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POSSIBILITIES TO USE NATURAL EXTRACTS FROM MEDICINAL AND AROMATIC PLANTS (MAP) LIKE BOTANICAL REPELLENT OR INSECTICIDE COMPOUNDS AGAINST PEST INSECTS IN ECOLOGICAL CROPS (II)

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Abstract

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health. The body of scientific literature documenting bioactivity of plant derivatives to arthropods pests continues to expand i.e. repellents based on essential oils extracted from Chenopodium ambrosioides, Eucalyptus saligna, Rosmarinus officinalis to mosquitoes, or cinnamon oil, sandalwood oil and turmeric oil are previously reported as insect repellents evaluatede in the laboratory conditions. With the constantly increasing problems of insecticide resistance and increasing public concerns regarding pesticide safety, new, safer active ingredients are becoming necessary to replace existing compounds on the market. The present study carried out in the period 2010-2012 comprises a review of two insect repellents, followed by some new research conducted in our laboratory on plant-derived insect repellents. The two alkaloids tested against the Colorado potato beetle, Leptinotarsa decemlineata Say in laboratory conditions was obtained by water and alchohol extraction from two vegetal species, Cichorium intybus L. (Asterales:Asteraceae) and Delphinium consolida L. (Ranales:Ranunculaceae). The tests carried out in laboratory and field experimentally plots under cages permit to evaluate several other compounds for repellent activity of lacctucin alkaloids.

Key words**:** *alkaloids, biorational insecticide, repellents*

INTRODUCTION

Biological resources and the potential for sustainable exploitation of crops of medicinal and aromatic plants in our country are huge and represent an important component of sustainable agricultural development in Romania. From existing data, our country has in his flora of over 3,700 different plant species, cultivated or spontaneous, with therapeutic action, of which 800 species have properties defined and phytotherapeutic, 370 species have been recognized as having the qualities of pharmacy-dynamic effects, but which have not yet been fully studied from the scientific point of view [1]. The European Pharmacopoeia is adopted in all EU Member States includes 98 species of plants. Minimum risk pesticides are exempt from EPA registration and they are currently listed at [12]. A result of various international research and traditional practices, medicinal and aromatic plant list is in constant dynamic; so that the European Association of producers and processors of medicinal and aromatic plants has expanded the number of plants to about 1,500. Pest control using chemical insecticides had been from many years a critical activity for environmental protection. For sustainable agriculture purposes the largest agricultural use of insecticides became undesirable and dramatically dangerous for entire wildlife and of course, human health. Considering unanimously out of question the toxicity of pesticide use “softer” category of some substances with potential insecticide effect would be desirable from an environmental standpoint. From theoretical point of view there are some terms which are trying to define such active substances against a large spectrum of agricultural pests [6]. In terms of chemical composition of these substances are secondary plant metabolism compounds such as: terpenoidele (mono-, sesqui-, di-), poliacetilene, alkaloids, flavonoids and polysaccharides [4]. From the multitude of attempts to name and to define of the natural products we select the two terms which are constantly found in the world scientific literature but used without been a unanimously accepted definition. In Europe more often is speaking about the botanical insecticides or botanicals which are according to Science Dictionary [11] an insecticide made from a substance extracted from plants, e.g. pyrethrum, derived from chrysanthemums, or nicotine, derived from tobacco plants. In USA pest control materials that are relatively non-toxic with few ecological side-effects are sometimes called ‘bio-rational' pesticides, although there is no official definition of this term. Often their effects are not as long lasting as those of synthetic pesticides and some of these products may be very difficult to find. Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health [10]. The body of scientific literature documenting bioactivity of plant derivates to arthropods pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products. There is no doubt that botanical insecticides are an interesting alternative to insect pest control, and on the other hand only a few of the more than 250,000 plant species on our planet have been properly evaluated for this purpose. Considering that more than 80,000 are MAP found in different agro-ecosystems around the world but only a few are used as green plants synthesize and preserve a variety of biochemical products for industry. Many of them are extractable compounds and used as chemical feed stocks or as raw material for various scientific investigations and industrial and medicine utilization. This means that the potential for the future may be huge. In fact, plants like neem *Azadirachta indica* (Meliaceae) have shown excellent results and there already are commercial products in the market made from it. Azadirachta indica and also many species of Zingiberaceae MAP from India or vetiver (*Vetiveria zizanioides* from Thailand) are also related groups of plants having in their part chemical constituents which are active like botanical insecticides or repellents activity.Insect repellents are an alternative to the use of insecticides. In general terms we can say that certain natural substances having a range of biological properties against insect pests, fungal, bacterial, and viral diseases, and weeds have been used for centuries to protect crops. These plant substances are more rapidly degraded in the environment, and some favor beneficial insects. Some plants can produce a diverse range of secondary metabolites such as terpenoids (mono-, sesqui-, and di-), alkaloids, polyacetylenes, flavonoids, and sugars. Terpenoids are some of the most successful examples of pesticides among these substances. The present paper have been resumed the older research carried out in period 2006-2008 in the RIDPP laboratory of entomology with the objective to indicate one important strategy to ecological use of botanical product in plant protection [7].

MATERIALS AND METHODS

The test species *L. decemlineata* was collected from the field by two methods and maintained on natural food under controlled conditions.
Experimental design in the Nicotox assay (laboratory conditions).
There were 7 variants with 3 replicates each/10 larvae by replicate:
V1 – larvae L1, 10 individuals; V2 – larvae L2, 10 individuals;
V3 – larvae L3, 10 individuals; V4 – larvae L4, 10 individuals;
Product: Nicotox (sulphate distilled)
Application mode:
a) sprayed on food substrates aqueous solution
b) sprayed directly on larvae
V5 – imago, 10 individuals;
Application mode:
a) sprayed on food substrates aqueous solution
b) sprayed directly on adults
V6 – eggs 1 egg cluster
Sprayed directly on eggs
V7 – check treated with distilled H_2O

Experimental design for the repellents assay

The *Cichorium intybus* and *Delphinium consolida* plants were collected from the field in the period 2.07-4.07.2010 when the flowering process is beginning and then all the vegetative organs of plants were completely developed. The organs of plants collected were separated, chopped and small mortared. From each organ, aqueous and alcoholic solutions were prepared at five different concentrations. According to the vegetative plant organs the plant pieces were either driest at 25 ± 1ºC, RH 60 ± 5% and photoperiod of 16 hours continuous light either fine small mortared like green mass. The dried plant parts were boiled at 100ºC and the decoction obtained were used in field experiment treatments. The experimental field were establishing like multifactor design including subdivided parcels (30 variants with 4 replicates each).
Four factors were tested:

- o the vegetative organ (3 hypothesis tested);
- o the plant processing mode (2 hypothesis tested);
- o the solution concentration (5 hypothesis tested);
- o the extract composition (2 hypothesis tested)

The vegetal extracts were administrated by spraying the plant leaves on the both sides. During the 10 days of experiments 2 splashing were made, one at the beginning of experiment and the second after 5 days. The observations were registered after 24, 48, 72 hours and 10 days after first splash.

RESULTS AND DISCUSSIONS

In connection with the preparation and extraction of plant insecticides at present intense research is conducted at various research centers around the world (being very advanced research from two American universities (Iowa and Cleveland), which is why research in this area should be developed, and the results should be achieved with a database [3], [9]. The toxicity of the extracts of plants on insect varies greatly even if the extracts are made of the same species. This is due to the different phases of the plant, the percentage moisture content of the plant at the time of collection, enforcement and the extraction method, different compounds have an extract can act synergistically or antagonist. The most important factor that determines the variation in the response of the various species of insects from the vegetal extracts is the native or acquired resistance against a product or another. The most conclusive example is that of the Colorado beetle whose digestive system is adapted to consume glycoalkaloyds of solanine type and, on the other hand this species is endowed with a series of enzymatic mechanisms that allow it to destroy all toxic compounds which are used in chemical control of this species [2], [8], [5]. The results obtained in the Nicotox assay were presented in the Tables 2 and 3. In the first table the variants with larvae and adults tested are included and in the table 2 are presented the variant with eggs tested. There was a special commentary related to this egg test. The Nicotox formulation was very active against the first instars larvae of Colorado potato beetle (V_1). The mortality percent registered was biggest after 24 hours after the product administration in all replicates. Related to the vegetal extract administration in the case of sub variants tested its mean that the lethal effect was predominant of ingestion effect in the L_2 – L_4 larvae case while at the first instar the mortality was especially by contact administration. The Nicotox 5 insecticidal activity was reduced during the development of the insect meaning that at the third instars (L_3) the mortality percent reach 100% only of a minim interval of 48 hours and maximum of 120 hours. In the case of L_4 larvae the mortality was at the level of standard chemical product meaning between 80-100% after 7 days from first application.

Table 1. List of the more important botanical insecticides used in pest control programs around the world

Common name	Chemical formula	Origin/plant species	Status
azadirachtin	C35H44O16	*Azadirachta indica*	Not official
anabasine	C10H14N2	*Nicotiana glauca*	Not official
Pyrethrin I	C21H28O3	*Chrysanthemum cinerariaefolium*	ISO 765
Pyrethrin II	C22H28O5	*Chrysanthemum cinerariaefolium*	ISO 765
Jasmolin I	C21H30O3	*Chrysanthemum cinerariaefolium*	Not official
Jasmolin II	C22H30O5	*Chrysanthemum cinerariaefolium*	Not official
Cinerin I	C20H28O3	*Chrysanthemum cinerariaefolium*	ISO 765
Cinerin II	C21H30O5	*Chrysanthemum cinerariaefolium*	ISO 765
nicotine	C10H14N2	*Nicotiana tabacum*	ISO 765
Matrine/China	C15H24N2O	*Sophora japonica, S. subprostrata*	Not official
rotenone	C23H22O6	*Tephrosia virginiana, Lonchocarpus utilis, L. nicou, L. urucu, Derris elliptica, D. involuta, Duboisia sp., Verbascum thapsus, Mundulea sericea, Piscidia piscipula*	Not official
sabadilla	C32H49NO9 C36H51NO11	*Schoenocaulon officinale*	Not official
quassia	Not yet establish	*Quassia amara, Q. indica*	Not official
d-limonene	C10H16	*Citrus medica, C. aurantifolia, C. maxima, C. reticulate, C. latifolia, C. limon, C. limonia, C. paradisi, C. sinensis, C. tangerina*	Not official
Ryania/ryanodine	Not yet establish	*Ryania speciosa*	Not official

The adults of *L. decemlineata* were totally immunes at the product Nicotox 5 action, whatever of sex and doses administrated the result confirming the fact that the resistance of this species of control products is biggest in the adult cases.

In the case of *L. decemlineata* a species with a pregnant defoliation activity the control product is very important to be efficient against the egg stage giving the possibility to prevent from the start the leaves consuming.

Table 2. Efficiency of treatments with alkaloid NICOTOX 5 against *Leptinotarsa decemlineata* Say under laboratory conditions

Variant	Replicate	% mortality after period of:						TOTAL	CHECK PLOT
		24 hour	48 hour	72 hour	96 hour	120 hour	7 days		
V1	R1	100,00	-	-	-	-	-	100,00	0
	R2	100,00	-	-	-	-	-	100,00	0
	R3	100,00	-	-	-	-	-	100,00	0
	Average	100,00	-	-	-	-	-	100,00	0
V2	R1	100,00	-	-	-	-	-	100,00	0
	R2	100,00	-	-	-	-	-	100,00	0
	R3	100,00	-	-	-	-	-	100,00	0
	Average	100,00	-	-	-	-	-	100,00	0
V3	R1	50,00	100,00	100,00	100,00	100,00	-	100,00	0
	R2	60,00	100,00	100,00	100,00	100,00	-	100,00	0
	R3	30,00	90,00	90,00	90,00	100,00	-	100,00	0
	Average	46,66	96,66	96,66	96,66	100,00	-	100,00	0
V4	R1	40,00	60,00	90,00	90,00	90,00	90,00	90,00	0
	R2	30,00	40,00	80,00	80,00	80,00	80,00	80,00	0
	R3	30,00	50,00	100,00	100,00	100,00	100,00	100,00	0
	Average	33,33	50,00	90,00	90,00	90,00	90,00	90,00	0
V5	R1	0	-	-	-	-	-	-	0
	R2	0	-	-	-	-	-	-	0
	R3	0	-	-	-	-	-	-	0
	Average	0	-	-	-	-	-	-	0

Table 3. Efficiency of treatments with alkaloid NICOTOX 5 against *Leptinotarsa decemlineata* Say stages under laboratory conditions

Variant	Replicate	% appearance after..................................days:					% appearance check plot after days:		
		9 days	13 days	14 days	TOTAL	% mortality L1	5 days	6 days	TOTAL
V6	R1	0	0	0	0	100,00	10,00	10,00	100,00
	R2	5,88	5,88	5,88	5,88	100,00	90,00	100,00	100,00
	R3	0	38,46	63,46	63,46	100,00	-	96,00	96,00
	Average	1,96	14,78	23,11	23,11	100,00	33,33	68,66	98,66

Fig. 1. *Leptinotarsa decemlineata* Say larvae L_3 dead after Nicotox 5 treatment

The tests experimented shows that the Nicotox 5 had a very good efficiency against the egg of Colorado potato beetle (Table 2).
The product action was manifested in two ways:
- by slowing the larvae appearance or by eggs incubation blocking;
- by mortality of L1 larvae induction

The preliminary tests performed in the case of the two lactucin extracts from *C. inthybus* and *D. consolida* shows that the effect of treatments with vegetal extracts on the feeding of the larval populations of *L. decemlineata* in the field cages was typical antifeedant in all cases of experiment conditions (fresh extract in water and fresh extract in alcohol) and dry extract in water and in the alcohol compared with check plot.

Fig. 2. *Leptinotarsa decemlineata* Say larvae L_4 dead after Nicotox 5 treatment

The future research will follow two new objectives: the necessity of purifying the vegetal extract to identify the specific active substance and in finding of the possibility of product conditioning for agricultural application.

CONCLUSIONS

Nicotox 5 formulation was very active against L1 instars of Colorado potato beetle the mortality rate registered being high after 24 hours from treatment;
The tests shows that the lethal effect is predominantly of ingestion in the L3- L4 instars facing with first instars (L1- L2) where the lethal effect was especialy of contact;
The insecticide activity of Nicotox 5 is reducing with the development period of the insect stages: at the first instars the mortality rate was 100% after 12 hours from treatment when in the L3- L4 instars the mortality rate reach 80-100% after 7 days from administration;
Nicotox 5 formulation was very active against Colorado potato eggs by slowing and blocking the incubation process;
The preliminary tests performed in the field cages with two lacctucin extracts from C. intybus and D. consolida shows a predominant antifeedant effect in all experiment conditions:
Fresh extract in water;
Fresh extract in alcohol;
Dry extract in water;
Dry extract in alcohol
Further researches will be addressed to other 3 objectives:
Biosynthesis of botanical alkaloids/insecticide from plant species: *Datura innoxia*, *Hyoscyamus niger*, *Atropa belladonna*, *Aconitum nepellus*, *Solanum nigrum*;
Extraction of vegetal proteins/antifeedants; species proposed: *Cichorium intybus*, *Delphinium consolida*, *Lavandula angustifolia*, *Alliaria officinalis*, *Fumaria officinalis*, *F. vaillantii*, *Achillea millefolium*;
Extraction of volatile oils from species *Valeriana officinalis*, *Capsicuum anuum*, *Armoracia rusticana*, *Anethum graveolens*, *Petroselinum hortense*, *Artemisia dracunculus*, *Ambrosia artemisiifolia*

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