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INSECTICIDAL AND ANTIFEEDANT ACTIVITY OF THE ETHANOLIC EXTRACTS FROM *ALLIUM ROTUNDUM* L.

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ABSTRACT

The species of the genus *Allium* L., one of the largest genera of higher plants, occupying a significant place in the modern plant world, are of great interest both from the theoretical and from the practical point of view. Plants of *Allium* spp. accumulate a large amount of carbohydrates, phenolic compounds (flavonoids and its glycosides, coumarins, anthocyanins, catechins), amino acids and organosulfur compounds, such as allicin – a precursor for alkaloids and saponins formation. Secondary metabolites of *Allium spp*. have been successfully used during the past few decades in plants protection against pests and pathogens. We have earlier reported about high insecticidal and antifeedant properties of extracts from *A. subhirsutum* L., *A. narcissiflorum* Vill. and *A. ramosum* L. The highest insecticidal properties against imago (20.0%) and larvae (60.0%) of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidaea) were demonstrated by extract from aerial part of *A. subhirsutum.* The purpose of this investigation was to determine the insecticidal, antifeedant and repellent properties of *A. rotundum* L. extracts against various species of insects. As a result, it was found that the ethanolic extracts from the aerial part possessed the moderate level (40.0%) of insecticidal properties against the larvae of the *L. decemlineata* and low – against the imago (6.7-13.3%). Moreover, the extracts of *A. rotundum* showed moderate level of insecticidal, antifeedant and repellent activity against the larvae of lepidopterans (cotton budworm *Helicoverpa armigera* and wax moths *Galleria mellonella*).

Keywords: *Allium rotundum L., extract, insecticidal, antifeedant, repellent.*

INTRODUCTION

The species of the genus *Allium* L. (Alliaceae J. Agardh.), one of the largest genera of higher plants, occupying a significant place in the modern plant world, are of great interest both from the theoretical and from the practical point of view. There are more than 900 species, which naturally grow in the temperate regions of the Northern Hemisphere. Representatives of the genus grow in meadows, steppes, forests (The Plant List, 2018; Block E., 2009). Wild onions are promising for use in

human economic activities; among them there are food, medicinal, honey, ornamental plants. Many of them occupy a limited area.

Plants of the *Allium* genus have long attracted the attention of a large number of researchers due to the high the content of biologically active substances (BAS) which possess a wide spectrum of action. Due to the high content of valuable BAS – steroidal glycosides and unsaturated fatty acids that were found in onions species such as: *Allium nutans* L., *A. narcissiflorum* Wells., *A. giganteum* Rgl., *A. jajlae* Vved., *A. komarovianum* Vved., *A. schoenoprasum* L., *A. schoenoprasum* L., *A. porrum* L., *A. angustifolium* L., *A. ramosum* (*odorum*) L., and because they use as a raw material for the production of valuable medicines, the active substances of which have steroid origin (Shirshova, Volkova, 2005). Many representatives of the Allium genus have demonstrated an insecticidal and antifeedant effect on phytopatogens (Elisovetcaia et al., 2012).

On the territory of Republic of Moldova, there are about 10 species of Allium, including Allium *oleraceum* L. (onion field), *Allium rotundum* L. (Valdstein's onion), *Allium ursinum* L. (onion bear) and some others that are spread wildly in natural ecosystems.

Round-headed leek (or purple-flowered garlic) *Allium rotundum* L. (Amaryllidaceae: Alliaceae) is a perennial herbaceous rhizome-bulbous plant, originally from the steppe and Mediterranean regions of Europe, Anatolia and North Africa. It was distributed in Western Asia, Central and Southern Europe, Central and Southern regions of the European part of Russia, the North Caucasus (Chadaeva, 2016). The species grows in dry sabulous steppes, on coastal sands, rocks, fields and vineyards. In the Republic of Moldova *A. rotundum* is commonly found. It is cultivated as decorative, vegetable, but can inhibit the crops of winter wheat, because its seeds fall easily to the ground. The round garlic has stems of up to 90 cm in height, bearing pink or purple flowers. Its name derives from the large rounded umbels, which are 2-3 cm across. The umbels lack bulbils, but the underground bulb is usually surrounded by purplish-black bulbils with long stalks. In addition to their nutritional effects, the antibacterial, antifungal and antioxidant activities is extensively investigated due to the content of steroidal saponins, sapogenins, steroidal glycosides and others chemicals (Assadpour et al., 2016; Isman, 2006; Maisashvili et al., 2008, 2012; Sobolewska et al., 2016). However, there is no report on pesticidal ability of extracts from this species, growing on the territory of the Republic of Moldova. For this reason, the purpose of our investigation was to evaluate the insecticidal and antifeedant activity of the ethanolic extracts from *Allium rotundum* L.

MATERIALS AND METHODS

Plant material and preparation of ethanolic extract.

Allium rotundum L. was collected from Central zone of the Republic of Moldova. Gathering of phytogenic raw materials has been made according to methods approved in botanical and biochemical researches. Aerial parts of the plant were collected during flowering stage when plants accumulated the highest quantities of

biologically active substances. Phytogenic raw materials were dried off at the temperature of 28-30°C until the hydroscopic moisture concentrations were reached 7-10% according to standard methods. Before extraction the dry materials were crushed using an electric laboratory mill (Type: MRP-1, asynchronous motor). Crushed materials were extracted by maceration using 96% ethanol for 24 hours (shaking on a laboratory horizontal shaker ARMED HY-2B). After that, the extract was separated from the residue by filtration through filter paper. The resulting extract was concentrated under vacuum to a crude solid extract which was then dissolved in 96% ethanol to obtain the alcohol extract containing 20% of solid extract. The concentration of the tested extract of A. rotundum was 2.5%.

Test objects and laboratory testing

Experiments have been made for larvae and imagoes of potato beetle – Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae) a natural population and larvae of lepidopterans – cotton budworm Helicoverpa armigera Hubner (Lepidoptera: Noctuidae) and wax moths Galleria mellonella L. (Lepidoptera: Pyrolidae) laboratory populations.

Contact, intestinal and contact-intestinal effects of extracts have been studied. Each variant consisted of nine replications using 5 insects per replication. Young potato leaves of standard size served as a substrate for feeding L. decemlineata. Artificial diet was used to feed the larvae H. armigera and G. mellonella. In variants with the determination of intestinal and contact-intestinal effects, leaves were treated by immersion into an extract and then were stored in an exhaust box for 1 hour to complete evaporation of solvent. The leaves, treated using 12.0% alcohol solution, were served as the standard. For Lepidoptera, an artificial nutrient medium was mixed with the extract (100 µl of extract per 2 g of medium). As a standard, the mixture of 12.0% water alcohol solution with artificial medium was used. After that, prepared substrates were placed into double (Petri) dishes with insects. During the determination of contact and contact-intestinal effects the tested extract was applied topically on dorsal area of insects. Insecticidal activity was evaluated by counting determined using number of dead insects for three days in comparison with the standard according to accepted equation (Elisovetcaia, 2010, Elisovetcaia et al., 2012). Antifeedant activity of extracts was estimated three days after beginning of each experiment according to the point scale (tab. 1).

Table 1. Scale of antifeedant activity.

Browsing of leaf surface, %	Level of antifeedant activity	Points
0 to 5%	very high	1
6 to 25%	high	2
26 to 50%	moderate	3
51 to 75%	low	4
76 to 100%	very low (zero)	5

The presence or absence of the repellent activity of the extract of *A. rotundum* was established according to standard techniques (Elisovetcaia, 2010) - on the movement of insects in the opposite direction from the artificial nutrient medium mixed with the extract.
Statistical analysis of obtained data has been made according to the one-factor dispersion method (Dospehov, 1979) using Microsoft Excel software.

RESULTS AND DISCUSSION

As a result of laboratory testing, it has been revealed that the extract of *A. rotundum* is the most effective against larvae of potato beetle and lepidopterans. It has been established that the character of effects of this extract on insects, in the main, is intestinal and contact-intestinal. For the imago *L. decemlineata* and larvae *G. mellonella* no contact action of the ethanolic extract *A. rotundum* was found (tab. 2).

Table 2. Insecticidal activity of ethanolic extract from *Allium rotundum* L. against *Leptinotarsa decemlineata* Say, *Helicoverpa armigera* Hbn. and *Galleria mellonella* L.

Tested effects of extracts	Insecticidal activity, %			
	Leptinotarsa decemlineata		*Helicoverpa armigera* Larvae of age II-III	*Galleria mellonella* Larvae of age II-III
	Larvae of age II-III	Imagoes		
Contact	13.3	0	6.7	0
Intestinal	26.7	6.7	40.0	26.7
Contact-intestinal	40.0	13.3	46.7	40.0

It should be noted that observed intestinal insecticidal activity of extract against imago (6.7%) and larvae (26.7%) of *L. decemlineata* and against larvae of *G. mellonella* (26.7%) is also very low and insufficient to reduce the number of these pests to an economically advantageous level. The same effect was obtained in variants with topical treatment or contact action of the ethanolic extract from *A. rotundum* against larvae *L. decemlineata* (13.3%) and *H. armigera* (6.7%) (tab. 2). At the same time, it has been discovered that larvae of potato beetle are more vulnerable to the effects of extracts in comparison with imagoes. The death of larvae *L. decemlineata* in the variant with contact-intestinal treatment reached 40.0%, while the number of imagoes decreased by no more than 13.3%. Thus, it has been established that the ethanolic extract from the aerial part of *A. rotundum* has both intestinal and contact-intestinal action against to both the coleopterans and lepidopterans larvae, mortality of which ranged from 40.0 to 46.6%.
The results of insecticidal activity of *A. rotundum* extract are in good agreement with the data obtained earlier on the predominantly intestinal action of extract from

onion *Allium odorum* L. (Elisovetskaya et al., 2012). It was found that extract from aerial parts of the *A. rotundum* showed the same level of insecticidal activity on the larvae of Colorado potato beetle as extract of *A. narcissiflorum* (46.6%) and was significantly poorer compared to *A. subhirsutum* extract (80.0%) (Elisovetskaya et al., 2012). The higher mortality of larvae under influences of *A. subhirsutum* extract is apparently explained by the fact that the extract has both an intestinal and a contact action in contrast to *A. rotundum* extract which has only intestinal action on the *L. decemlineata*.

It was established that the highest insecticidal properties of the ethanolic extract from the aerial part of *A. rotundum* was demonstrated against larvae *H. armigera*: contact activity consisted 6.7%, intestinal – 40.0% and contact-intestinal – 46.7%.

The antifeedant properties of *A. rotundum* L. ethanolic extracts against various species of insects were on the moderate level and reached 3 point for larvae lepidopterans (*H. armigera* and *G. mellonella*) and imago *L. decemlineata.* The higher level was against larvae *L. decemlineata* and reach 2 point (tab. 3).

Table 3. Antifeedant and repellent activity of ethanolic extract from *Allium rotundum* against *Leptinotarsa decemlineata*, *Helicoverpa armigera*. and *Galleria mellonella.*

Tested effects of extracts	Antifeedant (point) and repellent activity (presence or absence)			
	Leptinotarsa decemlineata		*Helicoverpa armigera* Larvae of age II-III	*Galleria mellonella* Larvae of age II-III
	Larvae of age II-III	Imagoes		
Antifeedant Repellent	2 +	3 –	3 +	3 +

Note: "+"effect presence, "–"effect absence.

It was found that ethanolic extract from *A. rotundum* had a repellent effect on the tested species of coleopterans and lepidopterans insects, except for the imago of the Colorado potato beetle (tab. 3). The insects (larvae) moved from the treated food in the opposite direction, long circling in the Petri dishes, apparently until the action of the volatile components of the onion was reduced, or because of hunger.

As a result, it was established that the ethanolic extracts from the aerial part possessed the moderate level of insecticidal (40%), antifeedant (2 point) and repellent properties against the larvae of the *L. decemlineata* and low – against the imago (6.7-13.3%, 3 point). Moreover, the extracts of *A. rotundum* showed moderate level of insecticidal, antifeedant and repellent activity against the larvae of lepidopterans (cotton budworm *H. armigera* and wax moths *G. mellonella*).

Some differences in the degree of insecticidal and antifeedant activity of extracts from *A. rotundum* and other species of onions can be explained by their diverse chemical composition. Thus, aerial parts of the plant *A. rotundum* contains

essential oil, ascorbic acid, saponins (steroidal glycosides), saponegins (taperogen, diosgenin, hekogenin, gytogenin, b-chlorogenin, yukkagenin, agigenin) and others (Maisashvili et al., 2007; Sobolewska et al., 2016). It is known that aboveground parts of *A. subhirsutum* and *A. narcissiflorum* contain steroid glycosides (0.2-0.3%), but *A. narcissiflorum* – saponins, phenols and coumarins as well (Kintea, Degteareva, 1989; Selyutina, 2007). In addition to steroid saponins and glycosides, a plant of *A. odorum* also accumulates 0.1 to 0.3% of alkaloids (Stearn, 1992). It is most likely that the various chemical compounds in extracts explain the different nature of the action of the extracts against *L. decemlineata, H. armigera* and *G. mellonella.*

CONCLUSION

On basis of laboratory testing, it has been proved that ethanolic extract obtained from aerial parts of the plant *A. rotundum* possess insecticidal, antifeedant and repellent properties against imagoes and larvae of Colorado potato beetle (*L. decemlineata*) and larvae of lepidopterans – *Helicoverpa armigera* and *Galleria mellonella*. The character of properties and activity level directly depend on nature of biologically active substances accumulated by plants of the species *Allium* as well depend on the age of phytophagous. Taking into account the obtained data, plant *A. rotundum* can be recommended as biological insecticide for reducing the number of pests.

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