BIOLOGICAL ACTIVITY OF THE EXTRACT OF *Veratrum lobelianum* BERNH. AGAINST HARMFUL SPECIES OF INSECTS AND MITES AND ITS IMPACT ON ENTOMOPHAGES

ELISOVETSKAYA Dina, NASTAS Tudor

Abstract. It has been experimentally proved the existence of high insecticidal activity of the extract of *Veratrum lobelianum* BERNH. (0.1 to 0.5% of active substance) against imagoes and larvae of *Leptinotarsa decemlineata* SAY, larvae of the age I-III of *Heliothis armigera* HBN., *Mamestra brassicae* L., as well as against *Acyrtho siphonpisum* HARR. The extract shows high ovicidal (61.5 to 100%) and antifeedant (1-2 points) activity against *L. decemlineata*. It has been experimentally confirmed that the extract shows repellent activity against the larvae of *G. mellonella*. It has been proved that carrying out a single treatment with 0.1% extract of *V. lobelianum* (water-alcohol solution, 7.5 l/ha, 40 g/l of active substance) is enough to reduce the numerosity of *L. decemlineata* of the first generation. On the 21st day after being treated, the efficiency remained at reference level (92.0%), and plant damage did not exceed 5% (1 point). It has been determined that the treatment of potato plants with the extract of *V. lobelianum* has no negative impact onentomophages of *Chrysopa carnea* STEPH., *Coccinella septempunctata* L. and *Zicrona caerulea* L.: their numerosity remained at reference level and significantly exceeded chemical reference (by 1.5-4.0 times).

Keywords: biological activity, extract, *Veratrum lobelianum,* entomophages.

Rezumat. Eficacitatea biologică a extractului din specia *Veratrum lobelianum* BERNH. împotriva speciilor de insecte dăunătoare şi acariene, şi influenţa lui asupra entomofagilor. Experimental s-a demonstrat că extractul din specia *Veratrum lobelianum* BERNH. (0,1-0,5% s.a.) posedă proprietăţi înalte insecticide asupra fazelor imago şi larvelor *Leptinotarsa decemlineata* SAY, larvelor de vârstele a II-III *Heliothi sarmigera* HBN., *Mamestra brassicae* L., precum şi asupra *Acyrtho siphonpisum* HARR. Extractul posedă o activitate înaltă ovicidă (61,5-100%) şi antifidantă (1-2 puncte) contra *L. decemlineata.* Experimental s-a confirmat faptul că extractul manifestă o activitate repelentă asupra larvelor de *G. mellonella*. S-a demonstrat că efectuarea unui tratament la cartof cu extractul de 0,1% din *V. lobelianum* (soluţie alcoolică apoasă, 7,5 l/ha, 40 g/l s.a.) este suficientă pentru diminuarea densităţii populaţiei *L. decemlineata* de prima generaţie. Eficacitatea la 21 de zile după tratament a fost menţinută la nivelul standardului (92,0%), precum şi defoliarea tufelor de cartofi nu a depăşit nivelul 5% (1 punct). A fost stabilit că tratarea cu extractul din *V. lobelianum* nu provoacă efecte negative asupra entomofagilor *Chrysopacarnea* STEPH., *C. septempunctata* şi *Z. caerulea* L.: densitatea populaţiei lor se menţine la nivelul variantei martor şi depăşeşte esenţial pe cel al etalonului chimic (1,5-4,0 ori).

Cuvinte cheie: eficacitatea biologică, extract, *V. lobelianum,* entomofagi.

INTRODUCTION

One of the most dangerous pests on solanaceous crops in all parts of constantly expanding wide area is *Leptinotarsa decemlineata* SAY (Coleoptera, Chrysomelidae). Potato beetle inflicts the largest harm to plants at the first development stages, starting from seedling stage till flowering phase. At bud stage, the nutrition of 10 pest larvae on a potato plant during a week results in a decrease of harvest quality by 40-45% – reduced plant size and changed biochemical parameters (KOVALI, 2005). At a density of 25 larvae per plant during flowering phase (tuber formation), the degree of leave damage can reach 50-80% with the harvest decreased by 25-52%. In areas where pest management is not held, the losses reach 70-80% (KOVALI, 2005). At present, there is a practical necessity to control resistant populations of potato beetle selected as a result of many years of pyrethroid products application (MOTA-SANCHEZ et al., 2006). One of the efficient ways to reduce pest numerosity is to use specialized entomophages. However, optimal combination of using specialized entomophages against *L. decemlineata* has proved to be problematic due to complex interspecific correlations, various adaptability degree and ecological valence of entomophage species and potato beetle acclimatized to conditions of immigration areas, as well as due to mismatched development phases of *L. decemlineata* and some of its predators in new areas (FILIPPOV et al., 1985). That is why it is so important to preserve natural populations of native entomophage species, such as ladybirds (Coleoptera, Coccinellidae), golden-eyes (Neuroptera, Chrysopidae), bugs (Heteroptera - Pentatomidae, Miridae, Nabidae), etc. Despite the fact that these predators do not influence on the reduction of pest population density significantly, however they are capable to restrain the numerosity of other harmful phytophagous species, contributing to the preservation of healthy phytosanitary environment of the agrocoenosis.

In this regard, the tendency of studying and applying herbal substances to reduce the density of pest populations is of current interest at present stage associated with the development of environmental systems for plant protection and ecological agriculture. Products based on the substances extracted from *Pyrethrum cinerariaefolium* TREV., *Nicotiana tabacum* L., *Quassia amara* L., *Anabasis aphylla* L., *Derrise lliptica* (WALL.) BENTH., *Lonchocarpus nicou* (AUBLET) DC., *Tephrosia vogelii* HOOKF. and *Azadirachta indica* A. JUSS. are the most studied and used in plant protection (HUMMELI, 2007; ISMAN, 2006; KHAMBAY& JEWESS, 2000). At this stage, some tests of plant extracts against the larvae of *L. decemlineata* are carried out in Germany, Spain, Turkey, Canada, USA, Japan, China, India and many

other countries (KUMAR & POEHLING, 2007; KOUL et al., 2004; ZABEL et al., 2002). Some researches of secondary plant metabolites with insecticidal activity are carried out in Russia and Belarus as well (LITVINOVA et al., 2004; ZOLOTAR et al., 2001).

We have also carried out a research to identify the substances of plant origin that show biological activity against various types of the phytophagous species – insects and mites. About 200 plant species from 50 families have been tested against 14 species of pests. As a result, a number of plants with extracts showing high insecticidal, antifeedant, deterrent, repellent, or ovicidal properties have been selected. The purpose of the present research is to determine biological activity of the extract of *V. lobelianum* against harmful insects and mites, as well as to study its impact on some species of entomophages.

MATERIAL AND METHODS

The extract of *Veratrum lobelianum* BERNH. (Liliaceae) has served as an object for the research. Collecting, drying, grinding, extraction of active substances from roots of *V. lobelianum*, as well as determination of the sum of active substances has been carried out according to standard methods (MURAVIOVA, 2007).

Experiments have been carried out using egg clutches, larvae and imagoes of potato beetle - *Leptinotarsa decemlineata* (SAY 1824) (Coleoptera, Chrysomelidae) due to its greatest damage in conditions of Moldova and quick development of the resistance to existing protection methods. As a feeding plant, it has been used "Amerikanka" potato variety with a medium ripening period (*Solanum tuberosum* L., Solanaceae), which is damaged by the potato beetle significantly. The extract has been also tested against the following types of phytophagous species: Insecta - *Heliothis armigera* (HÜBNER, 1808) (Lepidoptera, Noctuidae), *Mamestra brassicae* (LINNAEUS 1758) (Lepidoptera, Noctuidae), *Galleria mellonella* (LINNAEUS 1758) (Lepidoptera, Pyralidae), *Acyrtho siphonpisum* (HARRIS, 1776), (Homoptera, Aphididae), Arachnida - *Tetranychus viennensis* (ZACHER 1920) and *Tetranychus urticae* (KOCH 1836)(Acariformes, Tetranychidae).

Laboratory testing of the extract has been carried in a climate cell with adjustable conditions at set temperatures of +22…+24^{o}C, air humidity of 70-80% and 16-hour photoperiod. For *L. decemlineata*, insects of a natural population have been used. Other types of phytophagous species have been bred using an artificial nutrient medium or plants grown under laboratory conditions. Young leaves of potatoes and tomatoes with standard sizes have served as a substrate for feeding *L. decemlineata* and *H. armigera*. Young cabbage leaves cut in rectangles with a size of 5 x 5 cm have served as a substrate for feeding *M. brassicae*. Leaves have been treated using a method of immersing into the extract and then kept in an exhaust hood for 1 hour until full evaporation of the solvent, and then they have been placed intoPetri dishes with insects. Cut pea shoots have served as a substrate for feeding *A. pisum*. Shoots have been treated together with colonies of aphids using a standard manual sprayer at a rate of 5 ml per shoot. Variants of treating leaves with 12% alcoholic solution have served as the reference. An artificial nutrient medium (ANM) has served as a food for *G. mellonella* with the addition of 2 ml of 0.1% extract. Variants of feeding with the addition of 2 ml of 12% alcohol solution have been used as the reference. Insecticidal activity (efficiency) has been determined using the number of dead insects for three days in comparison with the reference according to standard methodology (DOLJENCO, 2004).

Young leaves of the plum and the soy with standard sizes has served as a substrate for feeding *T. viennensis* and *T. urticae*. They have been treated using a method of immersing into 0.1% extract for several seconds and then kept for 1 hour until full evaporation of the solvent. Treated leaves have been placed into Petri dishes with cotton matrasses wetted with distilled water according to standard methodology. Then female mites have been placed thereinto. Records of dead individuals have been made in a day. Variants of treating leaves with 12% alcoholic solution have served as the reference. Acaricidal activity has been determined on the basis of the number of dead mites in comparison with the reference according to standard methodology.

Under laboratory conditions antifeedant activity of the extract against potato beetle has been determined on thethird day after the beginning of the experiment. Antifeedant activity level has been assessed according to a standard scale in points (DOLJENCO, 2004).

Ovicidal activity of extracts of *V. lobelianum* has been tested using egg clutches of *L. decemlineata*, collected from untreated potato plants. Each variant consists of three replications, three egg clutches each. Leaves with egg clutches have been treated using a method of immersing into the extract for several seconds and then kept in an exhaust hood for 1 hour until full evaporation of the solvent. Then they have been placed into double dishes. Leaves with egg clutches treated with 12.0% alcoholic solution have served as the reference. Ovicidal activity has been determinedon the 10th day according to standard methodology (DOLJENCO, 2004).

Tests of the extract of *V. lobelianum* were carried out in 2012 on an experimental field of the Institute of Plant Protection and Ecological Agriculture of the ASM using 'Amerikanka' potato variety with a medium ripening period. The experiment has been made in three variants. Each variant included four replications, and the area of a single replication was 50 m^2. Experimental plots were positioned using randomization method. During vegetation period agrotechnical measures of weed protection were carried out on this potato field.

The extract of *V. Lobelianum* has been tested at a concentration of 0.1% (water-alcohol solution, active substances – sum of alkaloids, in equivalent of protoveratrine 40 g/l) with a dose of 7.5 l/ha. 2.5% alcohol solution has been used as the reference. 'Confidor Maxi 70 WG', an insecticide with systemic and contact action (water-soluble

granules, 70%, active substance – imidacloprid 700 g/kg), has been used as a chemical standard with a dose of 0.08 kg/ha. Treatment operations have been carried out using "KWAZAR COR" knapsack sprayer with a capacity of 12 l. The consumption of working solution was 300 l/ha.

Biological efficiency of the extract of *V. lobelianum* has been determined using a method of recording 15 plants for each replication. Pest population density has been recorded before treating plants and on the 1st, 3rd, 7th, 14th, 21st day after being treated. Biological efficiency has been determined according to standard methodology. Antifeedant activity (damage degree of potato plants) has been determined on the1st, 3rd, 7th, 14th, 21st day according to standard methodology (DOLJENCO, 2004).

The impact of the extract of *V. lobelianum* on Chrysopacarnea [Chrysoperlacarnea] (STEPHENS, 1836) (Neuroptera: Chrysopidae), *Coccinella septempunctata* (LINNAEUS, 1758) (Coleoptera: Coccinellidae) and *Zicronacaerulea*(LINNAEUS 1758) (Heteroptera: Pentatomidae) has been recorded simultaneously.The population density of areas treated with the extract has been compared with the reference and chemical standard.

Mathematical processing of obtained data has been carried out according to the method of unifactor variance analysis (DOSPEHOV, 1979).

RESULTS AND DISCUSSIONS

As a result of laboratory testing against imagoes of *L. decemlineata*, it has been found that at low concentrations of active substances of 0.05 to 0.025% the extract of *V. lobelianum* mostly suppresses the nutrition of insects, slightly reducing their numerosity from 6.7 to 20.0%. When raising the concentration of active substances up to 0.3-0.5%, the efficiency of the extract against imagoes of potato beetle increases and ranges from 66.7 to 73.3% (Table 1).

Table 1. Insecticid alandantifeedant activity of the extract of *V. lobelianum* against larvae andimagoes of *L. decemlineata* under laboratory conditions depending on the concentration, n= 5x4.

Variant	Concentration of active substances, %	Insecticidal activity, %				Antifeedant activity, points			
		Larvae (by ages)			Imagoes	Larvae (by ages)			Imagoes
		I-II	II-III	III-IV		I-II	II-III	III-IV	
Reference	-	0	0	0	0	5	5	5	5
Extract of *V. lobelianum*	0.5	100	100	100	73.3	1	1	1	1
	0.4	100	93.3	80.0	73.3	1	1	1	1
	0.3	100	93.3	80.0	66.7	1	1	1	1
	0.2	100	93.3	73.3	53.3	1	1	2	2
	0.1	100	86.7	60.0	40.0	1	1	2	2
	0.05	93,3	80.0	53.3	20.0	1	1	2	2
	0.025	86,7	80.0	26.7	6.7	1	1	2	2
$HSD_{0.05}$ = 14.1									

It has been determined that the larvae of *L. decemlineata* of younger ages (I-III) are the most sensitive to the action of the extract. Even at the lowest concentrations of 0.025%, their death has reached 80.0%-86.7%. At the same time, older larvae (III-IV) show a high resistance degree. At concentrations of the extract of 0.025%, only a quarter (26.7%) of insects died, and only after increasing the concentrations of the extract up to 0.1% the death has increased up to 60.0%.

Antifeedant activity of the extract against older larvae and imagoes of *L. decemlineata* depends on the concentration of active substances and slightly decreases with reduced concentration. Thus, at a concentration of 0.3-0.5% antifeedant activity against imagoes and larvae of the age III-IV has reached 1 point (browsing of leaf surface up to 5%). When lowering the concentration up to 0.025-0.2%, leaf damage has reached 15% that equals to 2 points. For younger larvae, antifeedant properties of the extract are not dependent on the concentration of active substances (within the range of tested concentrations), as leaf damage has not exceeded 5% – 1point (Table 1).

As a result of observing the behaviour of insects during laboratory tests, it has been noticed that after being placed into Petri dishes with treated food both larvae and imagoes did not immediately approach to potato leaves, but travelled across the perimeter of dishes for 10-15 minutes and more at the most remote distance. The nutrition was of a short duration, and individuals left leaves repeatedly and nervously moved across the perimeter of Petri dishes again. Insects eating treated potato leaves show signs of metabolic disturbance and diarrhoea symptoms. Insects became limp and stiff, and many individuals have shown complete or partial paralysis. Subsequently all paralyzed younger larvae died. After the death their bodies become very wrinkly due to the hydration, and coverings lose the elasticity. Our observations have revealed that imagoes are able to recover, as after 2-day paralysis with obvious signs of metabolic disturbance many individuals return to normal state on the third day, being ready to coupling and egg laying. After placing such insects (on the fourth day of the experiment) onto fresh untreated food and monitoring them for 3 more days, we have observed no death of any individual. Thus, it has been revealed that active substances contained in the extract of *V. lobelianum* come out with metabolism products of insects and do not accumulate in the body.

When determining the nature of the action of 0.1% extract of *V. lobelianum*, it has been noticed that it shows mostly intestinal action against insects. At the same time, the extract also shows contact action, as the death of insects has been recorded as a result of topical application on dorsal area. The highest death percentage as a result of topical method of treating insects has been recorded for larvae of the age I-III (80.0 to 100%) and the smallest one – for imagoes (up to 26.7%).

We have also determined ovicidal activity of the extract against potato beetle. As a result, it has been found that the treatment with the extract sets back terms of hatching out of eggs by 1-2 days compared to the reference. In addition, it has been noticed that depending on the concentration of active substances the extract suppresses hatching out of eggs by 5.5%-25.5%. Corrected to the number of dead eggs in the reference - 1.5%, the number of dead eggs in the experiment ranges from 4.0% to 24.0%. At the same time, it has been found that larvae of *L. decemlineata* die during hatching period when gnawing through the chorion. At high concentrations of the extract from 0.4 to 0.5%, most of larvae die before being able to completely break free out of chorions. At lower concentrations, larvae die later, in a range from 1 to 24 hours. As a result, it has been found that depending on the concentration of active substances under laboratory conditions total ovicidal activity ranges from 61.5 to 100% (Fig. 1). Thus, we have proved that ovicidal action of extracts of *V. lobelianum* comprises the ovicidity itself and the death of embryos at the time of hatching. It has been noticed that at concentrations of the extract from 0.05 to 0.5% major number of eggs in an egg clutch (83.5-100%) die.

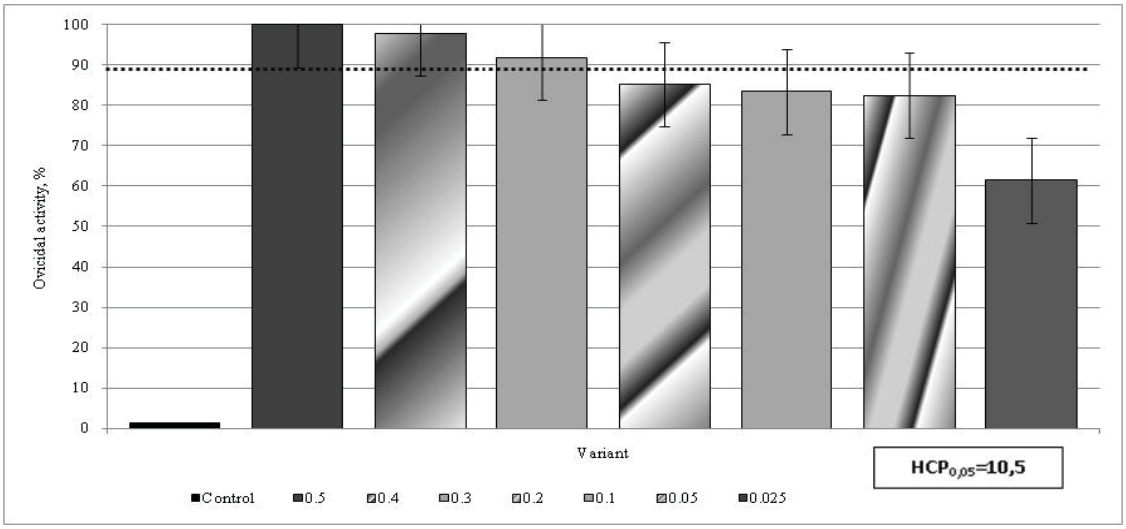

Figure 1. Ovicidal activity of the extract of *V. lobelianum* against *L. decemlineata* under laboratory conditions depending on the concentration.

Thus, it has been found that the extract of *V. lobelianum* possesses insecticidal, antifeedant and ovicidal activity against potato beetle (Coleoptera). In addition to potato beetle, we have also tested 0.1% extract of *V. lobelianum* against insects belonging to such orders as Lepidoptera and Homoptera, as well as against mites of Acariformes (Table 2).

As a result, it has been found that the extract shows high insecticidal activity (100%) against larvae of the age II-III of *M. brassicae* and *H. armigera*, as well as against colonies of aphids of *A. pisum* (Table 2).

Table 2. Insecticidal and acaricidal activity of the extract of *V. lobelianum* (0.1%) under laboratory condition sagainst various phytophagous species.

Class	Order	Pest species	Pest phase	Replication x times	Insecticidal activity, %	
					Reference	Extract of *V. lobelianum*
Insecta	Lepidoptera	*Mamestra brassicae* L. (Noctuidae)	II-III	4x5	0	100
		Heliothis armigera HBN.(Noctuidae)	Larvae of the age II-III	3x20	0	100
		Galleria mellonella L. (Pyralidae)	Larvae of the age II-III	4x5	0	47.0
	Homoptera	*Acyrthosiphon pisum* HARR. (Aphididae)	Colony	3x20	0	100
Arachnida	Acariformes	*Tetranychus viennensis* ZACHER. (Tetranychidae)	Imagoes	3x25	0	24.8
		Tetranychus urticae KOCH. (Tetranychidae)	Imagoes	3x25	0	23.4

The extract of *V. lobelianum* shows a medium level of insecticidal and antifeedant activity against larvae of the age II-III of *G. mellonella*. The death of insects reaches only 47.0% (Table 2), and the amount of eaten food is 25-30%.

We have noticed repellent activity of the extract, as in the first hours after being placed into Petri dishes with treated food insects travelled for a long time at the most remote distance from the food. Subsequently their behaviour also differed from that of reference individuals: most larvae in the experiment with the extract stayed at the edge of treated food, whereas larvae in the reference completely submerged into the food, embracing made holes with the silk.

It has been noticed that the extract of *V. lobelianum* possesses low acaricidal activity against both *T. viennensis* and *T. urticae*. The death of female mites reaches 23.4-24.8%. We have not revealed any repellent activity against both species of mites. It has been noticed that female mites lay eggs on leaves treated with the extract. The number of laid eggs in the experiment slightly differs from the reference.

Thus, we have not revealed any overall regularity proving that the extract of *V. lobelianum* possesses an activity at a certain level against insects belonging to a particular order. At the same time, it has been determined that the extract shows a quite low level of insecticidal activity against mites-phytophages and does not show any deterrent properties. This confirms the conclusions of other authors (HUMMELI, 2006; ISMAN, 2006; KHAMBAY & JEWESS, 2000) that plant extracts show selectivy action against different types of insects and mites and therefore may be low-toxic or non-toxic for entomophagous species.We have carried out studies to determine biological efficiency of the extract of *V. lobelianum* for reducing numerosity of *L. decemlineata* under field conditions. During vegetation period a single treatment was made, the terms of which have been determined depending on the numerosity of pests on tested plots. The average number of larvae on the date of June 4, 2012 was 55 individuals per 100 plants, exceeding economic limits of the harmfulness, and that was the reason for treating with the extract of *V. lobelianum* against potato beetle of the generation I. Plants were at the phase of budding-flowering.

Results show that the effeciency of tested extract of *V. lobelianum* is high (Figs.2a,b; 3a, b). Thus, it has been found that on the first day 100% of larvae died. On the 7th day after being treated, the effeciency of the extract against larvae of beetle was 99.6% and on the 21st– 92.0%, being at standard level (99.4 and 89.0% respectively). Three weeks after the treatment, potato leaf damage in the experiment did not exceed 5% (1 point). On reference plot the increase of pest number for this period resulted in complete destruction of leaf surface of plants (Table 3).

a)

b)

Figure 2. Death of larvae of *L. decemlineata* after treating with the extract of *V. lobelianum* (original).

a)

b)

Figure 3. Death of imagoes of *L. decemlineata* after treating with the extract of *V. lobelianum* (original).

Table 3. Biological efficiency of the extract of *V. lobelianum* against *L. decemlineata* under field conditions.

Variant	Biological efficiency on a corresponding day after being treated, %					Antifeedant activity on the 21st day after being treated, points
	1	3	7	14	21	
Reference	0	0	0	0	0	5
Standard - 'Confidor Maxi 70 WG'	100	100	99.4	95.0	89.0	1
Experiment - Extract of *V. lobelianum*	100	100	99.6	97.0	92.0	1
						$HSD_{0.05}$= 5.4

Statistical processing of the data obtained using variance analysis method allows us to reveal that the efficiency of tested extract of *V. lobelianum* against potato beetle larvae slightly differed from the standard and significantly differed from reference variant.

In field experiments we have also noticed that 80 to 95% of larvae hatch out of egg clutches treated with *V. lobelianum*. It has been found that in the experiment larvae hatched out of treated egg clutches do not spread across leaves and do not start eating but die (in the first 24 hours) when gnawing through and eating their chorions and unhatched eggs. It has been revealed that after the death larvae tend to blacken. At the same time, in the reference after hatching larvae eat their chorions and unhatched eggs and start eating first on same leaf and then on other leaves, spreading as a rule to younger top parts of plants.

As a result of the experiments made under field conditions, it has been found that the extract of *V. lobelianum* does not show any phytotoxicity. Plants in the experiment are well developed, with green leaves and growing young shoots. Potato plants do not retard in the growth compared to the standard.

One of the objectives of our research was to study the impact of the extract of *V. lobelianum* under field conditions on some species of predators and entomophagous species found on potato plants. As a result of field observations on a plot treated with the extract of *V. lobelianum*, we have found the following species of predators: egg clutches, larvae, pupae and imagoes of *C. septempunctata* egg clutches, larvae and imagoes *C. carnea*, egg clutches, larvae and imagoes of *Z. caerulea*.

According to our observations for the entire recorded period (21 days), on potato plants treated with the extract of *V. lobelianum* we have not found any dead larvae or imagoes of the golden-eye (*C. carnea*) or the ladybug (*C. septempunctata*). In addition, fresh egg clutches, hatching of healthy larvae, as well as further normal nutrition, development and pupation of larvae of both predator species has been observed (Figs. 4; 5; 6; 7). On the 7th day after being treated, in chemical standard the number of larvae of *C. septempunctata* was 0.1 individuals per plant, while in both the experiment and the reference their number was 5 times higher. On standard plot treated with 'Confidor Maxi 70 WG', a few death cases have been recorded for imagoes of *C. septempunctata*. On the 7th day after being treated, in the experiment and the reference the number of egg clutches of *C. carnea* (Fig. 4) reached 0.4 eggs per plant and larvae – 0.3 individuals per plant. The number in chemical standard – 0.1 eggs and 0.05 larvae per plant.

Figure 4. Eggs of *C. carnea* on potatoes (original).

Figure 5. Egg-laying of *L. decemlineata* attacked by larvae of *C. carnea* (original).

Figure 6. Larvae of *C. septempunctata* on aplot treated with the extract of *V. lobelianum* (original).

Figure 7. Pupa of *C. septempunctata* on a plot treated with the extract of *V. lobelianum* (original).

It has been noticed that *Z. caerulea*, a predatory bug, colonizes potato plants much later than terms of treating against the first generation of potato beetle. We have observed imagoes of this bug (Figs. 8; 9) in the third decade of June, with increased number of larvae of *L. decemlineata* on plants. At the same time, on the experiment plot and reference plot the numerosity of this predator was growing rapidly and by mid-July it reached 2 to 5 imagoes per plant. A few egg clutches of *Z. caerulea* have been observed. The number of imagoes was 4 times lower in chemical standard. It also has been noticed that imagoes and larvae started colonizing plants in chemical standard 7 days later than in the experiment and the reference.

Thus, we have found that at a concentration of 0.1% of active substance the extract of a plant of *V. lobelianum* species has intense insecticidal, antifeedant and ovicidal properties ensuring efficient protection of potatoes from summer generation of *L. decemlineata* for at least three weeks. For maximum effect, the treatment with plant extract is best done during mass hatching of larvae and not later than the apparition of larvae of the age II-III.

Our studies show that the treatment with plant extract of *V. lobelianum* has no negative impact on beneficial entomofauna that allows keeping natural balance of agricultural ecosystem.

Figure 8. Imago of *Z. caerulea,* eatinga larva of *L. decemlineata*. (original).

Figure 9. Coupling ofimagoes of *Z. caerulea* on a plot treated with the extract of *V. lobelianum*. (original).

CONCLUSIONS

As a result of laboratory testing, it has been proved that the extract of *V. lobelianum* shows high insecticidal (60.0-100%), antifeedant (1-2 points) and ovicidal (61.5 to 100%) properties that are dependent on the concentration of active substances. It has been revealed that the extract possesses both intestinal and contact action. However, contact action of the extract is weaker, so it is less toxic for imagoes of Coleoptera, as well as for individuals that are not exposed to the extract directly or do not eat treated food.

It has been determined that the extract shows a low level of insecticidal activity and does not show any deterrent properties against mites-phytophages (Tetranychidae). It has been experimentally confirmed that the extract possesses repellent activity against larvae of *G. mellonella*.

Made research has proved that a single treatment with 0.1% extract of *V. lobelianum* (water-alcohol solution, 7.5 l/ha, 40 g/l of active substance) is enough to reduce the numerosity of the first generation of potato beetle. On the 21st day after the treatment, the efficiency remained at standard level (92.0%), and plant damage did not exceed 5% (1 point).

It has been determined that the treatment of potatoes with the extract of *V. lobelianum* has no negative impact on entomophagous species (*C. septempunctata, C. carnea* and *Z. caerulea*), as their number remained at reference level and was significantly higher than chemical standard (1.5-4.0 times). Thus, it has been proved that the extract of *V. lobelianum* with a high biological efficiency can be used as a means of natural origin for reducing numerosity of potato beetle without any negative influence on beneficial entomofauna of the agrobiocoenosis of a potato field.

ACKNOWLEDGEMENTS

The authors thank Elena Iordosopol, Doctor of Biology, and Victoria Sumenkova, Doctor of Biology,for their helpwhen working with mites of Tetranychidae, Natalia Bradovskaya, research associate, forprovidingthe cultureof *Galleria mellonella.* We also express our gratitude to the reviewers that work together with the Editorial Board of the Journal "Oltenia. Studii şi comunicări. Ştiinţele Naturii" for their useful discussions and constructive suggestions.

REFERENCES

DOLJENCO V. I. 2004. *Metodiceskie ukazania po registraţionnym ispytaniam insecticţidov, acariţidov, moliuscoţidov i rodentiţidov v seliscom hozeaistve*. Edit. Rashn, Vniizr, Innovaţionnyi centr Zascitz Rastenii. S-P. 363 pp.

DOSPEHOV B. A. 1979. *Metodika polevogoopyta*. Edit. Kolos. Moskva. 338 pp. [In Russian].

FILIPPOV N. A., MATSIUC V. A., JAROVOI V. M. 1985. *Integrirovannaia zascita ovoşcnyh culitur i cartofelea v usloviah promyşlennyh tehnologhii ih vozdelyvania.* In: Integr. zascita ovoşcnyh i plodovyh culitur. Edit. Ştiinţa. Chisinev: 33-41. [In Russian].

HUMMELI A. 2007. *Primenenie biopesticidov Nimazal-T/C i Kvassia-MD protiv vreditelei v zasciscennom grunte.* În: Naucino-Informaţioonyi jurnal dlea speţialistov zasciscennogo grunta „Gavriş". **6**: 16-20. [In Russian].

ISMAN M. B. 2006. *Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world.* Annual Review of Entomology. Edit. Annual Reviews. USA. **51**: 45-66. [In Russian].

KHAMBAY B. P. S. & JEWESS P. 2000. *The potential of natural naphtoquinones as the basis for a new class of pest control agent.* Crop Protection. Edit. Elsevier Science Publishing Company, Inc., United Kingdom of Great Britain and Northern Ireland. **19**: 597-601.

KOUL O., SINGH G., SINGH R., SINGH J., DANIEWSKI W. M., BERLOZECKI S. 2004. *Activity of some nonazadirachtin limonoids from Azadirachta indica against Lepidopteran larvae.* Australian Journal of Entomology. Edit. John Wiley & Sons, Incorporated. Australia. **43**(2): 189-195.

KOVALI A. G. 2005. *Jujeliţy (Coleoptera, Carabidae) polei ovoşcnyh paslenovyhculitur (vidovoi sostav, ecologia, biologia, entomofaghi coloradscogo juka)*. Ph. D. Thesis, RashnVniizr. Sankt-Peterburg. 25 pp. [In Russian].

KUMAR P. & POEHLING H. M. 2007. *Effects of azadirachtin, abamectin, and spinosad on sweetpotato whitefly (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics*. In: Journal of Economic Entomology. Edit. Entomological Society of America. **100**(2): 411- 420.

LITVINOVA V. I., BABICEVA S. V., GOKOV A. V. 2004. *Ispîtanie preparatov prirodnogo proishojdenia dlea zascitz cartofelea ot coloradscogo juka.* Materialy mej dunaro dnoi nauchnoi conferenţii „Biologicescaia Zascita rastenii -osnova stabilizaţii ecosistem". Krasnodar. **(2)**: 322-324. [In Russian].

MOTA-SANCHEZ D., HOLLINGWORTH R. M., GRAFIUS E. J., MOYER D. D. 2006. *Resistance and cross-resistance to neonicotinoid insecticides and spinosad in the Colorado potato beetle, Leptinotarsadecemlineata (Say) (Coleoptera: Chrysomelidae).* Pest Management Science. Edit. John Wiley & Sons Ltd. United Kingdom of Great Britain and Northern Ireland. **62**: 30-37.

MURAVIOVA D. A. 2007.Farmacognozia. Edit. Medicina. Moskva. 654 pp. [In Russian].

ZABEL A., MANOJLOVIC B., RAJKOVIC S., STANKOVIC S., KOSTIC M. 2002. *Effect of Neem extract on Lymantria dispar L. (Lepidoptera: Lymantriidae) and Leptinotarsa decemlineata SAY (Coleoptera: Chrysomelidae).* Journal of Pest Science. Edit. Springer-Verlag GmbH. Germany. **75(1)**: 19-25.

ZOLOTAR R. M., BYKHOVETS A. I., KOVGANKO N. V. 2001. *Effect of certain phytoecdysteroids on larvae of Colorado beetle Leptinotarsa decemlineata*. Chemistry of Natural Compounds. Edit. Springer New York Consultants Bureau. Uzbekistan. **37**(6): 537-539.

Elisoveţcaia Dina
Institute of Plant Protection and Ecological Agriculture of the ASM
Chişinău. Moldova.
E-mail: dina.elis.s@gmail.com

Nastas Tudor
Institute of Plant Protection and Ecological Agriculture of the ASM
Chişinău. Moldova.
E-mail: tudor_nastas@mail.ru

Received: 28 March, 2013
Accepted: April 7, 2013