.6 ± 0.2
Misgar	22	I	11.7	1.6 ± 0.2	3.5 ± 0.5	3.0 ± 0.4	0.7 ± 0.1
Khisorak							
Archa boshi	18	II	8.1	1.0 ± 0.1	1.8 ± 0.3	1.6 ± 0.3	0.5 ± 0.1
Arpalisoy	35	I	9.3	1.4 ± 0.2	4.9 ± 0.5	4.2 ± 0.5	1.4 ± 0.2
Kul							
Khazratistonsoy	40	III	6.4	0.7 ± 0.1	2.8 ± 0.4	2.4 ± 0.3	0.7 ± 0.1
Maydanli	17	II	9.6	1.3 ± 0.1	2.1 ± 0.2	1.9 ± 0.2	0.40 ± 0.05
Kizilemgak							
Archa maydan	30	I	10.2	1.5 ± 0.2	4.5 ± 0.5	3.7 ± 0.5	0.9 ± 0.1
Zajrak	25	II	8.2	1.0 ± 0.1	2.5 ± 0.3	2.0 ± 0.3	0.50 ± 0.06
Maydantal	14	III	6.7	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.20 ± 0.03
Total:	239				27.3	24.5	6.9

Note. Category I—thickets easily accessible for the collection of raw materials and convenient for its transportation due to the proximity to roads; category II—thickets are 8 - 10 km from roads and on higher and less accessible slopes; category III—thickets are removed from roads by 12 - 20 km or more.

**Table 6.** Effect of the operating mode on the development of the above-ground mass *Rhaponticum integrifolium*

Indicator	Observation time	Experience option	Average by option	The accuracy of the impact
Number of shoots, copy/10 m <sup>2</sup>	The beginning of the experience	Control	40.2 ± 4.1	1.03
		I	41.1 ± 4.5	
		II	38.0 ± 4.0	
	The end of the experience	III	42.1 ± 4.6	18.6
		Контроль	40.9 ± 4.1	
		I	38.1 ± 4.0	
Phytomass, kg/10 m <sup>2</sup>	The beginning of the experience	II	30.0 ± 3.0	1.7
		III	25.4 ± 3.0	
		I	52.6 ± 5.1	
	The end of the experience	II	50.3 ± 5.0	3.3
		III	48.8 ± 4.8	
		I	46.3 ± 4.8	
		II	40.2 ± 4.1	
		III	35.1 ± 4.0	

Note. Option experience: I—two years of “rest” after cutting shoots; II—one year of “rest” after cutting shoots; III—annual cutting shoots; control— without cutting shoots.

**Table 7.** Content of 20-hydroxyecdysone (% of total dry weight) in different parts of *Rhaponticum integrifolium* by phases of development.

Phase of development	Underground part		Aboveground part		Fruit	
	Chodak	Gilon	Chodak	Gilon	Chodak	Gilon
Rosette plant	0.89	0.83	0.55	0.43	—	—
Budding	0.67	0.45	0.82	0.51	—	—
Flowering	0.44	0.74	0.98	0.94	—	—
Fructification	0.32	0.13	0.47	0.23	1.42	0.53
End of vegetation	0.43	2.05	0.21	0.06	—	—

#### 4. Conclusions

In conclusion, it should be noted that the duration of large life cycle of *Rh. integrifolium* in the territories of Gilan and Chodak is close to each other and there are no noticeable differences in the change of periods and stages of development. However, the change in morphological parameters in ontogenesis is associated with soil and climatic conditions of the territories.

Ontogeny *Rh. integrifolium* lasts 17 - 18 years. The first year of vegetation includes seedling and juvenile stage virginile period. In 1 - 2 years, the plant is not observed immature stage and 2 year *Rh. integrifolium* goes to Virginile stage. The absence of the immature stage in the conditions of Uzbekistan is associated with the manifestation of morpho-biological adaptations to arid conditions of the territory with the acceleration of the stages of ontogenesis of plants in hot weather conditions. The Virginile stage of the plant lasts 3 years, but the generative period begins with 3 years of vegetation. The generative period of the plant covers 10 - 11 years or almost 60% of ontogenesis. The old age of the plant begins in 13 - 14 years and lasts until 17 - 18 years. Plants growing on the territory of the Chodak are characterized by increased development of above-ground parts and for plants growing on the territory of Gilan active development of underground parts.

The area of thickets *Rh. integrifolium*, identified in Uzbekistan, is 239 hectares, the biological stock of air-dry above-ground mass—29.46 tons, operational—24.55 tons. Without prejudice to the restoration of thickets, the volume of annual harvesting can be 8.18 tons, after such exploitation, the thickets of this plant are restored within five years.

The amount of 20-hydroxyecdysone in phenological phases and parts of the plant varies from 0.21% to 1.42%. The accumulation of ecdysteroids in plants grown on the territory of Chodak (Namangan region) in the above-ground parts and in plants grown on the territory of Gilan (Kashkadarya region) in the underground parts is explained by the different development of the above-ground and underground parts of the plant in ontogenesis in these territories.

#### Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this



paper.

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