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EFFICACY OF SOME BIOPESTICIDES AND PLANT SECONDARY METABOLITES AGAINST FALL WEBWORM *HYPHANTRIA CUNEA* DRURY (*F. ARCTIIDAE-LEPIDOPTERA*) IN THE LAB CONDITIONS

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ABSTRACT. The paper presents the efficacy of the some biopesticides used in the experiments to control fall webworm (*Hyphantria cunea*), comparatively to some plant secondary metabolites from autochthonous flora. From the first category there were used: spinosad, a secondary metabolite produces by the fermentation from *Saccharopolyspora spinosa* mushroom and is the active principle of the commercial products of the *Naturalyte* class; azadyrachtines – a group of limonoids, obtained from the seeds of the Neem tree (*Azadirachta indica*), and milbecmectin, a product obtained from a metabolite of the *Streptomyces hygroscopicus* subsp. *aureolacrimans* bacteria. The results revealed the efficacy of all bio insecticides against fall webworm in 2-7 days period after treatment. Spinosad presented a quick action, comparatively to the other bio pesticides. The secondary metabolites, used into fall webworm control, were extracted from autochthonous plants: the common ladyfern (*Drioperis filix mas*), the perennial sage (*Salvia nemorosa*), the wormwood (*Artemisia dracunculus, A. vulgaris, A. absinthium*) the European birthwort (*Aristolochia clematidis*), Cow parsnip (*Heracleum spondylium*), the hedge nettle (*Stachis sylvatica*), the speedwell (*Tanacetum vulgare*), the nettle (*Urtica dioica*), the danewort (*Sambucus ebulus*) and the yew tree (*Taxus baccata*) to fall webworm. Plants extracts were obtained from dried ground plants, using 25 g/ 1 litter of cold water, stirred for 24 hours. The extracts in ethylic alcohol were made using the same method, 25 g dried plants in 200 ml alcohol and completed up to 1 litter with water. The experiments were carried out under laboratory conditions, treatments being applied on shoots with leaves affected by fall webworm, placed in growth boxes. Each variant had three replications and each replication contained three infested shoots. The treatments were applied with manual small pumps. Efficacy (E%) was calculated after Săvescu-Iacob formula. The majority of alcoholic plant extracts influenced the decrease of leafs consumption as extracts with water. Extracts of metabolites

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influenced the eating with repellent effects against larvae, no palatable etc. The future experiments must use more chemical analyses to discriminate the main metabolites, which influence the worm activities.

Key words: Biopesticides; Azadyrachtin; Spinosad; Milbecmectin; Autochthonous plant secondary metabolites.

REZUMAT: **Eficacitatea unor biopesticide şi metaboliţi secundari ai plantelor împotriva omizii *Hyphantria cunea* Drury (F. Arctiidae – Lepidoptera) în condiţii de laborator.** Lucrarea prezintă eficacitatea unor biopesticide folosite în experimente de combatere a omizii dudului (*Hyphantria cunea*), comparativ cu metaboliţi secundari, extraşi din plantele autohtone. Din prima categorie s-au folosit: spinosad, un metabolit secundar, produs prin fermentarea ciupercii *Saccharopolyspora spinosa*, şi care este substanţa activă a produselor comerciale din clasa Naturalyte; azadirachtinele – un grup de limonoide, obţinute din seminţele din arborii Neem (*Azadirachta indica*), şi milbecmectin, obţinut dintr-un metabolit al bacteriei *Streptomyces hygroscopicus*, subsp. *aureolacrimans*. Rezultatele au arătat eficacitatea biopesticidelor împotriva omizii, în perioada de 2-7 zile de la tratament. Spinosad a prezentat o acţiune rapidă, comparativ cu celelalte două biopesticide. Metaboliţii secundari, folosiţi în combaterea omizii, au fost extraşi din plante autohtone: feriga comună (*Driopteris filix mas*), salvie (*Salvia nemorosa*), pelin (*Artemisia dracunculus*, *A. vulgaris*, *A. absinthium*), mărul lupului (*Aristolochia clematidis*), brânca ursului (*Heracleum spondylium*), balbisă (*Stachis sylvatica*), ventrice (*Tanacetum vulgare*), urzică (*Urtica dioica*), boz (*Sambucus ebulus*) şi tuie (*Taxus baccata*). Extractele au fost obţinute din plante măcinate, folosind 25 g/l de apă rece, agitată timp de 24 de ore. Extractele în alcool etilic au fost făcute după aceeaşi metodă; 25 g de plante uscate în 200 ml alcool, completat până la 1 litru cu apă. Experienţele au fost realizate în condiţii de laborator, tratamentele fiind aplicate pe lăstari cu omizi, plasate în borcane de creştere. Fiecare variantă a avut trei repetiţii şi fiecare repetiţie a conţinut trei lăstari. Tratamentele au fost aplicate cu o pompă manuală. Eficacitatea a fost calculată după formula Săvescu-Iacob. Majoritatea extractelor în alcool a influenţat descreşterea consumului de frunze faţă de extractele în apă. Metaboliţii extraşi au influenţat hrănirea prin efect de repelenţă asupra larvelor, fără palatibilitate etc. Experimentele viitoare trebuie să utilizeze mai multe analize chimice pentru a discrimina metaboliţii principali, care influenţează activitatea omizilor.

Cuvinte cheie: biopesticide; azadyrachtin; spinosad; milbecmectin; Metaboliţi secundari din plante autohtone.

INTRODUCTION

The fall webworm is a dangerous pest, due to presence of two generation and explosive development. The paper presents some characteristics of the biopesticides used in the experiments. Spinosad is a secondary metabolite produces by the fermentation (Thomson *et al.*, 1997) from *Saccharopolyspora spinosa* mushroom and is the active principle of the commercial products of the *Naturalyte* class. This substance is introduced in the IPM due his action mode, the low toxicity against useful insects, animals and environment. Spinosad links himself on the protein part of the nicotine-acetylcholine receptor and induces a sodium ions flux that depolarizes the neurons which becomes hyperactive and induces paralysis of the muscles. Because it acts on a single receptor in the nervous systems of the insects it produces no cross-linked resistance with the known synthetic or biologically insecticides (Salgado, 1997, 1998). His efficacy is similar with those of the majority of the synthetic insecticides, but it acts more rapid as the *Bacillus thuringiensis* bacteria or the *Beuveria* fungus. The product is not systemic, but it translaminates, framing in the IV-th class of toxicity. It acts against Lepidoptera and against the leaf miner flies from orchards, vegetables and ornamental plants (Salat, 2000), against caterpillars of cotton and cabbage, and against citrus trips (Bret *et al.,* 1997; Thompson *et al.,* 1997, 2000).

Azadyrachtines, a group of limonoids obtained from the seeds of the Neem tree (*Azadirachta indica*), that disturbs or inhibits the egg development, blocks the larvae moulting and the development of pupas, disturbs the sexual communication with repellent effects against larvae and adults and also blocks the insect feeding. His efficacy was tested against more than 110 insects; for 75% of them the control was successful. It controls sucking insects and acarians due to his systemic effects. The azadyrachtines degrades oneself easily in the field, the optimal application time must be carefully chosen, usually in the early development stages (young larvae, colonies building, the apparition of the fundatrix). NeemAzal-T/S (1%) 3 l/ha has efficacy against Lepidoptera *Helicoverpa armigera* and *Spodoptera littoralis* from the green beans (Salat, 2000). In concentration of 10%, by applications on the trees stem he has an efficacy of 67-70% against *Chnetocampa processionea*, and by spraying (0.5 %), an efficacy of 50-60% against *Lymantria dispar* (Lehmann, 2000). NeemAzal-T/S in doses of 0.025-1g/l azadyrachtin has destroyed the eggs and larvae of miner moth (*Leucoptera coffella*) from the coffee leaves (Venzon *et al.*, 2004). It is now accepted that neem insecticides have a wide margin of safety for both user and consumer.

Milbecknok (milbecmectin) is a product obtained from a metabolite of the *Streptomyces hygroscopicus* subsp. *aureolacrimans* bacteria. His acting mode is unique, different from those of the conventional acaricides. The insects die without any move or spasm. It is effective against a broad spectrum of insects as: greenhouse whitefly, aphides, Lepidoptera, cicadae, miner insects, trips, nematodes etc; all the development stages of the spiders (egg, larvae, nymph, adult); has a long period of residual activity (about 40-50 days); needs a smaller pause until the harvesting, between 1 and 14 days, depending on the species; has a trans-laminarian effect and it is not affected by the rain; it is not influenced by the ambient temperatures. Is included in the fourth toxicity class and does not affect the bees.

The studies have as purpose the implementation of some biological products in the IPM of fall webworm: Laser 240 SC (spinosad), NeemAzal T-S (azadirachtin), Milbeknock (milbecmectin), in order to replace the chemical insecticides and to establishment efficacy of some vegetal metabolites.

MATERIAL AND METHODS

The experiments were carried out under laboratory conditions, treatments being applied on shoots with leaves affected by fall webworm, placed in growth boxes. The worms were collected from Adâncata stand, where a strong attack is produced and majority were in second and third-instars larvae. Each variant had three replications and each replication contained three infested shoots. The treatments were applied with manual small pumps. Efficacy (E%) was calculated using the Săvescu-Iacob formula:

$$E = [1-a2/(N-M)] x100,$$

where: a2= number of alive worms after treatment; N= total number of worms analysed; M= number of insect with natural death (untreated plot).

There were tested bio pesticides Laser 240 SC (active ingredient spinosad), NeemAzal – T/S (active ingredient azadirachtin) and Milbecknock (active ingredient milbecmectin). The extracts from autochthonous plants were made from dried plants, using 25 g/ 1 litter of cold water, stirred for 24 hours. The extracts in ethylic alcohol were made using the same method; 25 g dried plants in 200 ml alcohol and completed up to 1 litter with water.

The following plants were used: the common ladyfern (*Drioperis filix mas*), the perennial sage (*Salvia nemorosa*), the parsley (*Petroselenium crispum*), the wormwood (*Artemisia dracunculus, A. vulgaris, A. absinthium*) the European birthwort (*Aristolochia clematidis*), cow parsnip (*Heracleum spondylium*), the hedge nettle (*Stachis sylvatica*), the speedwell (*Tanacetum vulgare*), the nettle (*Urtica dioica*), the danewort (*Sambucus ebulus*) and the yew tree (*Taxus baccata*).

According to the literature (Glasby, 2005; Ciulei *et al.* 1993), the plant extracts contain the following secondary metabolites: *Driopterix filix-mas* (dimer: desaspidin); *Artemisia absinthium* (lignan: lirioresinol A, norsesquiterpenoide: 3,6-dihydrochamazulene, 5,6-dihydrochamazulene, diterpenoid: absinthin, sesquiterpenoide: anabsin, anabsinin, artabsin, artabsinolides A, B, C and D, artemolin-a, artemolin-b, 2,3-diepi-artabsinolide C, hydroxypelenolide, ketopelenolide, ketopelenolide a, ketopelenolide b; *A. dracunculus*: (isocumarine: (E)-artemidin, (Z)-artemidin, artemidinol, capillarin, sesquiterpenoid: pathulenol); *A. vulgaris* (monoterpenoid: vulgarole, sesquiterpenoide: spathulenol, vulgarin, triterpenoide: α-amyrin, α-amryin acetate, fernenol); *Aristolochia clematidis* (alkaloid: aristolochine); *Heracleum spondylium* (coumarine: angelicin, bergapten, byakangelicin, heraclesol, imperatorin, isobergapten, isopimpinellin, phellopterin, pimpinellin, xanthotoxin); *Taxus baccata* (alcaloids taxine: taxine I, taxine B, ester: ester myo-inositol-p-coumaric); *Stachis sylvatica* (quaternar alcaloids: betonicine, turicine, iridoide: harpagide, harpagide acetate, diterpenoid: acid stachysic); *Tanacetum vulgare* (sesquiterpenoid: crispolide); *Sambucus ebulus* (iridoid: ebuloside, steroid: stigmast-4-ene-3,6-dione); *Salvia nemorosa* (diterpenoid: nemorone); *Urtica dioica* (caffeic, p-cumarinic and ferulic acids, carotenoides and flavonoids of quercetol).

RESULTS AND DISCUSSION

The control of fall webworm used bio insecticides. After treatment application has been established the efficacy (*Table 1*). The microbial pesticides spinosad are presented a high mortality after two days and milbecmectin after seven days. Despite of these good results, the microbial biopesticides can not to be used in organic farms. Also, the plant metabolite azadirachtin produced maximal mortality after seven days period. It is known that the metabolite blocks the insect feeding, feeding deterrence (including cessation of feeding) and after that dying. Such secondary antifeedant effects result from the disturbance of hormonal and/or other physiological system, e.g. movement of food through the gut, inhibitions of digestive enzyme production, effects on the stomatogastric nervous system etc. (Trumm and Dorn, 2000).

A commercial formulation of neem seed extract, Margosan-O, containing 0.3% AI azadirachtin, were tested under laboratory and field conditions against the European leafroller, *Archips rosanus* L.; in laboratory tests, a 1% aqueous of solution of neem pesticide produced 100% larval mortality within 48 hours treatments (AliNiazee *et al.,* 1997). In the context of pest management, botanical (vegetal) pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post harvest protection of food in developing countries (Isman, 2006). The pyretroid – cipermetrin were very efficient after two days, but biopesticides, less toxic for medium and animal organisms produced also a good mortality.

Table 1 - Efficacy of some biopesticides against fall webworms *Hyphantria cunea*

Biopesticides	Percentage % of worms mortality after days			E%
	2	5	7	
Spinosad (Laser 240 SC) – 0.033%	100			100
Azadirachtin (NeemAzal T-S) – 0.5%	75.3	85.7	100	100
Milbecmectin (Milbeknock EC) – 0.075%	12.5	87.5	100	100
Cipermetrin (Faster 10 CE) – 0.02%	100			100
Untreated plot	0	0	0	

Influence of plant metabolites in leaves consumption by fall webworm. In each Petri dish were located three larvae of fall webworm, which were fed with treated leaves. Generally, all plant metabolites produce lower leave consumption comparative with untreated variants (*Tables 2, 3*).

In evaluation of mood of metabolites extraction, it can see that extracts in ethylic alcohol, were influenced more the feeding of larvae (*Table 2*). Excepted, metabolites of

Artemisia dracunculus, all extracts (*Sambucus ebulus, Artemisia vulgaris, A. absinthium, Tanacetum vulgare, Urtica dioica, Aristolochia clematidis, Heracleum spondylum, Taxus baccata, Salvia nemorosa*) are reduced the feeding which statistical assurance.

The vegetal extracts in cold water had a differential influence about leaves consumption, only four extracts are reduced the feeding which statistical assurance (*Artemisia vulgaris, A. dracunculus, Salvia nemorosa* and *Stachys silvatica*) (*Table 3*).

Table 2 - Influence of plant extracts in ethylic alcohol against feeding fall webworms of *Hyphantria cunea*

Plant metabolites sources	Leaves consumption percentage after number of days (%)		
	1	2	3
Sambucus ebulus	4	25	**33*****
Artemisia vulgaris	7	18	**20*****
Artemisia absinthium	9	23	**32*****
Artemisia dracunculus	22	33	**42**
Tanacetum vulgare	6	13	**27*****
Urtica dioica	4	18	**27*****
Aristolochia clematidis	3	15	**27*****
Heracleum spondylum	5	13	**27*****
Taxus baccata	7	15	**17*****
Salvia nemorosa	7	33	**37*****
Untreated plot	10	28	**83**

Anova test P<0,05

Table 3 - Influence of plant extracts in cold water against fall webworms *Hyphantria cunea*

Plant metabolites sources	Leaves consumption percentage, after number of days (%)		
	1	2	3
Sambucus ebulus	11.6	30	**43.3**
Artemisia vulgaris	20	25	**30*****
Artemisia dracunculus	11.6	20	**30*****
Tanacetum vulgare	16.6	28.3	**36.6**
Urtica dioica	13.3	26.6	**43.8**
Aristolochia clematidis	15	28.3	**36.6**
Heracleum spondylum	23.3	25	**45**
Taxus baccata	25	58.3	**56.6**
Salvia nemorosa	13.3	13,3	**30*****
Driopteris filis mas	21.6	25	**36.6**
Stachys silvatica	16.6	21.6	**26.6*****
Untreated plot	11.6	55	**91.6**

Anova test P<0,05

Many experiments confirm the influence of plant extracts against lepidopterous pests. Also, extracts of *Ocimum basilicum* L., *Origanum majorana* L. and *Salvia officinais* L. are presented toxicity against worms of *Spondoptera littoralis* Boisd. (Roman, 2004). Extract of dried leaves *Melia azedarach* L. mixed with distilled water filtered after 48 hours, applied to leaves of cabbage, destroyed 90% of *Plutella xylostella* L. caterpillars (Berg, 2000). Infusion of *Tanacetum vulgare* are used against codling moth *Cydia pomonella* L.; metabolites of *Urtica dioica* control mites (*Tetranichus utticae*) and aphids (*Macrosiphum rosae, Erisoma lanigerum*); extracts of *Allium cepa* has repellent action against *Pieris rapae* and *P. napi* (Toncea and Stoianov, 2002). Extracts of *Aristolochia baetica* inhibited completely F_1 progeny production of *Tribolium castaneum* Herbst (Jbilou *et al.*, 2006).

CONCLUSIONS

All biopesticides, microbial - spinosad, milbecmectin and botanical – azadirachtin, were very efficacy in the control of fall webworms.

Extracts of plants in ethylic alcohol, were influenced more the feeding of larvae than extracts in water.

It can be concluded that plant metabolites may influence the development of insect. Extracts in ethylic alcohol (*Sambucus ebulus, Artemisia vulgaris, A. absinthium, Tanacetum vulgare, Urtica dioica, Aristolochia clematidis, Heracleum spondylum, Taxus baccata, Salvia nemorosa*) are reduced the larvae feeding which statistical assurance, but four extracts in cold water are reduced the feeding which statistical assurance (*Artemisia vulgaris, A. dracunculus, Salvia nemorosa* and *Stachys silvatica*).

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